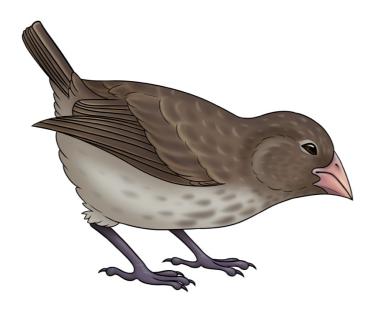


The Avian Vampire Fly, Philornis downsi:

A Parasite's Perspective of Co-evolutionary Dynamics





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Bachelor of Science (Animal Behaviour) (Honours)

Thesis

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Thesis Summary

Invasive species, particularly parasites and pathogens, are an increasing threat to human, animal, and ecosystem health. Although the impacts of invasive parasites on their novel hosts and environment are often known, less is understood about how invasive parasites are affected by invasion. This study explores changes in the accidentally introduced avian vampire fly, *Philornis downsi*, since its 1997 discovery in the nests of Darwin's finches on the Galápagos Islands.

Utilising a long-term dataset across multiple host species, this study explores changes in reproductive behaviour (Chapter 2 & 6), morphology (Chapter 3), mortality (Chapter 4), adult behaviour (Chapter 5) and genetic structure (Chapter 6). Coincident with decreasing body size across decades, *P. downsi* reproductive behaviour has changed. Females are now mating with fewer males, ovipositing earlier in the nesting cycle, and ovipositing fewer eggs per nest. Across time, patterns of intensity and mortality in *P. downsi* differ between host species, a potential precursor to host-specificity. Low genetic differentiation across time suggests that *P. downsi* populations on Floreana Island are not genetically isolated from other island populations. Despite this, island-specific patterns of adult *P. downsi* spatial and temporal abundance highlight the need to understand each island as a separate system, to ensure the success of targeted control techniques.

The use of long-term datasets and a combination of techniques has given unparalleled insight into change in a wild, dynamic host-parasite system. Under extreme selection pressures, *P. downsi* behaviour, genetics and patterns of host association have shifted across time. The results of this study present recommendations for the management of *P. downsi* populations and the conservation of avifauna of the Galápagos Islands.

Declaration

'I certify that this thesis: 1. does not incorporate without acknowledgment any material

previously submitted for a degree or diploma in any university 2. and the research within will

not be submitted for any other future degree or diploma without the permission of Flinders

University; and 3. to the best of my knowledge and belief, does not contain any material

previously published or written by another person except where due reference is made in the

text'

Lauren Kathleen Common

13/12/2022

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It has been an absolute privilege to live my life's dream of obtaining a PhD and studying such an incredible system, a wonderful mix of my favourite things – birds, insects, parasites, and invasive species.

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Statement of Authorship/Contribution

Chapters 1 & 7: 100% - LKC

Chapter 2:

Research design: 70% - LKC, 30% - SK

Manuscript writing: 100% - LKC

Manuscript editing: 85% - LKC, 5% - RYD, 5% - DCN, 5% - SK

Chapter 3:

Research design: 70% - LKC, 30% - SK

Data collection: 25% - SK, 25% - JAOC, 25% - KJP, 25% - RYD

Laboratory analysis: 100% - LKC

Statistical analysis: 100% - LKC

Manuscript writing: 100% - LKC

Manuscript editing: 80% LKC, 5% - JAOC, 5% - RYD, 5% - KJP, 5% - SK

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Research design: 70% - LKC, 30% - SK

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Manuscript editing: 80% - LKC, 5% - PS, 5% - RYD, 5% - DCN, 5% - SK

Chapter 5:

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Data collection: 100% - LKC

Statistical analysis: 80% - LKC, 10% - PS, 10% - SCS

Manuscript writing: 100% - LKC

Manuscript editing: 75% - LKC, 5% - PS, 5% - SCS, 5% - DCN, 5% - RYD, 5% - SK

Chapter 6:

Research design: 60% - LKC, 30% - RYD, 10% - SK

Data collection: 50% - LKC, 25% - SK, 25% - RYD

Statistical analysis: 100% - LKC

Manuscript writing: 100% - LKC

Manuscript editing: 85% - LKC, 5% - DCN, 5% - SK, 5% - RYD

All work presented within this thesis adhered to the legal and ethical requirements of the Government of Australia, the Government of Ecuador, Charles Darwin Research Station (Ecuador), the Galápagos National Park (Ecuador) and Flinders University (Australia).

Publications Associated with this Thesis

Information from this thesis has been published in peer-reviewed journals as follows:

Chapter 2 - Common, LK, Dudaniec, RY, Colombelli-Négrel, D and Kleindorfer, S.

Taxonomic Shifts in *Philornis* Larval Behaviour and Rapid Changes in *Philornis downsi*Dodge & Aitken (Diptera: Muscidae): An Invasive Avian Parasite on the Galápagos Islands.

In Life Cycle and Development of Diptera, 2019. IntechOpen.

Chapter 3 - Common, LK, O'Connor, JA, Dudaniec, RY, Peters, KJ and Kleindorfer, S. Evidence for rapid downward fecundity selection in an ectoparasite (*Philornis downsi*) with earlier host mortality in Darwin's finches. Journal of Evolutionary Biology. 2020; 33: 524–533.

Chapter 4 - Common, LK, Sumasgutner, P, Dudaniec, RY, Colombelli-Négrel, D and Kleindorfer, S. Avian vampire fly (*Philornis downsi*) mortality differs across Darwin's finch host species. Scientific Reports. 2021; 11(1): 1–12.

Chapter 5 - Common, LK, Sumasgutner, P, Sumasgutner, SC, Colombelli-Négrel, D, Dudaniec, RY and Kleindorfer, S. Temporal and spatial variation in sex-specific abundance of the Avian Vampire Fly (*Philornis downsi*). Parasitology Research. 2021; 121 (1), 63-74.

Chapter 6 - Common, LK, Kleindorfer, S, Colombelli-Négrel, D and Dudaniec, RY. Genetics reveals shifts in reproductive behaviour of the invasive bird parasite *Philornis downsi* collected from Darwin's finch nests. Biological Invasions. 2022; 1-19.

Chapter 1 – Introduction

Introduced species and the effects of invasion

In the current era of globalisation, increased rates of human travel and trade have led to a scale and diversity of invasive species not seen before in history (Hulme 2009; Chapman et al. 2017). Invasive species can pose significant threat to human health (Hulme 2014; Zhang et al. 2022), and the economy (Marbuah et al. 2014; Crystal-Ornelas et al. 2021). Invasive species can also have severe detrimental effects on native species and ecosystems, altering ecosystem services (Charles and Dukes 2007; Vilà and Hulme 2017), and reducing biodiversity via local extinction, for example, when an invasive species outcompetes a suite of local species, or an introduced predator consumes a range of prey and expands its range (Schirmel et al. 2016; Mollot et al. 2017). Given the breadth of potential impact, invasive species are considered a primary driver of extinctions (Clavero and García-Berthou 2005; Dueñas et al. 2021), particularly in delicate ecosystems that display high levels of endemism such as islands (Bellard et al. 2016; Spatz et al. 2017). Invasive species may also cause evolutionary changes in native species, through processes that select for host survival, reproductive success, and in some cases are associated with hybridisation (Mooney and Cleland 2001; Le Roux 2021). Understanding these changes is essential for the conservation of native species, and the control of invasive species (Leger and Espeland 2010; Chown et al. 2016; Mayer et al. 2021).

Given their potential severe impacts on ecosystems, invasive species offer an unparalleled opportunity to study adaptation and novel inter-species interactions (Huey et al. 2005; Prentis et al. 2008). Biotic and abiotic conditions in their novel environment can exert strong selection pressure on invasive species (Le Roux 2021), which can lead to rapid adaptation to

new prevailing conditions (Lee 2002; Prentis et al. 2008; Le Roux 2021). For example, dispersal rates in invasive species have been documented to increase significantly, particularly at the edge of a species' range, due to assortative mating of highly dispersive individuals (Shine et al. 2011; Lombaert et al. 2014; Dudaniec et al. 2021). Dispersal ability can also be lost rapidly, particularly on island ecosystems, due to their isolated nature and lower numbers of predators (Wright et al. 2016; Waters et al. 2020). Novel interactions between a range-expanding species and local species can be difficult to monitor and quantify, particularly in wild populations. Species interactions are dynamic and may fluctuate over short time scales, particularly in invasive species systems (Phillips and Shine 2004; Burdon et al. 2013; Bezemer et al. 2014). Therefore, studies of invasive and native species dynamics with fine-scale temporal resolution are useful to better understand the ecology and evolution of species after introduction.

Once established, invasive species are difficult to control and eradicate. The lag-phase that frequently occurs between species introduction and discovery cause significant delays in human response (Crooks et al. 1999; Sakai et al. 2001). Generally, after discovery, invasive populations are too dense and/or large to be easily managed, although eradication is still possible (Simberloff 2003). Control methods and the associated monitoring that is required before and after implementation are costly and time consuming, especially across large spatial scales (Hoffmann and Broadhurst 2016; Jardine and Sanchirico 2018). Many control methods, such as those based on mating systems (e.g., the Sterile Insect Technique: Lance and McInnis 2005; pheromone based mating disruption: Welter et al. 2005) require detailed ecological information to be successful (Simberloff 2003). Biological control, which has been successful in controlling or eradicating many invasive species (Stiling and Cornelissen 2005; Hoddle et al. 2013), similarly requires detailed knowledge about interactions between the

target species and the control agent, and thorough evaluation of risk to native species (Simberloff 2012). This information may not be readily available for the species, or the ecology of the species may have changed after invasion, changing the optimal control technique (Packer et al. 2017). Eradication also requires open collaboration between governments, national parks, landowners, the public and scientists across disciplines (Simpson et al. 2009; Johnson et al. 2017; Packer et al. 2017; Larson et al. 2020). The sharing of pertinent information, such as life history, native and invasive distribution and/or changes in reproductive behaviour, facilitates more effective response to invasive species (Simpson et al. 2009; Johnson et al. 2017; Larson et al. 2020). Long-term studies of wild populations therefore not only benefit our understanding of species' evolutionary trajectories after invasion but are critical in control and eradication efforts.

Host-parasite dynamics after invasion

The relationship between hosts and their parasites is a constantly fluctuating, dynamic system. Parasites impose pressures selecting for host resistance, while hosts impose pressures selecting for parasite infectivity, resulting in reciprocal selection often with coevolutionary outcomes (Woolhouse et al. 2002). These dynamics are often described as the metaphor of the Red Queen (Van Valen 1973; Gandon et al. 2008), where 'it takes all the running you can do, to keep in the same place'. This dynamic may be driven by two differing modes of evolution: arms race dynamics (i.e. directional, recurrent selective sweeps: Buckling and Rainey 2002) or the fluctuating selection dynamics (i.e. negative frequency-dependent selection: Decaestecker et al. 2007). Extreme antagonistic selection pressures can then lead to rapid adaptation in both systems (Duffy and Sivars-Becker 2007; Paterson et al. 2010; Schulte et al. 2010). Studying populations across multiple spatial and temporal scales is

necessary to fully understand host-parasite coevolution (Penczykowski et al. 2016). Much of the recent research into coevolution of dynamics between species use experimental approaches in microparasites and pathogens (Brockhurst and Koskella 2013; Papkou et al. 2019). Studies in wild populations of hosts and parasites are rare, and often lack the temporal component required to document and explore coevolution (Ebert and Fields 2020; Märkle et al. 2021). However natural populations give us the opportunity to understand adaptation and species dynamics in complex non-model systems, such as invasive host-parasite relationships (Feis et al. 2016; Kurtz et al. 2016).

Parasites can affect biological invasions in many ways, through direct and indirect effects, disrupting host-parasite dynamics (Dunn et al. 2012; Dunn and Hatcher 2015; Chalkowski et al. 2018; Llopis-Belenguer et al. 2020). Parasites can directly affect their hosts after invasion, whether they are native or introduced species (e.g., Marzal et al. 2011; Meeus et al. 2011; Keogh et al. 2017). Host population size reductions, parasite loss, and changes to host ecology, genetics, and behaviour due to parasitism may impact native and invasive species indirectly (Dunn et al. 2012). For example, invasive hosts can lose their parasites during invasion, resulting in enemy release (Heger and Jeschke 2014), and can be infected by native parasites (Schatz and Park 2021). If hosts are introduced with their parasites (co-invasion: Lymbery et al. 2014), invasive parasites can spill over into native populations (Daszak et al. 2000) and potentially amplify (spillback: Kelly et al. 2009) or decrease (dilution: Paterson et al. 2011; Poulin et al. 2011) infection rates in native hosts. Parasites can also be introduced without their hosts (e.g., Chapman et al. 2012), likely during free-living stages. The effects of parasitism by invasive parasites on native hosts can vary, depending on parasite virulence, but are often severe (Lymbery et al. 2014; Marzal et al. 2015; Schmid-Hempel 2021).

Extreme selection pressures associated with both introduction and host-parasite dynamics can lead to rapid changes in these systems and warrant in-depth study.

Islands invasions

Island ecosystems are particularly important systems to study and conserve due to their high levels of endemism (Kier et al. 2009). Population declines and extinctions occur on islands at a disproportionate rate compared to mainlands (Vitousek 1988; Tershy et al. 2015). Islands are more vulnerable to the effects of biological invasions (Vitousek 1988; Bellard et al. 2016), with invasive species being a key driver of extinctions on islands (Tershy et al. 2015; Bellard et al. 2016). Despite this, rates of evolution and adaptation are higher on islands (Millien 2006). The rapid adaptation or rapid extinction on islands after biological invasions offer a powerful lens in which one could study evolution. On the Hawaiian archipelago, avian malaria (Plasmodium relictum) was first detected in the 1940s after the introduction of invasive mosquitos (van Riper III et al. 1986; LaPointe et al. 2005). The endoparasite causes severe mortality in native birds, particularly in Hawaiian honeycreepers found at higher elevations (Passeriformes: Fringillidae, Carduelinae) (Samuel et al. 2015). Under extreme selection pressures enforced by avian malaria, some species such as the Hawai'i 'Amakihi (Hemignathus virens) have adapted, now able to tolerate a previously highly virulent parasite (Atkinson et al. 2013). The evolutionary consequences of parasites have been studied on islands of long human habitation. In the age of the Anthropocene, with increased globalisation, human habitation, and habitat loss, it is possible that islands with recent human habitation could be particularly susceptible.

Invasions and the Galápagos Islands

The UNESCO World Heritage Site of the Galápagos Islands, Ecuador, are known as a 'living museum and showcase of evolution' (UNESCO). Only recently inhabited by humans, the archipelago boasts exceptionally high levels of endemism in plants (Tye et al. 2011), reptiles (Torres-Carvajal et al. 2019), birds (Fessl et al. 2015), insects (Peck 2001; Peck 2008), and fishes (McCosker and Rosenblatt 2010). Of the 29 small, native or endemic land birds, seventeen are recognised as threatened with extinction by the IUCN (Fessl et al. 2015; Kleindorfer et al. 2019b). Due to its high endemism, increasing rates of tourism and human habitation, invasive species are the most significant threat to the biodiversity and the economy of the Galápagos (Toral-Granda et al. 2017; Shackleton et al. 2020). The economic impact of invasive species on the Galápagos Islands between 1983 and 2017 is conservatively estimated to be at least \$85 million USD (Ballesteros-Mejia et al. 2021). Invasive species have contributed to the decline and local extinction of many species, such as the Galápagos land iguana (Conolophus subcristatus) on Baltra Island and the Floreana mockingbird (Mimus trifasciatus) on Floreana Island (Curry 1986; Jiménez-Uzcátegui et al. 2006; Jiménez-Uzcátegui et al. 2011). Invasive insects are a group of particular concern, with 545 species introduced to the islands, representing 23% of insect species (Causton et al. 2006; Toral-Granda et al. 2017). Significant research is needed on each introduced species to better understand its ecological impacts, though currently there is a lack of baseline and long-term data on most of these species. Documenting the effects of invasive species on native species and ecosystems gives insights into evolutionary change since introduction and informs management and eradication.

Darwin's finches and Philornis downsi

The Darwin's finches (Passeriformes: Thraupidae) are a diverse group of birds endemic to the Galápagos (17 species; Figure 1.1) and Cocos Islands (1 species). Derived from a common ancestor, likely a grassquit of the genus *Tiaris*, which arrived on the islands ~2 million years ago (Sato et al. 2001), the finch-like tanagers underwent adaptive radiation into 18 species recognised today (Grant 1986; Fessl et al. 2015). Darwin's finches offer an unparalleled opportunity to study adaptation, speciation, and hybridisation in a natural system (Grant and Grant 2002; Kleindorfer et al. 2014a; Lamichhaney et al. 2015; Grant and Grant 2016; Lamichhaney et al. 2018). Populations of Darwin's finches face several threats to their survival (Fessl et al. 2001; Cimadom et al. 2014; De León et al. 2019), with genetic diversity decreasing significantly (Petren et al. 2010) and several species have become locally extinct (Dvorak et al. 2021). Two species are listed as critically endangered on the IUCN Red List: the medium tree finch (*Camarhynchus pauper*), endemic to only the highlands of Floreana Island (Kleindorfer et al. 2021b), and the mangrove finch (*C. heliobates*), which has a single breeding population in the mangrove forests of Isabela Island (Fessl et al. 2010).

All Galápagos land birds, including Darwin's finches, are currently facing their greatest threat, the avian vampire fly, *Philornis downsi* (Muscidae: Diptera; Dodge and Aitken 1968; Figure 1.2) (Causton et al. 2006; Causton et al. 2013; Fessl et al. 2015). *Philornis downsi* is a generalist myasis-causing ectoparasitic fly, whose larvae feed on the blood and tissue of nestlings (Fessl et al. 2006b; Figure 1.3). Accidentally introduced to the Galápagos Islands by 1964, *P. downsi* was not discovered on the Galápagos until larvae were found in the nests of Darwin's finches on Santa Cruz Island in 1997 (Fessl et al. 2001; Fessl et al. 2018). *Philornis downsi* is now widespread across the archipelago, found on 15 of the 17 islands (Figure 1.4), and documented to parasitise over 150 species of birds in both its native and invasive range

(Dudaniec and Kleindorfer 2006; Wiedenfeld et al. 2007; Fessl et al. 2018; McNew and Clayton 2018). Parasitism by *P. downsi* causes anaemia, permanent deformation, and mortality, killing 55% of nestlings across years and host species (Dudaniec et al. 2006; Fessl et al. 2006a; Kleindorfer et al. 2014b; Kleindorfer and Dudaniec 2016; Kleindorfer and Sulloway 2016). Indirect impacts of *P. downsi* via naris deformation in hosts that persists into adulthood include altered song (Kleindorfer et al. 2019a), altered incubation behaviour (Kleindorfer et al. 2021a) and altered foraging behaviour (Kleindorfer et al. 2022). We still have much to learn about *P. downsi* biology, especially regarding adult ecology and behaviour (Bulgarella et al. 2015; Kleindorfer et al. 2016; Lahuatte et al. 2016; Fessl et al. 2018; Pike et al. 2021; Bulgarella et al. 2022).

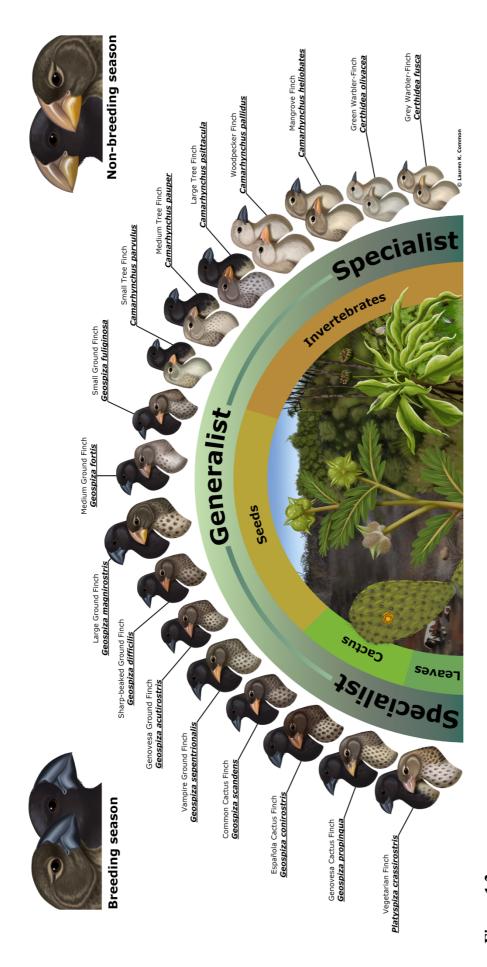


Figure 1.2

generalism or specialism, and preferred dietary item (leaves, cactus, seeds and invertebrates). Beak colour changes in both males and females The seventeen species of Darwin's finch (Passeriformes: Thraupidae) found on the Galápagos Islands. Species are arranged by foraging between seasons: black bills in the breeding season (upper left) and orange bills in the non-breeding season (upper right).

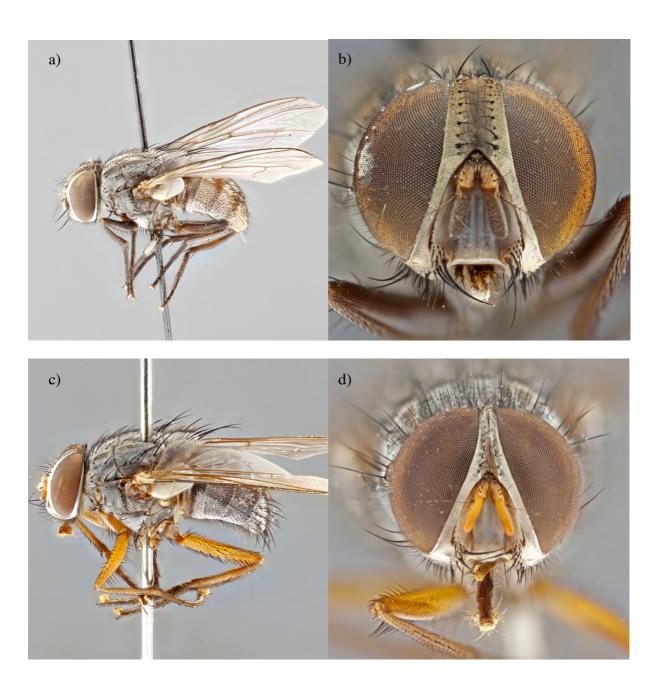


Figure 1.3

Adult *Philornis downsi* photographed by B. Sinclair: a) female lateral view, b) female anterior view, c) male lateral view, d) male anterior view.

Since its discovery on the Galápagos archipelago, there is evidence of shifting dynamics between *P. downsi* and its novel hosts. Coincident with nestlings dying at a younger age, the number of *P. downsi* found in each nest (intensity) has increased across years (Kleindorfer et

al. 2014b). Changes have also occurred between host species. For example, between 2000-2014, *P. downsi* intensity in the nests of warbler finches (*Certhidea olivaecaea*) decreased, with an associated decrease in host mortality (Kleindorfer and Dudaniec 2009; Cimadom et al. 2014). Across the same time period, *P. downsi* intensity and host mortality in the nests of small tree finches (*Camarhynchus parvulus*) increased (Kleindorfer and Dudaniec 2009; Cimadom et al. 2014). Currently, the different causes across species for change in number of *P. downsi* per nest is unknown. We do not have information on changes in *P. downsi* behaviour, morphology, or genetics since its introduction, which is a gap in knowledge this thesis aims to address. Given increasing impacts on threatened and endangered hosts, understanding *P. downsi* on the Galápagos Islands, and how the species has changed after introduction is imperative to inform management and control, and increase our understanding of host and parasite dynamics in general.

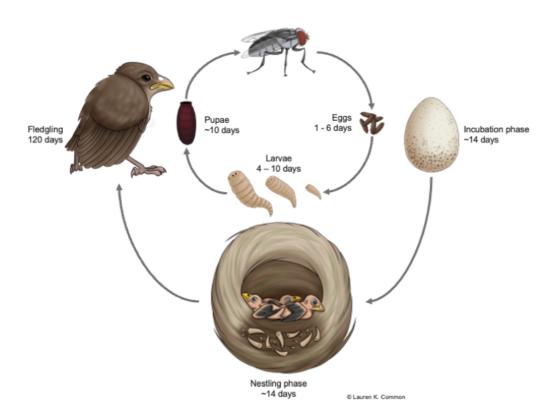


Figure 1.4 Life cycle of *Philornis downsi* in relation to the nesting phase of Darwin's finches. Female *P. downsi* lay their eggs in the base of nests during the incubation phase. *Philornis downsi* eggs hatch approximately simultaneously with the eggs of the host. The larval phase of *P. downsi* lasts between four to seven days, comprising of three larval instars. First and early second larvae reside in the nares and ear canals of nestlings, feeding on blood and keratin. Late second and third instar larvae reside in the base of the nest during the day, feeding on the nestlings both internally (in the nares and ear canals) and externally at night. Larvae then pupate in the base of the nest in a frothy cocoon for approximately 10 days.

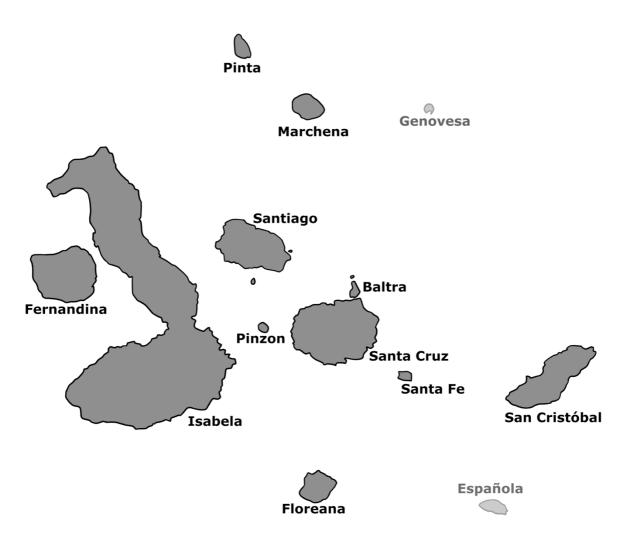


Figure 1.1 Map of the Galápagos Islands, Ecuador. *Philornis downsi* have been documented on all islands except Genovesa and Espanola, represented in pale grey.

Thesis scope and objective

The chapters of this thesis explore how the avian vampire fly (*P. downsi*) has changed since its introduction and discovery on the Galápagos Islands, Ecuador, as well as bettering our understanding of adult *P. downsi*. My thesis aims to explore temporal changes in various aspects of *P. downsi* occurrence at a landscape level, life history and genetics to inform management of invasive populations on the Galápagos and understand host-parasite dynamics in a novel system.

Specifically, this thesis aims to:

- 1. Review and synthesise the literature on the life cycle and behaviour of the genus *Philornis*, with particular focus on how *P. downsi* oviposition behaviour is changing during its invasion.
- 2. Explore changes in *P. downsi* pupae and adult size across time in relation to fecundity selection.
- 3. Determine rates of intensity and larval mortality in *P. downsi* from the nests of different host species.
- 4. Elucidate sex-specific spatial and temporal patterns of distribution of adult *P. downsi* across the breeding season.
- 5. Investigate changes in *P. downsi* genetic structure and reproductive behaviour across time.

Organisation of the thesis

This thesis is presented as a series of manuscripts that are published as a book chapter (Chapter 2) or in scientific, peer-reviewed journals (Chapters 3-6). This thesis is comprised of

five published papers (Chapters 2-6). As each chapter is intended as a separate manuscript, there is some repetition of content. Chapter 2 reviews the literature on *Philornis* systematics, taxonomy, and behaviour, with specific focus on *P. downsi* and evidence for its changing oviposition behaviour on the Galápagos. Chapter 3 explores fecundity selection and its relation to body size in *P. downsi*, and how pupae and adult body size have changed over time. Chapter 4 investigates how *P. downsi* intensity and larval mortality differs across time and different host species. Chapter 5 uses trapping data from the Darwin's finch breeding season to assess sex-specific temporal and spatial patterns in adult *P. downsi* abundance. Chapter 6 utilises genomic techniques to elucidate changes to genetic structure and reproductive behaviour across time. Discussion and synthesis of the main findings and suggestions for future investigation is included at the end of this thesis (Chapter 7).

Chapter 2 – Taxonomic shifts in *Philornis* larval behaviour and rapid changes in *Philornis downsi* Dodge & Aitken (Diptera: Muscidae): An invasive avian parasite on the Galápagos Islands

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Abstract

The parasitic larvae of *Philornis downsi* Dodge & Aitken (Diptera: Muscidae) were first discovered in Darwin's finch nests on the Galápagos Islands in 1997. Larvae of *P. downsi* consume the blood and tissue of developing birds, causing high in-nest mortality in their Galápagos hosts. In the wild, 1st instar larvae reside inside the nares of developing nestlings, while 2nd and 3rd instar larvae consume the blood and tissue of nestlings both externally and within the nares. The fly has been spreading across the archipelago and is considered the biggest threat to the survival of Galápagos land birds. Here, we review (1) *Philornis* systematics and taxonomy, (2) discuss shifts in feeding habits across *Philornis* species comparing basal to more recently evolved groups, (3) report on differences in the ontogeny of wild and captive *P. downsi* larvae, (4) describe what is known about adult *P. downsi* behaviour, and (5) discuss changes in *P. downsi* behaviour since its discovery on the Galápagos Islands. From 1997 to 2010, *P. downsi* larvae have been rarely detected in Darwin's finch nests with eggs. Since 2012, *P. downsi* larvae have regularly been found in

the nests of incubating Darwin's finches. Exploring *P. downsi* ontogeny and behaviour in the larger context of taxonomic relationships provides clues about the breadth of behavioural flexibility that may facilitate successful colonisation.

Introduction

Three genera of flies within the order Diptera have larvae that parasitise avian hosts: *Protocalliphora* Hough (Calliphoridae), as well as *Passeromyia* Rodhain & Villeneuve (Muscidae) and *Philornis* Meinert (Muscidae). The adult flies in these genera are free-living and do not parasitise birds, but their larvae develop in the nests of altricial birds, feed on their avian hosts, and exhibit feeding behaviours from hematophagy to coprophagy (Couri 1999; Teixeira 1999). Most larval infestations have been documented in host nests of the order Passeriformes, but larvae have also been found in nests of Accipitriformes, Apodiformes, Strigiformes and other avian taxa (*Protocalliphora*: (Sabrosky et al. 1989); *Passeromyia*: (Pont and AC 1974); *Philornis*: (Bulgarella and Heimpel 2015; McNew and Clayton 2018)). The effect of these parasitic fly larvae on host survival can be severe to mild, depending on many factors including host population size, body size, nesting density and the presence of behavioural or immunological defence mechanisms (Dudaniec and Kleindorfer 2006; McNew and Clayton 2018; Bulgarella et al. 2019).

Protocalliphora is widely distributed throughout the Holarctric and contains 40+ species with obligate avian parasitic larvae (Sabrosky et al. 1989). Within Muscidae, only *Passeromyia* and *Philornis* larvae parasitise birds (Pont and AC 1974; Skidmore 1985; Couri and Carvalho 2003). Both *Passeromyia* and *Philornis* are members of the subfamily Cyrtoneurininae, however their complete evolutionary relationships have yet to be resolved (Kutty et al. 2014;

Haseyama et al. 2015). Due to the similarities between *Passeromyia* and *Philornis*, many workers regarded the two genera as close relatives, including Skidmore (Skidmore 1985), who stated that their similarities could not be based on convergent evolution alone. The five Passeromyia species include P. steini (Pont), P. heterochaeta (Villenueve), P. indecora (Walker), P. longicornis (Macquart) and P. veitchi (Bezzi), and are distributed throughout Europe, Africa, Asia and Australasia (Pont and AC 1974; Edworthy et al. 2019). Passeromyia species differ in their larval habits. For example, P. steini larvae scavenge nests for organic matter and *P. indecora* larvae consume host resources as subcutaneous parasites. The 52 Philornis species are distributed primarily in Neotropical South America and southern North America (Couri 1999; Teixeira 1999; Couri and Carvalho 2003). Philornis species also show a wide range of feeding habits, including free-living coprophagous larvae, free-living semi-hematophagous larvae, and subcutaneous hematophagous larvae (Table 2.1). One species, P. downsi, is a recently discovered invasive species on the Galápagos Islands (Fessl et al. 2001; Fessl et al. 2018). Its semi-hematophagous larvae cause significant in-nest host mortality in their novel Galápagos land bird hosts (Kleindorfer and Dudaniec 2016). Cladistics and molecular phylogenetic analyses suggest that the parasitic larval habits of Passeromyia and Philornis evolved independently (Couri and Carvalho 2003; Haseyama et al. 2015) despite the similarities between both genera including cocoon-wrapped puparia, life history, and clade.

The Galápagos Islands have been listed as a World Heritage site in 1978. Given a suite of threats, including introduced species, the archipelago was added to the 'List of the World Heritage in Danger' in 2007 and then removed from this list in 2010 because of actions by the Government of Ecuador to reduce invasions (Toral-Granda et al. 2017; Lethier and Bueno 2018). Biological invasion is considered the greatest threat to biodiversity in the Galápagos 17

Islands (Watkins and Cruz 2007). Currently, 543 terrestrial species have been introduced, of which 55 are considered harmful or potentially harmful to native biodiversity (Lethier and Bueno 2018).

In this chapter, we consider changes in the development and behaviour of the accidentally introduced fly P. downsi Dodge and Aitken (Diptera: Muscidae), that is now considered the biggest threat to the survival of Galápagos land birds (Causton et al. 2013). The first *P*. downsi larvae were collected from Galápagos land bird nests on Santa Cruz Island in 1997 (Fessl and Tebbich 2002). From examination of museum specimens collected in 1899 (during the Stanford University Expedition led by Robert Snodgrass and Edmund Heller), in 1905– 1906 and 1932 (during expeditions sponsored by the California Academy of Sciences), and in 1962 (by Robert Bowman) on Floreana Island, there is no current evidence to suggest P. downsi was present or abundant on the Galápagos Islands prior to 1964, though this is possible (Causton et al. 2006; Kleindorfer and Sulloway 2016). By collating information from a range of researchers investigating *Philornis* in general and *P. downsi* in particular, we aim to improve our understanding of the ontogeny and behaviour of an invasive *Philornis* species within the larger context of Dipteran parasites of birds. We review *Philornis* systematics and taxonomy, discuss feeding habits across *Philornis* species, report on differences in the ontogeny of wild and captive P. downsi larvae, report on adult P. downsi behaviour, and describe changes in *P. downsi* behaviour since its discovery on the Galápagos Islands.

Philornis systematics and taxonomy

Macquart (Macquart 1854) provided the first description of *Philornis* larvae when he described Aricia pici; a subcutaneous larval parasite found on an adult Hispaniolan woodpecker (Melanerpes striatus; previously Picus striatus) Muller (Piciformes: Picidae). Meinert (Meinert 1889) erected the genus *Philornis* for the single species, *P. molesta*, based on larvae with distinctive posterior spiracles found parasitising nestlings. At this time, Philornis was suggested to be a synonym for Protocalliphora and assigned to the family Calliphoridae (Becker 1907). In 1921, Malloch (Malloch 1921) proposed the genus Neomusca based on adult specimens, whereas the genus Philornis was based on larval characters. Aldrich (Aldrich 1923) revised this group and synonymized Neomusca with Philornis as independent genera, assigning both within the family Muscidae (Anthomyiidae at the time). This revision was supported by further work on *Philornis* species, as new and previously described species were transferred from other genera including Hylemyia, Mesembrina, Neomusca and Mydaea (Aldrich 1923; Dodge 1963; Dodge and Aitken 1968; Couri 1984; Skidmore 1985). *Philornis* adults are distinguished from other muscid genera by the presence of hair on the anepimeron and the postalar wall (Couri 1999; Savage and Vockeroth 2010).

Using morphological and ecological data, *Philornis* can be divided into three phylogenetic groups: the 'aitkeni-group', the 'falsificus-group' and the 'angustifrons-group' (Couri et al. 2007a). Male characters (given few female specimens) generally define the most basal lineage of *Philornis*, the 'aitkeni-group', including enlarged upper eye facets in holotypic males (Dodge 1963; Couri et al. 2007a). The members of this group display adult character states that are considered primitive among muscids (i.e., enlarged upper eye facets and

presence of cilia on the surface of the wing vein R_{4+5}) (Couri et al. 2007a). This group includes P. aitkeni (Dodge), P. rufoscutellaris (Couri), and P. fasciventris (Wulp). The phylogeny of the aitkeni-group is not completely resolved because of missing information about life history and morphology, as female and larval specimens are not available for many species. The second group, the falsificus-group, is defined primarily by P. falsificus (Dodge and Aitken), whose larvae are free-living (Skidmore 1985). Common morphological characters include five scutellar marginal setae that also place P. fumicosta (Dodge), P. univittatus (Dodge), P. grandis (Couri) and P. sabroskyi (Albuquerque) within this group (Couri et al. 2007a); however, data on the ecology of these species are missing. More information on larval life history is necessary to confirm whether species other than P. falsificus belong in this lineage. Despite a similar life history to P. falsificus, P. downsi is not within the falsificus-group (Skidmore 1985; Couri 1999; Couri et al. 2007a), but forms a sister-group to all species within the angustifrons-group for which larval habits have mostly been documented (Table 2.1). The angustifrons-group is the most recently evolved and largest of the three *Philornis* lineages and contains species with subcutaneous hematophagous larvae as well as *P. downsi* with semi-hematophagous larvae.

Comparative taxonomic analyses of *Philornis* species have been hampered by a lack of specimens and information (Skidmore 1985). For several species of *Philornis*, their morphological descriptions are based solely on one sex, generally males. In others, the holotype is missing or destroyed, and so other traits and ecological information are missing. *Philornis blanchardi* (Garcia) has been originally identified and described in Argentina from a single female specimen, which has since been lost (Couri et al. 2009). This specimen may belong to a previously described species as it has not been captured and identified since, however the original description is considered sufficiently unique that it may be a separate 20

species (Couri et al. 2009). The single male holotype used to describe *P. umanani* (Garcia) has also been lost and due to the lack of detail provided in the original description, this species is deemed unrecognisable and is now considered a *nomen dubium* (Couri et al. 2009). Evidence of a *Philornis* species complex within specimens of *P. seguyi* (Garcia) and *P. torquans* (Nielsen) in Argentina throws further doubt on the original taxonomic characterisation of many *Philornis* species (Quiroga et al. 2016). These issues highlight the need for more extensive molecular and morphological analysis of currently recognised *Philornis* species to confirm species classifications and their evolutionary relationships.

Larval feeding habits across *Philornis* species

Philornis larval behaviour

Philornis species differ in their larval feeding habits, which include coprophagous and hematophagous diets (Table 2.1). Larval habits have been documented for 30 out of 52 described species (Table 2.1). The most basal group in the Philornis phylogeny (aikteni) have free-living coprophagous larvae (Couri et al. 2007a). These larvae parasitise cavity nesting host species that do not remove waste, such as the rufous-tailed jacamar (Galbula ruficauda) Cuvier (Piciformes: Galbulidae) and appear to be specific to this type of nest (Dodge and Aitken 1968; Teixeira et al. 1990; Teixeira 1999; Couri et al. 2007b; Bulgarella and Heimpel 2015). Free-living saprophagous larvae in the nest are regarded as the ancestral trait, evolving into coprophagous larvae, semi-hematophagous larvae and then subcutaneous larvae (Skidmore 1985; Couri et al. 2007a). This transition is also supported in Passeromyia where species show a similar order of descent (Pont and AC 1974; Couri and Carvalho 2003; Couri et al. 2007a). Two documented species, P. downsi (angustifrons-group) and P. falsificus (falsificus-group), have free-living and semi-hematophagous larvae, although other

undescribed species within the *falsificus*-group may also have free-living larvae (Dodge and Aitken 1968; Couri 1999; Couri et al. 2007a).

Most *Philornis* species (83%) have larvae with subcutaneous hematophagous feeding habits, which is also the primary larval strategy in the angustrifrons-group. Within this group, only P. downsi has non-subcutaneous larvae. The semi-hematophagous P. downsi larvae may be similar to P. falsificus (falsificus-group), which is also suspected of having free-living semihematophagous larvae (Couri et al. 2007a) – but not enough is known about the biology of the falsificus-group. While P. falsificus is considered a free-living ectoparasite (Dodge and Aitken 1968), this assessment is limited by the observations to date of later instars and puparia (Leite et al. 2009; Bulgarella et al. 2017). On the other hand, in two species with subcutaneous feeding habits in the angustifrons-group, a few Philornis larvae have been also observed in avian nares. Specifically, P. mimicola larvae have been found in the nares of ferruginous pygmy-owl nestlings (Glaucidium brasilianum) Gmelin (Strigiformes: Strigidae), but most larvae occurred subcutaneously (Proudfoot et al. 2006). Larvae of *P. porteri* (Dodge) have been found in the nares and ear canals of some nestlings (Spalding et al. 2002; Le Gros et al. 2011), and 3rd instar larvae observed to feed externally on the abdomen and wings of their hosts (Kinsella and Winegarner 1974; Spalding et al. 2002). In the semihematophagous P. downsi larvae, 1st instars regularly reside within the avian nares (Fessl et al. 2006b; O'Connor et al. 2010a; O'Connor et al. 2014) and later instars move to the base of the nest where they emerge at dusk and dawn to feed externally on the blood and tissue of the developing birds (O'Connor et al. 2010a; O'Connor et al. 2014). Lineages with free-living larvae have been far less studied than lineages with subcutaneous larvae (Table 2.1). Freeliving larvae move freely within the host nest, detach from the host at various times and reside in the nest base during the day, making them less conspicuous to human observers 22

(O'Connor et al. 2010a; O'Connor et al. 2014). In contrast, subcutaneous larvae reside under the skin of the host and hence can be detected when nestlings are examined.

Philornis downsi larval development in the wild and in the laboratory

Philornis downsi larval instars

Philornis downsi larval development is split into three instar development stages. 1st instar larvae generally reside in the naris and ear canals of developing nestlings, but some have also been found moving freely within the nesting material (Fessl and Tebbich 2002; Silvestri et al. 2011; Cimadom et al. 2014). First instars are commonly collected from 2-3 day old nestlings (Kinsella and Winegarner 1974). Late 2nd and 3rd instar larvae are generally free-living, residing within the base of the nest and feeding externally on nestlings at night (Fessl et al. 2001; O'Connor et al. 2010a; O'Connor et al. 2014). These later instar larvae feed on the blood and fluids of their host by penetrating the skin of the nestlings (Dodge and Aitken 1968; Teixeira 1999). Larval instar morphological descriptions are given by Fessl et al. (Fessl et al. 2006b). The most distinct character between the instars is the posterior spiracles, which change in colour, shape and number of spiracular slits present throughout larval development (Fessl et al. 2006b).

Figure 2.1.1 (A) shows the posterior spiracles of a 1st instar *P. downsi* larva, characterised by their light pigmentation and two oval slits present (Fessl et al. 2006b). The spiracles of a 1st instar larva are separated by slightly more than their diameter. First instar larvae lack anterior spiracles (Figure 2.1.1 B). The posterior spiracles of a 2nd instar larva are similarly round with two oval slits, however the distance between them is two to three times of their diameter (Figure 2.1.2 D; (Fessl et al. 2006b)). Anterior spiracles are present during the 2nd instar, and

semicircular in shape, lightly pigmented and visible in Figure 2.1.2 (E). 3rd instar posterior spiracular plates are darkly pigmented and round in shape, distinct C-shaped spiracular slits radiate from median ecdysial scar (Figure 2.1.3 G). Pigmentation of the median ecdysial scar is light in early 3rd instar larvae and becoming darkly pigmented later in the stage. Semicircular anterior spiracles are retained in 3rd instar larvae (Figure 2.1.3 H). Cephaloskeleton morphology differs between instars as outlined in Fessl et al. (Fessl et al. 2006b). Recent studies report a decrease in *P. downsi* puparia size across 2004 to 2014 (Kleindorfer et al. 2014b; Common et al. Chapter 3), and hence body size is certainly not a useful method to classify instars. In general, it is recommended to use a suite of morphological characters, including anterior and posterior spiracular morphology, to determine the larval instar.

Larval development

The developmental period of *Philornis* larvae is associated with the species' larval feeding habit. For example, time to pupation in coprophagous species takes up to 29 days, but only 4-8 days in subcutaneous species (Uhazy and Arendt 1986; Teixeira 1999). Larval development periods in free-living species such as *P. downsi* are difficult to determine in the wild as the host nest needs to be dismantled to observe the larvae. Early studies of abandoned or failed nests found 1st instar larvae in nests with 1-3 day old nestlings, 2nd instars in nests with 3-6 day old nestlings and 3rd instars in nests with 3-10 day old nestlings (Fessl et al. 2006b). Larval collections following the cessation of activity at host nests suggest that the minimum time for pupation in *P. downsi* is 4-7 days (Kleindorfer et al. 2014b).

Compared with larval development times in the wild, larval development times under laboratory conditions are longer. First attempts to rear *P. downsi* larvae in the absence of a

living host had a low success rate, with only three larvae out of 477 reaching the adult stage after a 36 day development time (mean 18 day larval development, 12 day pupation)

(Lincango and Causton 2008). As the diet for rearing larvae in captivity was refined, the success rate increased to 10% and larval development time decreased (Lahuatte et al. 2016). Development time in the laboratory ranged from 9-10 days from larva to pupa (Lahuatte et al. 2016) with even faster development times occurring as the rearing conditions have improved [pers. comm. P. Lahuatte]. Egg hatch rates in captivity have been high (96%), with most mortality in 1st instar larvae (77%) (Lahuatte et al. 2016). Laboratory-based diets that have been developed in the absence of a bird host are primarily based on chicken blood, with more successful diets including hydrolysed protein and vitamin fortification (Lahuatte et al. 2016). The lack of keratin in the diet may be causing elevated 1st instar mortality, as 1st instars consume the keratin of the beak in which they reside (Fessl et al. 2006b), however the true cause is unknown.

Philornis downsi adult behaviour

The behaviour of adult *P. downsi* is much less understood than that of the larvae. The adult fly is vegetarian, feeding on decaying fruits and flowers, including the invasive blackberry (*Rubus niveus*) Thunb (Rosales: Rosaceae) (Couri 1984; Skidmore 1985; Fessl et al. 2018). *Philornis downsi* is commonly attracted to a mix of blended papaya and sugar, which is used to trap adult flies (developed by P. Lincango and C. Causton; Kleindorfer et al. 2016; Causton et al. in review). This mix is particularly attractive to adult flies due to the presence of yeast and fermentation products such as ethanol and acetic acid (Cha et al. 2016).

A one-year study on Floreana Island found that male and female *P. downsi* display dimorphic flight patterns, with females more likely to be caught in high and low traps (2 metres, most common at 6-7 metres), and males more likely to be caught in traps of intermediate height (4-5 metres) (Kleindorfer et al. 2016). As the pattern of male and female abundance are quadratic opposites, this has tentatively been suggested to be an advantage for females to avoid male flies, as frequent mating in other Dipterans has been found to decrease female reproductive success and lifespan (Bateman 1948; Fowler and Partridge 1989). This flight pattern may also explain why certain host species experience higher parasite intensities, such as the medium tree finch (*Camarhynchus pauper*) Ridgway (Passeriformes: Thraupidae) that has an average nest height of 6 m, thus making it more susceptible to being encountered by female *P. downsi* (O'Connor et al. 2010b; Kleindorfer et al. 2014a; Kleindorfer et al. 2016). However, the factors that cause bird species to experience differing intensities of *P. downsi* are complicated and vary between years. Comparison of flight height in *P. downsi* on different islands is needed to test the generality of this pattern, which may be influenced by average tree height and/or other ecological variables.

Mating behaviour

The mating behaviour of *Philornis* in general is not well understood, though there are some insights into $P.\ downsi$ mating patterns. While mating has not been observed at or inside the nest, multiple $P.\ downsi$ flies have been video recorded to enter host nests concurrently (O'Connor et al. 2010a; Ramirez 2018). Analysis of offspring genetic relatedness has provided estimates of the re-mating frequency of $P.\ downsi$ (Dudaniec et al. 2010). Evidence for multiple mating by females has been frequently detected, and each larval infrapopulation (i.e., within nests) is sired by 1 to 5 males (average \sim 1.9 males per female) (Dudaniec et al.

2010). How *P. downsi* adults find each other to initiate mating is unknown. Pheromones for attraction and aggregation in muscid flies have been identified and studied (Carlson et al. 1971; Chapman et al. 1998; Jiang et al. 2002). Cuticular compounds show promise for determining if *P. downsi* produces pheromones, as females and mature males showed distinct cuticular profiles and females respond to chemicals produced by males (Collignon 2011; Doherty 2012; Collignon et al. 2014). Cuticular profiles could be developed as an attractant to capture flies in the field (Lance and McInnis 2005; Causton et al. 2013).

Oviposition behaviour

Studies into oviposition in the genus *Philornis* have revealed that species spanning diverse larval feeding habits are oviparous (Couri 1984; Skidmore 1985; Couri 1999; Meier et al. 1999; Patitucci et al. 2017). This current view has previously been hotly debated, in part because the majority of species remain unstudied. Laboratory rearing and field observation have confirmed that *P. downsi* is oviparous (Lincango and Causton 2008; O'Connor et al. 2010a; Lahuatte et al. 2016). *Philornis* flies enter and oviposit in nests regardless of nesting phase or nestling age but have not been observed to enter nests abandoned by the parent birds during the incubation phase (Young 1993; O'Connor et al. 2010a). From in-nest video recordings, *P. downsi* flies have been observed entering nests throughout the day, but generally during dusk between 1500 and 1800, with visiting rates peaking around 1700 (O'Connor et al. 2010a; Ramirez 2018). Visit length averaged 1.3-1.5 minutes and occurred most commonly when the adult host is away from the nest and completed once the adult host returned (O'Connor et al. 2010a; Ramirez 2018). Eggs have been generally deposited on nesting material and the base of the nest (O'Connor et al. 2010a; Lincango et al. 2015), however on one occasion, eggs have been also laid directly by the naris of a nestling

(O'Connor et al. 2010a). A genetic study of *P. downsi* larvae estimated that 1 to 6 adult females (average ~3 females) oviposit within a single nest, supporting previous observations of different sized larval groups within nests and suggesting repeated nest infestations throughout the nestling period (Dudaniec and Kleindorfer 2006; Dudaniec et al. 2010).

Effects of host species on Philornis behaviour and microbiome

Philornis downsi is one of the most generalist species within the genus, known to infest 38 host species across avian taxa (Bulgarella and Heimpel 2015; Couri et al. 2018; McNew and Clayton 2018). However, this high host number may reflect the large number of studies focused on *P. downsi* due to its invasive status on the Galápagos Islands (Kleindorfer and Dudaniec 2016; Fessl et al. 2018).

It is currently unclear how *Philornis* species in general or *P. downsi* in particular find their hosts. Preliminary studies into the role of semiochemicals and volatiles in host nests as an attractant for *P. downsi* have produced inconclusive results (Doherty 2012). Long-term ornithological field studies have provided some hints that the intensity of host cues may be relevant for *P. downsi* search behaviour, or alternatively that the density of host nests influences *P. downsi* oviposition behaviour. Aggregated host nests may attract *P. downsi* females due to an increase in olfactory or visual cues. These aggregated nests also provide a greater opportunity for *P. downsi* females to infest multiple nests. Indeed, small tree finch nests (*Camarhynchus parvulus*) Gould (Passeriformes: Thraupidae) with close neighbours contained more *P. downsi* larvae compared to solitary, more isolated nests (Kleindorfer and Dudaniec 2016). Nests in areas of lower nesting density (i.e., lowlands) have been more likely to contain the offspring of a single *P. downsi* female than nests in areas of higher

nesting density (i.e., highlands) that are more likely to contain the offspring of many *P*. *downsi* females (Dudaniec et al. 2010). Video recordings of adult *P. downsi* have been made inside the nests of the small ground finch (*Geospiza fuliginosa*) Gould (Passeriformes: Thraupidae), medium ground finch (*G. fortis*) Gould (Passeriformes: Thraupidae), small tree finch (*C. parvulus*) and Galápagos flycatcher (*Myiarchus magnirostris*) Gould (Passeriformes: Tyrannidae) (O'Connor et al. 2010a; Ramirez 2018; Pike et al. in prep). However, despite a combination of video recorders inside or outside the nest across studies, the recordings did not reveal information about *P. downsi* search behaviour from its flight behaviour.

A metagenomic study into *P. downsi* larval microbiome sampled from different host species found an effect of host diet on the gut bacterial community of *P. downsi* larvae (Ben-Yosef et al. 2017). Larvae retrieved from strictly insectivorous warbler finch (*Certhidea olivacea*) Gould (Passeriformes: Thraupidae) nests have a different microbiome structure compared with larvae parasitising hosts with broader dietary preferences (ground and tree finches, *Geospiza* and *Camarhynchus* sp., respectively) (Ben-Yosef et al. 2017). The gut microbiome also differed between *P. downsi* larvae (blood diet) and adults (plant diet), supporting the hypothesis that *P. downsi* microbiome changes during development and according to diet (Ben-Yosef et al. 2017). Further behavioural, biochemical and genetic studies are needed to understand *P. downsi* oviposition across host species, host locating behaviour and host specificity.

Changes in *P. downsi* behaviour since colonising the Galápagos Islands

Age of larval cohort in host nests

There is evidence that the oviposition behaviour of female *P. downsi* has changed since its discovery on the Galápagos archipelago. *Philornis downsi* flies are now known to oviposit during any stage of the nesting cycle (O'Connor et al. 2010a). In the first decades following initial discovery of *P. downsi* in Darwin's finch nests, changes in the proportions of instar classes amongst *P. downsi* have been observed, with evidence that oviposition occurred earlier and more synchronously in the nesting phase in the later years of the study (Kleindorfer et al. 2014b). Synchronisation in oviposition date may lead to an increase in larval competition for host resources, and as a consequence result in increased virulence for nestlings that must contend with a greater number of large, mature larvae at a younger age (Kleindorfer and Dudaniec 2016). The fitness consequences of female oviposition behaviour are further supported by observations in other *Philornis* systems. Host nests that are infested later in the nesting cycle are more likely to have higher fledging success than nests parasitised early in the nesting cycle (Rabuffetti and Reboreda 2007; Segura and Reboreda 2011).

Larval feeding on adult birds

Philornis larvae are generally exclusive parasites of developing nestlings, whether they be subcutaneous or free-living semi-hematophagous species. Infestation of host nests can happen quickly and is often observed within 24 hours of the first nestling hatching (Kinsella and Winegarner 1974; Spalding et al. 2002; Couri et al. 2005; Rabuffetti and Reboreda 2007). Many studies on *Philornis* species in their native range found no evidence of larvae present during incubation (Arendt 1985a; Young 1993; Nores 1995; De la Peña et al. 2003).

There have been a few cases of larvae feeding on adults in subcutaneous species (Oniki 1983; Mendonça and Couri 1999; Herrera and Bermúdez 2012), however these reports are rare, with generally only a few larvae per adult. For this reason, larval feeding on adults is generally regarded as opportunistic (Teixeira 1999). More data are needed to examine the oviposition behaviour of *Philornis* species to determine whether larvae are present during the incubation phase.

On the Galápagos Islands between 1998 and 2005, there have been no reported cases of P. downsi larvae present in host nests with eggs that would suggest that larvae also feed on incubating females. Two studies during this time period specifically stated that no P. downsi larvae have been found during host incubation (Table 2.2) (Fessl and Tebbich 2002; Cimadom et al. 2016). On Santa Cruz Island during 1998 to 2010, published studies report findings for 38 nests with eggs that have been inspected for the presence of P. downsi and found no larvae (Table 2.2) (Fessl and Tebbich 2002; Cimadom et al. 2016). In 2012, Cimadom and colleagues first observed P. downsi larvae in host nests during incubation where larvae have been found present in 17 of the 26 nests inspected (Cimadom et al. 2016). Since this initial observation, the prevalence of *P. downsi* in host nests with eggs has increased to 80% in some species and years on Santa Cruz Island, with larvae and puparia found in 70 of 177 nests inspected with eggs (Cimadom et al. 2019). Concurrently across this time period, brooding Darwin's finch females have P. downsi antibodies that are associated with decreased *P. downsi* intensity, but not increased fledging success (Huber et al. 2010; Koop et al. 2013b). This suggests that *P. downsi* larvae on the Galápagos Islands may have switched to feed on adult finches at some stage (Huber et al. 2010). On Floreana Island, inspection of nests that failed during incubation during 2006 and 2016 found P. downsi larvae in 4 of 72 (5.6%) nests with host eggs (Table 2.2). In 2006, three medium ground finch (G. 31

fortis) nests with eggs in the arid lowlands have *P. downsi* larvae and puparia, and in 2010 one highland small tree finch (*C. parvulus*) has *P. downsi* larvae during the egg stage. During a period of intense drought from 2003 until 2006 with less than 300 mm of rain per year in the lowlands, there were very few active host nests available for oviposition, which may be an explanation for a shift in *P. downsi* female oviposition and larval feeding on incubating females at the end of the drought during 2006. Notably, smaller larvae and eggs are not easily visible in nests and it is possible that *P. downsi* is present, but not detected during incubation in the early years of study.

In laboratory trials, P. downsi hatching success is found to be the same in nests with host eggs and nests with finch hatchlings (Lonchura striata domestica) Linnaeus (Passeriformes: Estrildidae) (Sage et al. 2018). In these trials, there is even a fitness benefit for *P. downsi* that hatched during incubation and hence earlier during the host cycle, as they survived for longer (Sage et al. 2018). Other than P. downsi, there is one report of an unidentified Philornis species parasitising adults in the pearly-eyed thrasher (Margarops fuscatus) Vieillot (Passeriformes: Mimidae) studied in Puerto Rico (Arendt 1985b). About 46% of incubating and brooding females and 13% of attending adult males sustained subcutaneous *Philornis* (Arendt 1985b). It has been suggested that this *Philornis* species may have invaded Puerto Rico, as the patterns of prevalence and host mortality mirror that of the P. downsi invasion in the Galápagos Islands (Arendt 1985a, b; McNew and Clayton 2018). Philornis consumption of attending adult hosts may be an oviposition tactic that is more prevalent under conditions of resource limitation. Resource limitation could be influenced by resource termination such as early host death, resource availability when there is a limited supply of host nests (e.g., during drought), and resource accessibility, for example when competition within and between fly cohorts changes (Kleindorfer et al. 2014b).

Conclusions

As one of three avian nest parasitic genera in Diptera, the genus *Philornis* provides a useful system to explore shifts in larval feeding behaviour in native and invasive species. *Philornis* downsi has been accidentally introduced to the Galápagos Islands and first observed in the nests of Galápagos land birds in 1997. In this chapter, we explored similarities and differences between P. downsi larval development and behaviour with what is known from the other 52 *Philornis* species. More basal *Philornis* (aitkeni-group) species have free-living coprophagous larvae and more recently evolved *Philornis* (angustifrons-group) tend to have subcutaneous hematophagous larvae with the exception of P. downsi that has free-living semi-hematophagous larvae. Since its introduction to the Galápagos Islands, there have been documented changes in the behaviour of P. downsi. During the early years after initial discovery of *P. downsi* on the Galápagos Islands, oviposition behaviour was asynchronous across the nesting cycle and larvae appeared to have fed exclusively on developing nestlings until 2005. In later years, P. downsi oviposition behaviour was earlier in the nesting cycle and more synchronous, and since 2006, larvae have also been recorded to feed on incubating females. The first records of *P. downsi* larvae in host nests with eggs rather than hatchlings occurred at the end of a four-year drought on the Galápagos in 2006. Since 2012, up to 80% of host nests with eggs may contain P. downsi larvae on Santa Cruz Island. Larval feeding by P. downsi on adult birds has been observed in laboratory finches and in one Philornis system (species unknown) in Puerto Rico. In light of changes in *P. downsi* larval feeding behaviour, we provided a description and photos of the larval instars for use in field identification. We compiled the observations to date of *Philornis* behaviour and ontogeny within a broad taxonomic framework and summarised patterns of change in the oviposition behaviour of P.

downsi in its (presumably) novel habitat on the Galápagos Islands. By examining *P. downsi* in relation to other *Philornis* species, we provided a broad phylogenetic context for the potential behavioural repertoire of an invasive species under conditions of intense natural selection in a novel environment.

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Table 2.1

Philornis species ordered according to taxonomy, from the most basal 'aitkeni-group' to the most recently evolved 'angustifrons-group' (groups from (Couri et al. 2007a)). Larval feeding habits are shown where known and abbreviated as follows: free-living coprophagous larvae (FLC), free-living semi-hematophagous larvae (FLSH), subcutaneous hematophagous larvae (SubH). The following nine species are not included in the list as they are currently not assigned to a taxonomic group (Couri et al. 2007a) given insufficient information: P. molesta, P. nielseni, P. blanchardi, P. cinnamomina, P. convexus, P. mima, P. obscurus, P. steini, P. umanani.

ʻaitkeni' group	Larval	'falsificus'	Larval	'angustifrons' group	Larval
	Habits	group	Habits		Habits
P. fasciventris (Couri et al. 2007b)	FLC	P. fumicosta		P. downsi (Dodge and Aitken 1968)	FLSH
P. schildi		P. univittatus		P. niger (Dodge and Aitken 1968; Couri 1999)	SubH
P. lopesi		P. grandis		P. porteri ¹ (Kinsella and Winegamer 1974)	SubH
P. aikteni (Dodge and Aitken 1968)	FLC	P. sabroskyi		P. mimicola ^{2(Proudfoot et al.} 2006)	SubH
P. zeteki		P. falsificus (Dodge and Aitken 1968; Couri 1999)	FLSH	P. spermophilus (Couri 1999)	SubH
P. rufoscutellaris (Teixeira et al. 1990)	FLC		1	P. carinatus (Young 1993)	SubH
P. rettenmeyeri				P. deceptiva (Arendt 1985a, b)	SubH
P. setinervis				P. trinitensis (Dodge and Aitken 1968)	SubH
	1			P. glaucinis (Dodge and Aitken 1968)	SubH
				P. pici (Macquart 1854)	SubH
				P. vespidicola ^{3(Teixeira} 1999)	SubH
				P. medianus (Couri et al. 2007a)	SubH
				P. vulgaris (Couri 1999)	SubH
				P. diminutus (Couri 1999)	SubH
				P. querulus (Dodge and Aitken 1968)	SubH

P. albuquerquei	
P. frontalis (Couri 1999)	SubH
P. gagnei (Couri et al. 2007a)	SubH
P. insularis (Couri et al. 2007a)	SubH
P. obscurinervis	
P. petersoni	
P. torquans ¹	SubH
P. angustifrons (Dodge and Aitken 1968)	SubH
P. bellus(Teixeira 1999)	SubH
P. sanguinis (Dodge and Aitken 1968)	SubH
P. seguyi ⁴ (Rabuffetti and Reboreda 2007; Quiroga and Reboreda	SubH
2012)	

¹ Some *P. porteri* larvae found in ear canals and nares of nestlings; some later instars found feeding externally on abdomen and wings (Kinsella and Winegarner 1974; Spalding et al. 2002)

² *P. mimicola* larvae found in the nasal cavity of owls, mainly subcutaneous on body (Proudfoot et al. 2006)

³Only known specimens of *P. vespidicola* collected from nests of the wasp *Paracharitopus* frontalis (Hymenoptera: Vespidae) (Dodge 1963; Teixeira 1999)

⁴P. nielseni proposed synonym of P. seguyi (Couri et al. 2009)

Table 2.2 Evidence of *Philornis downsi* larvae present in nests during incubation and before nestling hatching in studies on the Galápagos Islands. The islands are abbreviated as Santa Cruz (SC), Floreana (FL), Isabela (IS), Daphne Major (DMj). The 'total number of nests examined' refers to all active nests monitored over the course of the study and 'number inspected during egg phase' is the sample size for the sub-set of nests examined during host incubation (usually following abandonment or predation) where 'na' denotes that nests have been not sampled during the egg phase. The column 'P. downsi larvae during the egg phase' states 'yes/no' referring only to nest inspections that occurred during the egg phase. Host species are abbreviated as small tree finch (ST), large tree finch (Camarhynchus psittacula) (LT), small ground finch (SG), medium ground finch (MG), woodpecker finch (Cactospiza pallida) (WP), warbler finch (Certhidea olivacea) (WF), cactus finch (Geospiza scandens) (CF), Galápagos mockingbird (GM), smooth-billed Ani (Crotophaga ani) (SBA), yellow warbler (Dendroica petechia) (YW), dark billed cuckoo (Coccyzus melacoryphus) (DBC), vermillion flycatcher (Pyrocephalus rubinus) (VF), vegetarian finch (Platyspiza crassirostris) (VGF), and Galápagos flycatcher (Myiarchus magnirostris) (GF).

Ref	Year	Island	Host species	Total no. of nests examined/no. inspected during egg phase	P. downsi larvae during the egg phase	Comments
Fessl, Tebbich (2002)	1998, 2000	SC	ST, LT, SG, MG, WF, WP, CF, SBA, YW, VF, DBC, GM	105/17	No	Larvae not found in 17 SG, ST, WF and WP nests that failed during incubation
Cimadom et al. (2016)	1998 to 2010	SC	ST, WF	na/21	No	Larvae not found in 21 ST and WF nests abandoned during

						incubation (reported as part of a study during 2012-2015 listed below)
Dudaniec et al. (2006)	2004	SC, FL, IS	SG	24/na		
Fessl et al. (2006a)	2000, 2004	SC	SG, MG	27/na		Larvae not found in SG and MG nests depredated shortly after host hatch
Fessl et al. (2006b)	2000, 2004, 2005	SC	SG, MF, CF	63/na		
Dudaniec et al. (2007)	1998, 2000, 2001, 2002, 2004, 2005	SC	SG, MG, ST, LT, WP, WF	249/na		
Wiedenfeld et al. (2007)	1998, 2000, 2003 - 2005	islands incl. SC and FL		515/na		
Huber (2008)	2004 - 2006	SC	MG	63/na		
Kleindorfer, Dudaniec (2009)	2000 - 2002, 2004	SC	ST, LT, SG, WF, WP	43/na		
Huber et al. (2010)	2008	SC, DMj	MG			Brooding female MG had <i>P. downsi</i> -specific antibodies, suggesting nesting females are parasitised
O'Connor et al. (2010a)	2008	FL	ST, SG, MG	11/5	No	Larvae not found in 4 SG and 1 ST nests abandoned with eggs
O'Connor et al. (2010b)	2006, 2008	FL	ST, MT	63/2	No	Larvae not found in 2 MT nests depredated during egg phase
O'Connor et al. (2010)	2004 - 2006	FL	SG	71/na		

Kleindorfer (unpubl. data)	2006	FL	MT, SG, MG	129/27	Yes	Larvae and puparia found in 3 MG nests abandoned with eggs in the arid lowlands
Kleindorfer (unpubl. data)	2010	FL	ST, MT, SG	153/38	Yes	Larvae found in 1 ST nest depredated with eggs in the highlands
Koop et al. (2011)	2008	SC	MG	48/na		
Koop et al. (2013a)	2009	SC	MG	61/na		
Koop et al. (2013b)	2010	SC	MG	43/na		Female MG in parasitised nests had more <i>P. downsi</i> antibodies and spent more time standing upright when brooding than non-parasitised nests
Knutie et al. (2013)	2010	SC	MG	30/na		
Kleindorfer et al. (2014a)	2005- 2010	FL	ST, MT	43/na		
Kleindorfer et al. (2014b)	2004, 2006, 2008, 2010, 2012, 2013	FL	ST, MT, SG	561/na		Evidence that <i>P</i> . downsi oviposition behaviour occurred more synchronously and earlier in nesting phase in later years of the study
Knutie et al. (2014)	2013	SC	ST, SG, MG, VGF	26/na		
O'Connor et al. (2014)	2010	FL	SG	14/na		
Lincango et al. (2015)	2014	SC	GF	1/na		
Knutie et al. (2016)	2012, 2013	SC	MG, GM	127/na		
Kleindorfer et al. (2016)	2004 - 2006, 2008, 2010,	FL	ST, MT, SG	254/na		

Heimpel et al. (2017) Ramirez (2018)	2012 - 2014 2013, 2014 2015	SC SC	VGF GF	11/na 2/na		
Knutie (2018)	2013	SC	MG, GM	37/na		
Cimadom et al. (2019)	2012, 2014 - 2017	SC	ST, WF	850/177	Yes	Larvae and puparia found in 18/72 ST nests and 52/105 WF nests that failed during egg phase; range in prevalence across species and years was 0% to 80% of nests
McNew et al. (2019)	2012, 2013, 2015, 2016	SC	GM	131/na		
Peters et al. (2019)	2010, 2013, 2014	FL	ST, MT	27/na		

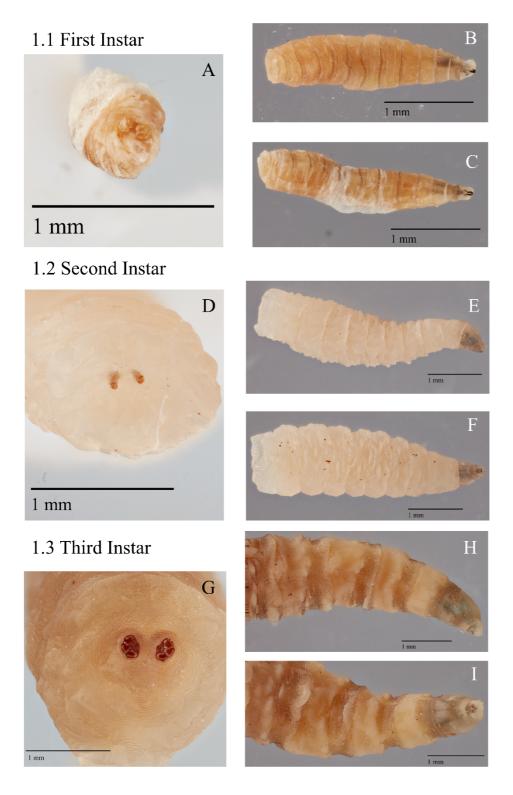


Figure 2.1 Three larval stages of *Philornis downsi*. 1.1 First instar: (A) Posterior spiracles, (B) lateral view, (C) ventral view. 1.2 Second instar: (D) Posterior spiracles, (E) lateral view, (F) ventral view. 1.3 Third Instar: (G) Posterior spiracles, (H) lateral view, (I) ventral view.

Obtained by the authors from larvae collected on Floreana Island, Galápagos, Ecuador between 2010 and 2014. The photographs were taken using a Visionary Digital LK imaging system (Dun, Inc) with a Canon EOS 5DsR camera and Capture One Pro 11.3.1, Phase One (Flinders University). Images were produced using Zerene Stacker 1.04, Zerene Systems LLC, software, and cropped and resized in Photoshop CS5.

Chapter 3 – Evidence for rapid downward fecundity selection in an ectoparasite (*Philornis downsi*) with earlier host mortality in Darwin's finches

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Abstract

Fecundity selection is a critical component of fitness and a major driver of adaptive evolution. Trade-offs between parasite mortality and host resources are likely to impose a selection pressure on parasite fecundity, but this is little studied in natural systems. The 'fecundity advantage hypothesis' predicts female-biased sexual size dimorphism whereby larger females produce more offspring. Parasitic insects are useful for exploring the interplay between host resource availability and parasite fecundity, because female body size is a reliable proxy for fecundity in insects. Here we explore temporal changes in body size in the myiasis-causing parasite *Philornis downsi* (Diptera: Muscidae) on the Galápagos Islands under conditions of earlier in-nest host mortality. We aim to investigate the effects of decreasing host resources on parasite body size and fecundity. Across a 12-year period, we observed a mean of $\sim 17\%$ *P. downsi* mortality in host nests with $55 \pm 6.2\%$ host mortality, and a trend of $\sim 66\%$ higher host mortality throughout the study period. Using specimens from 116 Darwin's finch nests (Passeriformes: Thraupidae) and 114 traps, we found that over

time, P. downsi pupae mass decreased by ~32%, and male (~6%) and female adult size (~11%) decreased. Notably, females had ~26% smaller abdomens in later years, and female abdomen size was correlated with number of eggs. Our findings imply natural selection for faster P. downsi pupation and consequently smaller body size in P. downsi, and consequently lower parasite fecundity in this newly evolving host-parasite system.

Introduction

Fecundity selection affects fitness by favouring traits associated with increased reproductive output (Roff 2001). Few studies examine fecundity selection (Pincheira-Donoso and Hunt 2017), and those that do generally focus on traits that increase fecundity (upward selection) (Orozco and Bell 1974; Preziosi and Fairbairn 1996; Välimäki and Kaitala 2007; Saino et al. 2017). Although traits that increase or decrease fecundity covary, far fewer studies have observed downward selection on traits leading to decreased fecundity (Orozco and Bell 1974; Nunney 1996; Reeve and Fairbairn 1999; Quintero-Fong et al. 2018). To better understand the role of fecundity selection on variation in biological fitness we need case studies that identify temporal patterns and processes of fecundity change. Host-parasite systems make excellent candidates for such case studies given their tight co-evolutionary interactions that depend on fecundity and survival. Thus, the relationship between parasite virulence and host mortality can be explored to understand the drivers and direction of fecundity selection.

The 'fecundity advantage hypothesis' was originally formulated by Darwin (1896) to explain the common occurrence of large female body size (Shine 1989; Cox et al. 2003). Across taxa, female body size is positively associated with fecundity (Pincheira-Donoso and Hunt 2017), as larger-bodied females can physically accommodate more offspring and can store more

energy to invest in reproduction (Calder 1996). Strong positive fecundity selection can generate directional selection for increased female body size in insects (Sivinski and Dodson 1992; Andersen 1994; Teder and Tammaru 2005; Hurlbutt 2008) and other taxa (Braña 1996; Scharf and Meiri 2013), and can also result in the increased size of particular body regions (i.e. trunk or abdomen) that are functionally linked to fecundity (Preziosi et al. 1996; Olsson et al. 2002; Parker et al. 2011; Winkler et al. 2012). Parasitic insects provide useful systems to test ideas about effects of body size on fecundity because parasite diets can be tracked through host availability (Nijhout, 2003; Lahuatte et al., 2016). In this way, parasitic insects can provide insights into changing body size and fecundity with altered nutritional conditions.

Parasites must balance virulence and fitness with maximising host resource use to ensure life cycle completion before host death (Hatcher et al. 2012). Increased host exploitation may lead to larger body size and higher fecundity, but could result in early termination of host and eventually population collapse as host populations are exhausted (Hatcher et al. 2012). Recent host-parasite associations undergoing co-evolutionary interactions are therefore ideal case studies for examining changing fecundity selection under unstable host resource pressures.

Here we focus on natural selection for small body size in the fly, *Philornis downsi* (Diptera: Muscidae) (Dodge and Aitken), which is an invasive myiasis-causing parasite of Darwin's finches on the Galápagos Islands. *Philornis downsi* larvae consume the blood and tissue of nestling birds, causing up to 100% in-nest mortality in some of its Darwin's finch hosts (Dudaniec and Kleindorfer 2006; O'Connor et al. 2010b; Kleindorfer et al. 2014b; Fessl et al. 2018). The adult fly has been present in the Galápagos since at least 1964 (Causton et al. 45

2006), but its larvae were first reported in Darwin's finch nests on Santa Cruz Island in 1997 (Fessl et al. 2001) despite long-term field study into Darwin's finches on other islands since 1973 (Grant and Grant 2002). Field research found P. downsi requires ~4-7 days to develop through three instar stages and reach pupation (Kleindorfer et al. 2014b; Common et al. 2019). In this newly evolving host-parasite system, mortality has been high in both P. downsi, and its Darwin's finch hosts. On average, about 17% of P. downsi larvae die in the host nest and about 55 ± 6.2 % of Darwin's finch nestlings die in the nest from P. downsi parasitism (Kleindorfer and Dudaniec 2016). In addition to the high mortality it exerts, P. downsi parasitism has on average been killing nestling hosts at an earlier age of 5.4 ± 0.3 days post-hatch in 2014 compared to 10.6 ± 0.5 days post-hatch in 2004 (O'Connor et al. 2010b; Kleindorfer et al. 2014b). Questions remain as to how this earlier termination in parasite resources (nestling hosts) affects life-cycle completion, body size and fecundity in P. downsi, and in turn, how the evolution of virulence may be affected.

In this study we use nine years of field data spanning a 12-year period to examine changes in body size (an indirect measure of fecundity) in the dipteran ectoparasite, *P. downsi*, in response to the increasingly earlier death of its host. Given that there is a near perfect correlation between insect body size and fecundity (Honěk 1993; Preziosi et al. 1996; Armbruster and Hutchinson 2002; Tammaru et al. 2002), we analyse body size in adult *P. downsi* flies and pupae as indicators of *P. downsi* fecundity across years. If natural selection favours faster pupation and smaller body size as the consequence of earlier host mortality, we predict (a) smaller size in *P. downsi* pupae and adult flies from 2004 to 2016. If natural selection for smaller body size favours lower fecundity via trade-offs between virulence and host resources, then we predict (b) a larger decrease in female body size relative to male body size in *P. downsi* adults. Together, this knowledge contributes to our understanding of how 46

shifting host mortality in the natural environment directly selects for parasite body size as the consequence of faster pupation, which may lead to an indirect selection pressure on female fecundity.

Materials and methods

Study site and study species

We collected data from long-term field study sites on the islands of Santa Cruz (Kleindorfer et al. 2006; Kleindorfer 2007a; Cimadom et al. 2014) and Floreana (O'Connor et al. 2010b; Kleindorfer et al. 2014b) in the Galápagos Archipelago. We conducted field work during nine Darwin's finch breeding seasons spanning the months of February to April over 12 years: 2004, 2005, 2006, 2008, 2010, 2012, 2013, 2014, 2016. On each island, study sites were located in both the arid lowland zone (El Garrapatero, -0.686479, -90.223775, and El Barranco, -0.739068, -90.301467 on Santa Cruz; habitat surrounding the town of Puerta Velasco Ibarra and La Loberia, -1.279932, -90.485927, on Floreana Island) and in highland Scalesia forest (Los Gemelos, -0.625982, -90.384829, on Santa Cruz; sites along the trail at the base of Cerro Pajas volcano, -1.299974, -90.452710, on Floreana Island). We sampled P. downsi from the following host species: small tree finch (Camarhynchus parvulus), hybrid Camarhynchus tree finch (cross between C. pauper and C. parvulus as well as introgressed individuals) (Kleindorfer et al. 2014a; Peters et al. 2017), medium tree finch (C. pauper), woodpecker finch (C. pallidus), small ground finch (Geospiza fuliginosa) and medium ground finch (G. fortis) (Table 3.S1). For analysis, we tested effects of host species and host genus (Camarhynchus, Geospiza) on P. downsi body size.

Adult *P. downsi* flies are vegetarian and feed on decaying plant material, so they do not pose a direct threat to Darwin's finches (Couri 1985; Skidmore 1985). However, the fly oviposits in active finch nests when the attending female is absent (O'Connor et al. 2010a; O'Connor et al. 2014; Lahuatte et al. 2016), and multiple female flies may oviposit in a single nest (Dudaniec et al. 2010). After P. downsi eggs hatch, 1st instar larvae enter the nares and body cavities of the nestling and reside there to feed on blood and tissue (Fessl et al. 2006b). During the night, 2nd and 3rd instar larvae emerge from the nest base to feed internally and externally on the body of nestlings (Fessl et al. 2006b; O'Connor et al. 2014; Kleindorfer and Sulloway 2016). After feeding for ~4-7 days, 3rd instar larvae pupate in the nest base, forming a frothy cocoon, and adult flies emerge after 7-14 days (Kleindorfer et al. 2014b; Lahuatte et al. 2016). Although field research has found that P. downsi requires ~4-7 days to develop through three instar stages and reach pupation (Kleindorfer et al. 2014b); lab studies have found that pupation occurs at \sim 7-10 days (Lahuatte et al. 2016; Bulgarella et al. 2017). P. downsi parasitism causes higher than average nestling mortality in 10 out of 17 Darwin's finch species in which the interaction has been studied (Kleindorfer and Dudaniec 2016; Fessl et al. 2018), with surviving nestlings commonly showing physical deformation of the naris into adulthood (Galligan and Kleindorfer 2009; Kleindorfer and Dudaniec 2016; Heimpel et al. 2017; Kleindorfer et al. 2019a).

Philornis downsi collection from Darwin's finch nests

We monitored 116 Darwin's finch nests for nesting outcome using our well-established field protocols (Kleindorfer et al. 2014b) in all sampling years except 2005. Upon nesting termination (fledging or death of the last nestling), each nest was collected in a sealed plastic bag, and all *P. downsi* larvae, pupae, empty puparia and adult flies were counted within 1-24

hours of collection. All *P. downsi* samples were stored in 90% ethanol immediately after counting. *Philornis downsi* intensity in the nest was measured as the total number of larvae, pupae, puparia and adult flies present upon collection of the nest. The sample size per year and host genus (*Camarhynchus*, *Geospiza*) is provided in Table 3.S1.

Philornis downsi collection from McPhail traps

We placed a total of 114 McPhail Traps in the lowlands and highlands of Santa Cruz and Floreana Island to sample adult *P. downsi* flies in the years 2004, 2005, 2012, 2013 and 2014 (for details see Table 3.S1). The McPhail traps were baited with a liquid lure of blended papaya, water and white sugar (following trapping protocol developed by P. Lincango and C. Causton) that was replaced every 7 days. Traps were hung in trees along 4 x 90m transects and flies were collected twice per week and stored in ethanol. In 2014 on Floreana Island, we placed 28 McPhail traps along four transects, seven traps per transect, at heights of 2 to 7 metres. In other years and locations, traps were placed *ad hoc* every 50 m within 100 m x 200 m plots spanning a 2 km transect within study sites. We analysed data from 46 lowland traps and 68 highland traps (Table 3.S1).

Pupa mass and size

Mass (g), length and width (mm) were measured for each pupa, as these measurements are known to be highly correlated with adult fly size (Gauld and Fitton 1987; Shingleton et al. 2008; Stillwell et al. 2011; Quiroga and Reboreda 2013), and can therefore be an indirect indicator of an individuals' fecundity upon maturity (Orozco and Bell 1974; Preziosi and Fairbairn 1996; Välimäki and Kaitala 2007; Saino et al. 2017). Pupae cannot be sexed, therefore these data could not be used for sexual dimorphism analysis but are useful when

looking at general temporal shifts in body size in the *P. downsi* population. All pupae were removed from ethanol and placed on filter paper to dry for 30 seconds before taking measurements (Armbruster and Hutchinson 2002). We measured the total mass of all intact pupae per nest and divided this by the number of pupae to calculate average pupa mass (Thomas et al. 2018). The pupae were weighed to the nearest 0.001 g using an A&D HR-200 Digital Analytical Balance. The length (mm) and width (mm) of the largest pupa per nest was measured using digital callipers. For analysis we used the average mass per nest. Pupa mass was measured from the nests of 19 *C. parvulus* (268 pupae), 10 hybrid *Camarhynchus* tree finch (55 pupae), 25 *C. pauper* (332 pupae), 57 *Geospiza fuliginosa* (816 pupae) and 5 *G. fortis* (52 pupae) (Table 3.S1).

Adult P. downsi size

We measured body size for 38 male and 38 female adult *P. downsi* from nests, and 34 male and 85 female adult *P. downsi* from McPhail traps. From the 43 nests and 114 McPhail traps sampled, we measured one male and one female adult fly unless there was only one sex present, in which case we used one sample per nest or trap. We visually sorted all fly specimens per sex for each nest or trap from smallest to largest and selected the median-sized fly as the specimen for analysis. This approach was used because we measured the average pupa mass per nest, and also to avoid any possible pseudoreplication due to genetic relatedness among the fly specimens. For each specimen, we used callipers with 0.1 mm accuracy to measure head length (mm), thorax length (mm) and abdomen length (mm), all measured with the specimen ventral side up; wing length (mm), measured from the base of the basicosta to the tip of the wing; and body length (mm), which was calculated from the values of head, thorax and abdomen length combined. For seven specimens, the head was

missing due to previous DNA extractions; for 23 specimens we only have data on body length as the specimens were destroyed for a separate study (Dudaniec et al. 2010). Therefore, sample size for head length (N=188) and body length (N=211) versus thorax, abdomen and wing length (N=195) differ.

Philornis downsi body size and fecundity

To assess if the overall pattern of association between abdomen size/body size and number of eggs in *P. downsi* is comparable with the pattern reported in other Diptera studies, we collated published r and r² values across 17 studies (Table 3.S2). Collated values were used to calculate average r² and 95% CI, and compared to the pattern found in *P. downsi*. We randomly sampled and dissected 10 female *P. downsi* specimens collected from McPhail traps at 4m in the study area on Floreana Island in 2014 (Kleindorfer et al. 2016). One specimen was collected from a different trap and/or different collection week to ensure independence of data. Specimens were stored in 70% ethanol at room temperature for at least 24 hours before dissection, and were dissected under a stereomicroscope at 16x magnification to count the total number of eggs present in ovaries (Malmqvist et al. 2004). We limit the sample size as the specimens are valuable intact for our long-term study and our aim is to test for an already established pattern of association in Diptera.

Statistical analysis

Data were analysed with SPSS version 25.0. The summary data are presented as mean ± standard error, unless otherwise stated. Data were checked for normality to satisfy requirements of parametric tests. We tested the association between abdomen size/body size and the number of eggs present in ovaries using linear regression analysis. We completed

Principal Component Analysis (PCA) on mean pupae mass, length and width to assess overall changes in pupae size. One Principal Component was retained, Pupae Size, which explained 85.96% of the total variation within these variables (Eigenvalue = 2.579) (Table 3.S4). We used a Generalized Linear Mixed Model (GLMM) to test for an effect of year on pupae size with PC Pupae size as the dependent variable, year, island and habitat as fixed factors, and species as a random factor. We then used linear regression to test for changes in pupae mass, length and width separately to investigate whether each variable displays a different pattern of change across time.

We explored adult fly size across years and in relation to sex (male, female). To assess overall changes in adult body size, we completed a PCA on abdomen length and body length. One Principal Component, fly size, was extracted which explained 91.4% of the variation within these three variables (Eigenvalue = 1.828) (Table 3.S5). We used a GLMM to test for an effect of year on adult fly size using PC Fly size as the dependent variable, and year, sex, year \times sex as fixed factors, and island as a random factor. There was no difference in body size of adult flies collected from Darwin's finch nests or McPhail traps (independent t-test: head length: t = -0.003, P = 0.998; thorax length: t = -0.188, P = 0.851; abdomen length: t = 1.804, P = 0.074; wing length: t = 0.629, P = 0.530). Therefore, nest and trap data were pooled to test for the effect of year on *P. downsi* head, thorax, abdomen, wing and body length separately using linear regression analysis. We conducted linear regression analyses separated by sex to examine for sex differences. We derive all statistical conclusions from the GLMM analyses, but present individual regression analyses for comparative purposes.

Results

Pupae size and mass

Only the fixed factor year had a significant effect on pupae size ($F_{1,112} = 30.814$, P < 0.001); no other covariate or interaction term was related to P. downsi size (Table 3.1). There was no effect of species on pupae size (Wald Z = 0.540; P = 0.589). Using regression analysis, P. downsi pupae mass was negatively correlated with year ($F_{1,115} = 30.709$, r = -0.461, P < 0.001, N = 115) (Figure 3.1), as was length ($F_{1,115} = 12.086$, r = -0.310, P = 0.001, N = 115) and width ($F_{1,115} = 33.450$, r = -0.476, P < 0.001, N = 115). Pupae mass decreased up to 32.9% (0.073 ± 0.003 g to 0.049 ± 0.006 g), pupae length by 5.8% (10.09 ± 0.12 mm to 9.50 ± 0.39 mm) and pupae width by 10.6% (4.17 ± 0.06 mm to 3.73 ± 0.15 mm). Since 2004, P. downsi pupa have become significantly lighter, shorter, and narrower (Table 3.S3). We found the same pattern when analysing the data separately for pupa collected from the nests of Camarhynchus finches (N = 53; mass: $F_{1,53} = 17.476$, r = -0.502, P < 0.001; length: $F_{1,53} = 10.476$, r = -0.409, P = 0.002; width: $F_{1,53} = 28.045$, r = -0.592, P < 0.001) and Geospiza finches (N = 61; mass: $F_{1,61} = 15.286$, r = -0.451, P < 0.001; length: $F_{1,61} = 4.197$, r = -0.256, P = 0.045; width: $F_{1,61} = 13.219$, r = -0.425, P = 0.001).

Adult P. downsi size

We found a correlation between number of eggs and female body length ($F_{1,9} = 7.085$, $r^2 = 0.47$, P = 0.029, N = 10) (Figure 3.S1) and abdomen length ($F_{1,9} = 5.917$, $r^2 = 0.43$, P = 0.041, N = 10) (Figure 3.S2). We calculated the coefficient of determination (r^2) from 17 studies on Diptera (3.S2) that published the association between abdomen size and body size and the number of eggs. The overall r^2 in Diptera was 0.39 (95% CI 0.28 – 0.50), and hence our values are in line with previous studies.

We found an effect of year and sex on P. downsi adult size (Year: $F_{1,184} = 19.435$, t = -4.972, P < 0.001; Sex: t = -2.075, P = 0.039) (Table 3.1). Next, we explored each *P. downsi* body size variable. Combining adult males and females, there was a significant negative correlation between year and *P. downsi* head length ($F_{1,187} = 8.394$, r = -0.208, P = 0.004, N = 188), thorax length $(F_{1,194} = 12.438, r = -0.246, P = 0.001, N = 195)$, abdomen length $(F_{1,194} =$ 13.321, r = -0.254, P < 0.001, N = 195) (Figure 3.2), wing length ($F_{1,194} = 33.335$, r = -0.384, P < 0.001, N = 195) and total body length ($F_{1,187} = 18.459$, r = -0.650, P < 0.001, N = 211). Adult flies were 7.6% smaller across the study period (8.44 \pm 0.05 mm to 7.80 \pm 0.17 mm), with abdomen length decreasing 7.2% (3.74 ± 0.11 mm to 3.47 ± 0.33 mm). We found similar patterns when analysing the data separately for P. downsi adults collected from McPhail traps (sexes pooled; N = 119; head: $F_{1,114} = 4.356$, r = -0.193. P = 0.039; thorax: $F_{1,114} = 4.356$, r = -0.193. P = 0.039; thorax: $_{118} = 3.423$, r = -0.169, P = 0.067; abdomen: $F_{1,118} = 21.433$, r = -0.393, P < 0.001; wing: $F_{1,118}$ = 6.236, r = -0.225, P = 0.014; total body length: $F_{1,75} = 23.140$, r = -0.412, P < 0.001) and P. downsi adults reared from pupae collected from Darwin's finch nests (sexes pooled; N = 72; head: $F_{1,72} = 5.263$, r = -0.263. P = 0.025; thorax: $F_{1,75} = 14.598$, r = -0.406, P < 0.001; abdomen: $F_{1,75} = 3.397$, r = -0.210, P = 0.069; wing: $F_{1,75} = 24.595$, r = -0.499, P < 0.001; total body length: $F_{1,72} = 8.503$, r = -0.327, P = 0.005).

Male vs. female adult fly size across years

Due to the significant interaction of year \times sex on fly size (F _{1,184} = 4.336, t = 2.082, P = 0.039), we explored the differences in body size between the sexes in more detail. From 2004 to 2016, adult male *P. downsi* wing length and head length became smaller (wing: F_{1,72} = 19.147, r = -0.463, P < 0.001, N = 72; head: F_{1,69} = 4.989, r = -0.261, P = 0.029, N = 70) but

there was no effect of year on male thorax length ($F_{1.69} = 2.393$, r = -0.182, P = 0.126, N = 72), or abdomen length ($F_{1.71} = 3.223$, r = -0.210, P = 0.077, N = 72) (Figure 3.2). Male head length decreased 15.0% across the study period (1.53 \pm 0.10 mm to 1.30 \pm 0.10 mm), and wing length decreased 7.0% (8.70 \pm 0.25 mm to 8.09 \pm 0.07 mm). There was no trend for smaller body length in adult males ($F_{1.69} = 3.866$, r = -232, P = 0.53, N = 82). In adult female P. downsi, there was a negative correlation between year and wing length ($F_{1.121} = 20.045$, r = -0.378, P < 0.001, N = 122), thorax length ($F_{1.121} = 12.776$, r = -0.310, P = 0.001, N = 122), abdomen length ($F_{1.121} = 12.591$, r = -0.308, P = 0.001, N = 122) (Figure 3.2), and body length ($F_{1.117} = 20.058$, r = -0.384, P < 0.001, N = 129), but no effect of year on female head length ($F_{1.117} = 3.533$, r = 0.172, P = 0.063, N = 118). Across the study period, female thorax length decreased 18.0% (3.21 \pm 0.18 mm to 2.63 \pm 0.06 mm), abdomen length decreased by 25.6% (3.83 \pm 0.14 mm to 2.85 \pm 0.06 mm) and wing length decreased by 12.9% (8.59 \pm 0.17 mm to 7.48 \pm 0.09 mm).

Discussion

Our findings show a change in *P. downsi* pupae and adult body size between 2004 and 2016 that is coincident with increasing in-nest mortality in both parasite and host (Kleindorfer and Dudaniec 2016) in a newly evolving host-parasite system. Across the time-period sampled, we found up to a 25% reduction in *P. downsi* pupae and adult size but greater size reduction in females than males. Therefore, these results support evidence that natural selection favours faster pupation and smaller body size as the consequence of earlier host mortality in both sexes, and also that natural selection for smaller body size may favour lower fecundity because only abdomen size was smaller in females. Abdomen length in female insects is a trait functionally linked with fecundity. Female abdomen length decreased across years,

while male abdomen length did not, which underscores fecundity changes in this system. Under conditions of early host death and high risk of in-nest *P. downsi* mortality, natural selection favours larvae that pupate earlier and at smaller a body size, and this smaller size at pupation results in adult flies with lower fecundity, supported by a correlation between female body size and the number of eggs. We do not know whether environmental plasticity or genetic changes explain variation in pupa and adult size, but both processes can be shaped by natural or sexual selection (Blanckenhorn 2000; Perry et al. 2018). Smaller *P. downsi* body size and lower fecundity may have implications for parasite competition within host nests and the evolution of host virulence in Darwin's finches.

The impact of *P. downsi* on native and endemic Galápagos bird species cannot be overstated: nestlings are being heavily parasitised, nestling hosts experience intense competition within nests to avoid being parasitised, and most die in the nest (O'Connor et al. 2010a; O'Connor et al. 2014). An average of 45% of parasitised birds fledge (Kleindorfer and Dudaniec 2016) but those that survive often have bill abnormalities due to early instar larval feeding, which has implications for song characteristics and mate choice (Kleindorfer and Dudaniec 2016; Kleindorfer and Sulloway 2016; Kleindorfer et al. 2019a). With the prediction that lower parasite fecundity should covary with lower virulence, Kleindorfer and Dudaniec (2016) found that the number of *P. downsi* in finch nests increased by 46% across the decade but that patterns of host mortality on both Floreana and Santa Cruz Island remained stable at a high ~55% per year (Kleindorfer et al. 2014a; Kleindorfer et al. 2014b; Kleindorfer and Dudaniec 2016). This suggests that forms of parasite resistance could be evolving in the host, or *P. downsi* is evolving to be less virulent – perhaps with the benefit of securing host resources for longer. Our data support the latter suggestion, with evidence for lower *P. downsi* fecundity corresponding with earlier pupation in more recent years.

Given that P. downsi requires between 4-7 days to pupate in the field, the early death of host nestlings at ~ 5 days post-hatch is likely to exert strong selection pressures on larval development (Kleindorfer et al. 2014b; Lahuatte et al. 2016). Insect larvae are generally required to reach a critical mass in order to pupate, after which they can pupate immediately or continue to grow (Nijhout and Callier 2015). Larvae can pupate faster and at a smaller size when starved after reaching that critical mass (Nijhout and Callier 2015). Shorter development times have been linked with decreased body sizes in Dipterans (Butlin and Day 1984; Lehmann et al. 2006), and larvae with resource termination or fewer resources during development were smaller as adults (Williams and Richardson 1983; Singh and Bala 2009). In P. downsi, earlier termination of host resources has likely led to shorter developmental periods, resulting in the smaller pupa size we observed. Understanding *P. downsi* developmental biology is critical for developing control strategies, with recent research gaining new insights into conditions that stimulate egg hatching in the field (Sage et al. 2018), and the effect of larval diet on pupal mass and developmental duration in a laboratory setting (Lahuatte et al., 2016). In the absence of a host, first instar P. downsi survived for up to five days, suggesting that larvae have the capacity to exploit and survive under conditions of unpredictable resources (Sage et al. 2018), however body size and condition after starvation are not yet known.

Although decreasing *P. downsi* body size is coincident with early host termination, there may be other factors driving body size in this system. Density-dependent parasite competition for limited resources may also affect developmental rate, body size and hence fecundity, a process that is well documented in Dipteran flies (Peckarsky and Cowan 1991; Lieske and Zwick 2008; Shiao and Yeh 2008). In nests of Darwin's finches, *P. downsi* intensity varies 57

considerably (Kleindorfer and Dudaniec 2016), and the genetic relatedness of larvae indicates that multiple adult female flies oviposit eggs in a single nest (mean = 3.04 ± 0.21), and multiple males (mean = 1.97 ± 0.08) sire the offspring of each female, with an average of five offspring per female (range 1-24 offspring per female) (Dudaniec et al. 2010). Relatedness among larvae in finch nests is therefore very low, while studies have found that decreased genetic relatedness can increase competitive interactions within species, which in turn may compromise fitness (Frank 1994). However, such interactions and any concurrent shifts in the genetic relatedness of *P. downsi* are yet to be examined.

Host switching by parasitising more than one host life stage may increase development time due to suboptimal resources. Previously, *P. downsi* larvae were only present in Darwin's finch nests once the host nestlings had hatched (Fessl and Tebbich 2002; O'Connor et al. 2014). However, in recent years, there have been a growing number of observations of *P. downsi* larvae in nests during the incubation phase suggesting that larvae are feeding on incubating females (Cimadom et al. 2016; Common et al. 2019). Incubating female finches have been found to express *P. downsi*-specific antibodies (Huber et al. 2010), and females with higher antibody levels were found to have fewer parasites in their nest (Koop et al. 2013b; Knutie et al. 2016). Parasitising incubating female finches may provide compromised nutrition for larvae due to the presence of *P. downsi*-specific antibodies, protective feathers, and behavioural adaptations such as the consumption and removal of larvae from nests (O'Connor et al. 2010a). Despite these potential costs, earlier host infestation during female incubation may be an attempt to prolong larval developmental period due to the narrowing window of nestling resources imposed by earlier host mortality.

Male and female P. downsi showed different size trajectories as adults, with evidence for strong downward selection on abdomen size in females, but not in males. Findings from multiple studies have reported substantial benefits of being a larger-bodied female (Blanckenhorn 2000; Esperk 2006), such as the associated increase in fecundity (Honěk 1993; Head 1995; Calder 1996; Tammaru et al. 2002). Notably, the significant decrease in female abdomen length we observed suggests an impact of earlier pupation on fly fecundity. Natural selection for faster pupation can have fecundity impacts when body size and specific body regions linked with reproductive output are affected by development time (Wickman and Karlsson 1989; Olsson et al. 2002; Winkler et al. 2012; Pincheira-Donoso and Hunt 2017). Downward fecundity selection has been documented far less frequently than upward fecundity selection (Nunney 1996; Preziosi and Fairbairn 1996; Reeve and Fairbairn 1999; Quintero-Fong et al. 2018), and most evidence for downward selection comes from laboratory studies rather than natural systems (Preziosi and Fairbairn 1996). It is important to note limitations with using the magnitude of female-biased sexual size dimorphism to determine the strength of fecundity selection as discussed in Pincheiro-Donoso and Hunt (2017). Due to the effects of sexual selection on sexual size dimorphism (Cox and Calsbeek 2009), research into the strength of sexual selection in P. downsi populations should be conducted to determine the driving factors for changing male and female body size.

Host parasite co-evolution is rarely observed in natural systems, and biological invasions by parasites offer an opportunity to explore co-evolutionary processes (Feis et al. 2016).

Understanding the effects of downward fecundity selection on female oviposition behaviour, larval competition within nests, and virulence patterns in Darwin's finches will further unravel the host-parasite co-evolutionary dynamics occurring in this system. This study

provides further understanding of host-parasite coevolution during invasion and parasite trade-offs of fecundity and nutrition under strong natural selection.

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Table 3.1

Coefficients of the generalized linear mixed model of pupae size and adult fly size. The test statistic was t for fixed factors and Z for random factors.

PC Pupae Size	Estimate	SE	Test statistic	P-value
Intercept	286.370	51.596	5.550	<0.001
Year	-0.143	0.026	-5.551	<0.001
Island	0.501	0.348	1.438	0.153
Habitat	0.103	0.227	0.453	0.651
Species	0.021	0.038	0.540	0.589
PC Adult Size	Estimate	SE	Test statistic	P-value
Intercept	325.560	65.523	4.969	<0.001
Year	-0.162	0.033	-4.972	<0.001
Sex (female) ¹	-211.557	101.934	-2.075	0.039
Year×Sex	0.105	0.051	2.082	0.039
Island	0.090	0.142	-0.629	0.530

¹ For Sex, male was set to zero

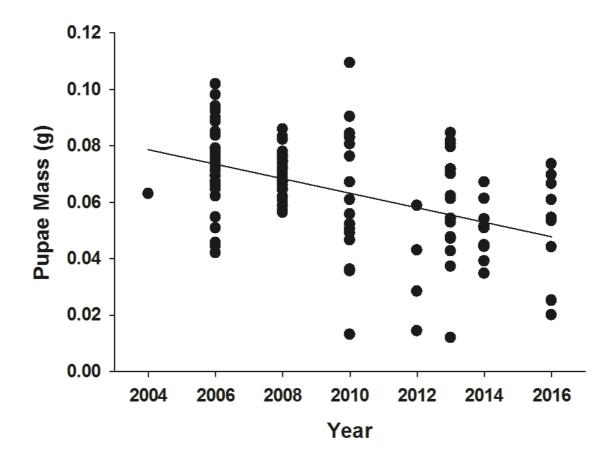


Figure 3.1

Mass (g) of *Philornis downsi* pupae collected from the nests of Darwin's finches between 2004 and 2016.

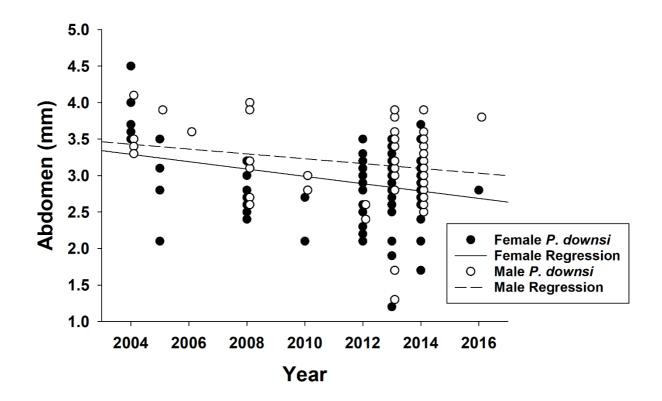


Figure 3.2

Abdomen length (mm) of male and female *Philornis downsi* adult flies collected from the nests of Darwin's finches and McPhail Traps between 2004 and 2016.

Summary data for all samples used in analysis. ID, N = nest, T = trap, S = sample of unknown origin (either nest or trap). Island: FL = Floreana, SC = Santa Cruz. Habitat: HL = highlands, LL = lowlands.

ID	Year	Island	Habitat	Species	Pupae	Male Adult	Female Adult
N1	2004	FL	HL	G. fuliginosa	1	0	0
N10	2006	FL	HL	C. parvulus	1	0	0
N100	2013	FL	HL	C. pauper	1	1	1
N101	2013	FL	HL	C. hybrid	1	0	0
N102	2013	FL	HL	C. parvulus	1	0	0
N103	2013	FL	HL	C. hybrid	1	0	0
N104	2013	FL	HL	C. hybrid	1	1	1
N105	2013	FL	HL	C. hybrid	1	0	0
N106	2013	FL	HL	C. hybrid	1	0	0
N107	2013	FL	HL	C. hybrid	1	0	0
N108	2013	FL	HL	C. hybrid	0	1	0
N109	2013	FL	HL	C. pauper	0	1	1
N11	2006	FL	HL	C. parvulus	1	0	0
N110	2013	FL	HL	C. parvulus	0	1	1
N111	2013	FL	HL	C. pauper	1	0	0
N112	2013	FL	HL	C. hybrid	0	1	1
N113	2014	FL	HL	C. parvulus	0	1	1
N114	2014	FL	HL	C. pauper	1	1	1
N115	2014	FL	HL	C. hybrid	1	1	1
N116	2014	FL	HL	G. fortis	1	0	0
N117	2014	FL	HL	G.fuliginosa	1	1	1
N118	2014	FL	HL	G.fuliginosa	0	1	1
N119	2014	FL	HL	G.fuliginosa	1	1	1
N12	2006	FL	HL	C. parvulus	1	0	0
N120	2014	FL	HL	G.fuliginosa	1	1	1
N121	2014	FL	HL	C. parvulus	0	1	1
N122	2014	FL	HL	C. hybrid	0	1	1
N123	2014	FL	HL	C. hybrid	1	1	0
N124	2014	FL	HL	C. hybrid	1	0	0
N125	2014	FL	HL	C. pauper	1	0	1
N126	2016	FL	LL	G.fuliginosa	0	0	1
N127	2016	FL	LL	G.fuliginosa	0	1	0
N128	2016	FL	HL	G.fuliginosa	1	0	0
N129	2016	SC	LL	G. fuliginosa	1	0	0

Table S3.1

N13	2006	SC	LL	G. fuliginosa	0	1	0
N130	2016	FL	LL	G.fuliginosa	1	0	0
N131	2016	SC	LL	G.fuliginosa	1	0	0
N132	2016	FL	LL	G.fuliginosa	1	0	0
N133	2016	SC	LL	G.fuliginosa	1	0	0
N134	2016	SC	HL	G.fuliginosa	1	0	0
N135	2016	SC	LL	G.fuliginosa	1	0	0
N136	2016	FL	LL	G.fuliginosa	0	1	0
N137	2016	FL	LL	G.fuliginosa	1	0	0
N138	2016	FL	HL	G.fuliginosa	1	0	0
N139	2006	FL	HL	G.fuliginosa	1	0	0
N14	2005	SC	HL	C. parvulus	0	0	1
N15	2006	FL	LL	G. fortis	1	0	0
N16	2006	FL	LL	G. fortis	1	0	0
N17	2006	FL	HL	C. pauper	1	0	0
N18	2006	FL	HL	C. pauper	1	0	0
N19	2006	FL	HL	C. pauper	1	0	0
N2	2004	SC	HL	C. pallidus	0	1	1
N20	2006	FL	LL	G.fuliginosa	1	0	0
N21	2006	FL	LL	G.fuliginosa	1	0	0
N22	2006	FL	HL	G.fuliginosa	1	0	0
N23	2006	FL	LL	G.fuliginosa	1	0	0
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N25	2006	FL	HL	G.fuliginosa	1	0	0
N26	2006	FL	HL	G.fuliginosa	1	0	0
N27	2006	FL	HL	G.fuliginosa	1	0	0
N28	2006	FL	LL	G.fuliginosa	1	0	0
N29	2006	FL	LL	G.fuliginosa	1	0	0
N3	2006	SC	LL	G. fortis	1	0	0
N30	2006	FL	LL	G.fuliginosa	1	0	0
N31	2006	FL	LL	G.fuliginosa	1	0	0
N32	2006	FL	HL	G.fuliginosa	1	0	0
N33	2006	FL	HL	G.fuliginosa	1	0	0
N34	2008	FL	HL	C. pauper	1	0	0
N35	2008	FL	HL	C. parvulus	1	0	0
N36	2008	FL	HL	C. parvulus	1	1	1
N37	2008	FL	HL	C. parvulus	1	0	0
N38	2008	FL	HL	C. parvulus	1	0	0
N39	2008	FL	HL	C. parvulus	1	0	0

N4	2006	FL	HL	C. parvulus	1	0	0	
N40	2008	FL	HL	C. parvulus	1	0	0	
N41	2008	FL	HL	G.fuliginosa	1	0	0	
N42	2008	FL	HL	G.fuliginosa	1	0	0	
N43	2008	FL	HL	C. pauper	1	0	0	
N44	2008	FL	HL	C. pauper	1	0	0	
N45	2008	FL	HL	C. pauper	1	0	0	
N46	2008	FL	HL	C. pauper	1	0	0	
N47	2008	FL	HL	C. pauper	1	0	0	
N48	2008	FL	HL	C. pauper	1	1	1	
N49	2008	FL	HL	C. pauper	1	0	0	
N5	2006	FL	HL	C. parvulus	1	0	0	
N50	2008	SC	HL	G.fuliginosa	1	0	0	
N51	2008	FL	LL	G.fuliginosa	0	1	0	
N52	2008	FL	HL	G.fuliginosa	1	0	0	
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N54	2008	FL	HL	G.fuliginosa	0	0	1	
N55	2008	FL	HL	$G.\ fuliginosa$	1	0	0	
N56	2008	FL	HL	$G.\ fuliginosa$	1	0	0	
N57	2008	FL	HL	$G.\mathit{fuliginosa}$	0	0	1	
N58	2008	FL	HL	$G.\ fuliginosa$	1	1	1	
N59	2008	FL	HL	$G. \mathit{fuliginosa}$	1	1	1	
N6	2006	SC	LL	G. fortis	1	0	0	
N60	2008	FL	HL	$G. \mathit{fuliginosa}$	1	1	1	
N61	2008	FL	HL	$G.\mathit{fuliginosa}$	1	0	0	
N62	2008	FL	HL	$G. \mathit{fuliginosa}$	1	0	0	
N63	2008	FL	HL	$G.\mathit{fuliginosa}$	1	0	0	
N64	2008	FL	HL	$G.\mathit{fuliginosa}$	1	0	0	
N65	2010	FL	HL	C. pauper	1	0	0	
N66	2010	FL	HL	C. pauper	1	0	0	
N67	2010	FL	HL	C. parvulus	0	1	1	
N68	2010	FL	HL	C. parvulus	1	0	0	
N69	2010	FL	HL	C. parvulus	1	0	0	
N7	2006	FL	HL	C. parvulus	1	0	0	
N70	2010	FL	HL	C. pauper	1	0	0	
N71	2010	FL	HL	C. pauper	1	0	0	
N72	2010	FL	HL	C. pauper	1	0	0	
N73	2010	FL	HL	C. pauper	1	0	0	
N74	2010	FL	HL	C. pauper	0	1	1	

N75	2010	FL	LL	C. pauper	1	0	0	
N76	2010	FL	HL	G.fuliginosa	1	0	0	
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N82	2010	FL	HL	G.fuliginosa	1	0	0	
N83	2010	FL	HL	G.fuliginosa	1	0	0	
N84	2012	FL	HL	C. pauper	0	0	1	
N85	2012	FL	HL	C. pauper	1	0	0	
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N87	2012	FL	HL	G.fuliginosa	1	0	0	
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N89	2013	FL	LL	G.fuliginosa	0	1	1	
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N92	2013	FL	HL	G.fuliginosa	1	0	0	
N93	2013	FL	HL	G.fuliginosa	1	0	1	
N94	2013	FL	HL	G.fuliginosa	1	0	0	
N95	2013	FL	HL	C. hybrid	1	0	0	
N96	2013	FL	HL	C. hybrid	0	1	1	
N97	2013	FL	HL	C. hybrid	0	1	1	
N98	2013	FL	HL	C. pauper	1	1	1	
N99	2013	FL	HL	C. hybrid	1	1	1	
S 1	2004	SC	LL	Unknown	0	1	1	
S10	2004	SC	LL	Unknown	0	1	1	
S11	2005	SC	LL	Unknown	0	1	1	
S12	2004	SC	LL	Unknown	0	1	1	
S2	2004	SC	LL	Unknown	0	1	1	
S 3	2004	SC	LL	Unknown	0	1	1	
S4	2004	SC	LL	Unknown	0	1	1	
S5	2004	SC	LL	Unknown	0	1	1	
S 6	2004	SC	LL	Unknown	0	1	1	
S7	2004	SC	LL	Unknown	0	1	1	
S8	2004	SC	LL	Unknown	0	1	1	
S 9	2004	SC	LL	Unknown	0	1	0	

T1	2004	FL	LL	Trap	0	1	1
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T100	2013	SC	HL	Trap	0	0	1
T101	2013	SC	HL	Trap	0	0	1
T102	2013	SC	HL	Trap	0	0	1
T103	2013	SC	HL	Trap	0	0	1
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T105	2013	SC	HL	Trap	0	0	1
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T108	2013	SC	HL	Trap	0	0	1
T109	2013	SC	HL	Trap	0	0	1
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T112	2013	SC	HL	Trap	0	0	1
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T25	2014	FL	HL	Trap	0	1	0
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T30	2014	FL	HL	Trap	0	1	0

T31	2014	FL	HL	Trap	0	0	1
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T52	2014	SC	HL	Trap	0	1	0
T53	2014	SC	HL	Trap	0	0	1
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T59	2014	SC	LL	Trap	0	1	0
T6	2005	SC	LL	Trap	0	0	1
T60	2014	SC	LL	Trap	0	0	1
T61	2014	SC	LL	Trap	0	1	0
T62	2014	SC	LL	Trap	0	0	1
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T64	2014	SC	LL	Trap	0	0	1
T65	2014	SC	LL	Trap	0	0	1
T66	2014	SC	LL	Trap	0	0	1

T67	2014	SC	LL	Trap	0	0	1
T68	2014	SC	LL	Trap	0	0	1
T69	2014	SC	LL	Trap	0	0	1
T7	2005	SC	LL	Trap	0	1	0
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T71	2012	SC	LL	Trap	0	0	1
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T74	2012	SC	LL	Trap	0	0	1
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T76	2012	SC	LL	Trap	0	0	1
T77	2012	SC	LL	Trap	0	0	1
T78	2012	SC	LL	Trap	0	0	1
T79	2012	SC	LL	Trap	0	0	1
T8	2005	SC	LL	Trap	0	0	1
T80	2012	SC	LL	Trap	0	0	1
T81	2012	SC	LL	Trap	0	0	1
T82	2013	SC	LL	Trap	0	0	1
T83	2013	SC	LL	Trap	0	0	1
T84	2013	SC	LL	Trap	0	0	1
T85	2013	SC	LL	Trap	0	0	1
T86	2013	SC	LL	Trap	0	1	0
T87	2013	SC	LL	Trap	0	0	1
T88	2013	SC	LL	Trap	0	0	1
T89	2013	SC	LL	Trap	0	0	1
Т9	2005	SC	LL	Trap	0	0	1
T90	2013	SC	LL	Trap	0	0	1
T91	2013	SC	LL	Trap	0	0	1
T92	2013	SC	LL	Trap	0	0	1
T93	2013	SC	LL	Trap	0	0	1
T94	2013	SC	LL	Trap	0	0	1
T95	2013	SC	LL	Trap	0	0	1
T96	2013	SC	HL	Trap	0	1	1
T97	2013	SC	HL	Trap	0	0	1
T98	2013	SC	HL	Trap	0	0	1
T99	2013	SC	HL	Trap	0	0	1

Collated published coefficients of determination of the association between body size and fecundity.

Reference	\mathbf{r}^2	r
Honěk (1993)	0.856	
McCann et al. (2009)	0.05, 0.73	
Armbruster, Hutchinson (2002)	0.61, 0.83	
Blackmore, Lord (2000)	0.071, 0.12	
Frankino, Juliano (1999)	0.636	
Blay, Yuval (1999)	0.25, 0.6	
Malmqvist et al. (2004)	0.312, 0.783	
Briegel (1990)		0.60
Sivinski (1993)		0.86
Steinwascher (1984)		0.34, 0.64
Xue, Ali (1994)		0.39
Reigada, Godoy (2005)		0.30
Kolluru, Zuk (2001)		0.32, 0.36
Riback, Godoy (2008a); Riback,		0.55, 0.56
Godoy (2008b)		
Gião, Godoy (2006)		0.63, 0.73

Table S3.3

Table S3.2

The mean mass and size of *Philornis downsi* pupae per year sampled from Darwin's finch nests, whereby the genus of the host species was either *Camarhynchus* or *Geospiza*. The data are shown for P. downsi pupa mass (g), length (mm) and width (mm). Data are shown as mean \pm SE calculated from the average per nest.

	P. do	P. downsi collected from			wnsi collecte	d from		
	Ca	marhynchus 1	nests		Geospiza nests			
	Mass	Width	Length	Mass	Width	Length		
2006	0.07 ± 0.00	4.27 ± 0.08	10.21 ± 0.21	0.07 ± 0.00	4.11 ± 0.09	10.02 ± 0.15		
2008	0.07 ± 0.00	3.91 ± 0.07	9.69 ± 0.16	0.07 ± 0.00	3.98 ± 0.07	10.21 ± 0.16		
2010	0.06 ± 0.01	3.54 ± 0.18	9.14 ± 0.55	0.07 ± 0.01	3.81 ± 0.09	9.74 ± 0.27		
2012	0.03 ± 0.01	2.90 ± 0.32	7.70 ± 0.51	- .	-	_		
2013	0.06 ± 0.01	3.58 ± 0.12	9.24 ± 0.31	0.07 ± 0.01	3.74 ± 0.12	9.84 ± 0.15		
2014	0.05 ± 0.00	3.38 ± 0.17	8.88 ± 0.37	0.05 ± 0.01	3.63 ± 0.22	9.23 ± 0.27		
2016	-	_	-	0.05 ± 0.01	3.73 ± 0.15	9.50 ± 0.39		

Table S3.4

Component coefficients and communalities of variables *Philornis downsi* pupae mean mass (g), length (mm) and width (mm) loaded with extracted Principal Component Pupae Size. Major loadings for each item are in bold.

	Component coefficients	Communalities
Mean mass	0.932	0.869
Length	0.931	0.867
Width	0.918	0.843

Table S3.5

Component coefficients and communalities of variables *Philornis downsi* adult abdomen length (mm) and body length (mm) loaded with extracted Principal Component Adult Fly Size. Major loadings for each item are in bold.

	Component coefficients	Communalities
Abdomen length	0.956	0.914
Body length	0.956	0.914

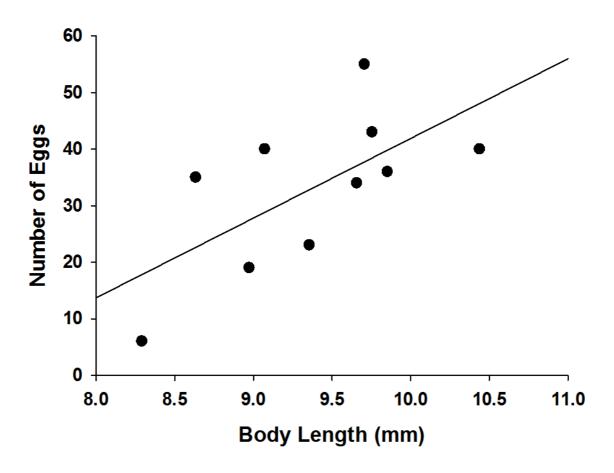


Figure S3.1

Correlation between body length (mm) and number of eggs of female *Philornis downsi* adult flies collected from 4m McPhail Traps on Floreana Island in 2014.

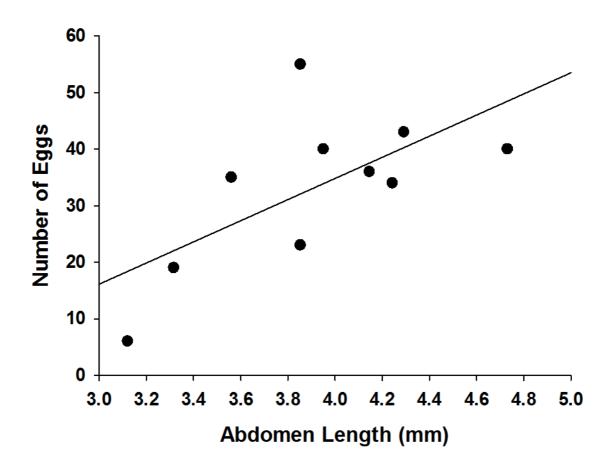


Figure S3.2

Correlation between abdomen length (mm) and number of eggs of female *Philornis downsi* adult flies collected from 4m McPhail traps on Floreana Island in 2014.

Chapter 4 – Avian vampire fly (*Philornis downsi*) mortality differs across Darwin's finch host groups

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Abstract

In invasive parasites, generalism is considered advantageous during the initial phase of introduction. Thereafter, fitness costs to parasites, such as host-specific mortality, can drive parasites towards specialism to avoid costly hosts. It is important to determine changes in host specificity of invasive populations to understand host-parasite dynamics and their effects on vulnerable host populations. We examined changes in mortality in the introduced avian vampire fly (*Philornis downsi*) (Diptera: Muscidae), a generalist myasis-causing ectoparasite, between 2004 and 2020 on Floreana Island (Galápagos). Mortality was measured as the proportion of immature larvae found upon host nest termination. Over the time period, the avian vampire fly was most abundant and had low mortality in nests of the critically endangered medium tree finch (*Camarhynchus pauper*) and had the highest mortality in nests of hybrid tree finches (*Camarhynchus* spp.). Low larval mortality was also found in small tree (*C. parvulus*) and small ground finch (*Geospiza fuliginosa*) nests. Selection could favour avian vampire flies that select medium tree finch nests and/or avoid hybrid nests. Overall, the finding of differences in avian vampire fly survival across host species is parsimonious with the idea that the introduced fly may be evolving towards host specialisation.

Introduction

Niche breadth is a fundamental concept that underpins key hypotheses in species ecology(Hutchinson 1957; Smith 1982; Leibold 1995). The breadth of a niche is the set of conditions in which a species can persist, and can include dimensions such as habitat diversity and climatic variation (Sexton et al. 2017). In parasitic organisms, niche breadth is often synonymous with host specificity – i.e., the number of host species a parasite can infect, and parasites range from highly host specific to generalist (Jaenike 1990; Thompson 1994; Krasnov et al. 2004). Host specificity is mediated by host-parasite co-evolutionary processes (Poullain et al. 2008). Hosts and parasites enter an arms race in which they adapt and counteradapt reciprocally at the expense of the other (Whitlock 1996). Hosts are selected to evade or resist the parasite, whereas parasites evolve to more efficiently exploit their host (Thompson 1994). Higher virulence (damage to the host) and host specificity can lead to increased exploitation of the host by the parasite (Gandon 2002). Selection for greater host exploitation may break down when parasite fitness is reduced, whereby high exploitation of hosts leads to premature host mortality, leading to decreased parasite growth and fecundity, or increased parasite mortality (Frank and Schmid-Hempel 2008; Alizon and Michalakis 2015).

Host specificity presents trade-offs for the parasite. Generalist parasites tend to occur on host species that are phylogenetically closely related (Krasnov et al. 2004; Beadell et al. 2006; Krasnov 2008; Poulin 2011). Nonetheless, they incur the cost of maintaining variation in life history, genetic and behavioural traits that enable exploitation of different host species (Välimäki et al. 2011). This relationship can be further complicated in host hybrid zones, where hybrids can be more or less resilient to parasite populations (Theodosopoulos et al. 2019). Despite high host encounter rates due to wide host ranges, generalist parasite

populations exhibit slower geographic expansion rates compared to specialist populations (Välimäki et al. 2011). The occurrence of parasite generalism or specialism is influenced by the costs and benefits inherent to occupying different host ranges, including mortality rates in parasite populations associated with particular host species (Mackenzie 1996). For blood feeding parasites, for example, the costs of generalist feeding can push species towards specialisation because of the variation in host blood properties and nutritional value for the parasite (Harrington et al. 2001). High mortality risk or a lack of nutritional value in specific hosts can drive parasites to specialise on hosts that optimise their fitness (Dick and Patterson 2007). Generalist parasites may have a selective advantage when colonising novel environments given their capacity to switch hosts if a primary host population declines, which can increase their chance of persistence despite a range of establishment challenges (Torchin and Mitchell 2004; Clark and Clegg 2015).

When a generalist parasite colonises a novel environment and suite of potential host species, the differences in fitness due to altered selection creates a window of opportunity to study niche and host specialisation shifts under changing evolutionary pressures. While selection may initially favour a generalist strategy to maximise initial spread upon colonisation, specialisation is favoured in parasites that are capable of host choice (Kawecki 1998; Egas et al. 2004). In fact, generalism is rare compared to specialism, with most species parasitising only one or a few host species (Dick and Patterson 2007; Poulin and Keeney 2008; Lyimo and Ferguson 2009). Compared with generalists, specialist parasites have been shown to evolve more quickly in response to evolving host defences and have fewer deleterious alleles present in their gene pool (Whitlock 1996; Visher and Boots 2020). Fitness differences and resulting mortality of parasites in certain host species can drive parasites to specialise on ideal hosts that minimise energetic and fitness costs. When a given optimal host species is

abundant, predictable (Sarfati et al. 2005), accessible (Lyimo and Ferguson 2009), and energetically efficient for the parasite (Egas et al. 2004), host specialisation is expected to evolve rapidly (Kawecki 1998). Host specialisation can be favoured when fitness trade-offs are present and in the presence of multiple viable hosts that vary in fitness costs for the parasite (Fry 1996; Kawecki 1998).

Here, we consider the case of the avian vampire fly (*Philornis downsi*) (Diptera: Muscidae) (Dodge and Aitken 1968), a generalist myiasis-causing invasive parasitic fly of the Galápagos Islands. First observed in a Darwin's finch nest in 1997 on Santa Cruz Island, the avian vampire fly is currently known to parasitise nestlings in all 18 studied bird species on the Galápagos Islands (Fessl et al. 2015; Fessl et al. 2018). Low host defences due to immunological naivety to this type of parasite (Frankham 1997; Reichard et al. 2010) and close taxonomic clustering of Darwin's finches (Krasnov et al. 2004) may have facilitated its rapid spread across hosts and the archipelago (Wiedenfeld et al. 2007; Fessl et al. 2018). Avian vampire fly larvae consume the blood and tissue of developing birds in the nest (Fessl et al. 2006b), causing high in-nest mortality in their hosts (Kleindorfer and Dudaniec 2016; Fessl et al. 2018). Nestlings that survive the parasitism often have permanently deformed nares, affecting their song and foraging strategy (Galligan and Kleindorfer 2009; Kleindorfer et al. 2019a, Kleindorfer et al. unpublished). Given the apparent ubiquity of the avian vampire fly across Galápagos passerine species and the increasing number of avian vampire fly larvae and pupae per host nest in studies carried out between 2000 to 2013 (Kleindorfer et al. 2014b; Kleindorfer and Dudaniec 2016), theory predicts that parasite generalism should prevail if there are negligible resource differences (i.e. nutritional value or fitness costs to parasites) between host species. However, host specialisation or host preference should occur if there are differences in fitness costs for the avian vampire fly between host species. 78

Nestling mortality in Darwin's finches caused by blood-sucking avian vampire fly larvae can be high (55% on average), with hosts dying younger in recent years (Kleindorfer et al. 2014b; Kleindorfer and Dudaniec 2016). Yet, some Darwin's finch species appear better able than others to tolerate the impacts of avian vampire fly parasitism (O'Connor et al. 2010b; Kleindorfer and Dudaniec 2016; Knutie et al. 2016; Peters et al. 2019), perhaps because of differences in brood size, such as between ground (Geospiza) and tree (Camarhynchus) finches (Kleindorfer 2007a). Smaller broods have higher parasite loads per nestling and hence suffer higher nestling mortality (Fessl and Tebbich 2002; Dudaniec et al. 2007). On Santa Cruz Island, nestling mortality caused by avian vampire fly larvae has shifted across the past decade in warbler finch (Certhidea olivaceae) and small tree finch (Camarhynchus parvulus) (Cimadom et al. 2014; Cimadom et al. 2019). Initially, during 2000-2005, warbler finches had on average more larvae per nest than small tree finches (41 \pm 6 compared to 23 \pm 3) (Dudaniec et al. 2007; Kleindorfer and Dudaniec 2009), with the pattern reversing during 2010-2014 (Cimadom et al. 2014). During 2012-2014, 56% of small tree finch nestlings died due to vampire fly parasitism, 71% of nests lost the whole brood before nestlings reached 7 days old, compared to 37% mortality in warbler finch nestlings (Cimadom et al. 2014). The differences in host mortality and intensity (total number of parasites present in the nest) between warbler finches compared with small tree finches on Santa Cruz Island might be due to changes in the oviposition behaviour by the vampire fly or the behaviour of the host, which remains to be further explored (but see Common et al. 2019).

Host tolerance of parasitism is also dependent on environmental conditions. For example, droughts and heavy rainfall may exacerbate the negative impact of avian vampire fly parasitism when hosts are unable to compensate with increased nestling feeding rates or 79

experience elevated numbers of parasites in nests (Cimadom et al. 2014; McNew et al. 2019). Periods of high rainfall, such as El Niño years, have been associated with increased numbers of avian vampire fly larvae in nests across host species (Dudaniec et al. 2007; O'Connor et al. 2010b; McNew and Clayton 2018). High rainfall years have also been associated with increased hybrid recruitment in *Camarhynchus* tree finches on Floreana Island (Kleindorfer and Dudaniec 2020). Current hybridisation patterns occur as female medium tree finches (*C. pauper*) pair with male small tree finches (*C. parvulus*), resulting in sex-specific gene flow and the existence of a hybrid swarm (Peters et al. 2017). Hybrid nests have significantly fewer avian vampire fly larvae with up to 60% fewer parasites per nest than their parental species (Peters et al. 2019). Parents of nests that had the greatest genetic admixture (therefore, greater hybrid assignment probability) had the fewest avian vampire fly larvae. However, the mechanisms driving these intensity differences are not yet known (Peters et al. 2019).

Here, we explore changes in avian vampire fly larval mortality across time and host species. We suggest that if there are changes in avian vampire fly larval mortality in different host species, this could indicate selection on the avian vampire fly to diverge and specialise, or to avoid particular hosts. Our long-term data offer a unique opportunity to describe early coevolutionary processes in a generalist parasite across a suite of hosts in its invasive range, which has implications for the evolution of host specificity. We analyse data spanning non-consecutive 17-years of avian vampire fly specimens collected from Floreana Island, Galápagos, to examine changes in in-nest mortality and survival when parasitising three host species and a hybrid cluster: small ground finch (*Geospiza fuliginosa*), small tree finch (*C. pauper* × *C. parvulus*), medium tree finch (*C. pauper*), and the hybrid tree finch (*C. pauper* × *C. parvulus*).

including hybrids that have backcrossed to one of the parent species (Peters et al. 2017)). In a comparison of samples collected from host nests between 2004 and 2020, we predict that (1) avian vampire fly intensity (i.e. total number per nest) has increased over time regardless of the host species based on previous research (Kleindorfer et al. 2014b); (2) avian vampire fly larval mortality has increased since 2004 - we predict a positive relationship between vampire fly larval mortality and year, and mortality and nestling age at death (the age at which the last nestling dies), as early host death (i.e. early termination of resources) (Kleindorfer et al. 2014b) results in younger parasites upon nest termination; (3) avian vampire fly larval mortality will increase with increasing annual rainfall, as heavy rainfall decreases host survival (Cimadom et al. 2014; McNew et al. 2019) and, (4) avian vampire fly larval mortality differs between host species due to (a) differences in brood size between small ground finch and the tree finch species (Kleindorfer 2007a) and (b) differences in parasite-induced nestling mortality between the small and medium tree finches and the hybrid tree finch given differences in parasite intensity between these host species (Peters et al. 2019).

Methods

Study system

This study was conducted on Floreana Island, Galápagos Archipelago, and followed long-term field protocols as described below (Kleindorfer et al. 2014b). The field work was conducted during the Darwin's finch breeding season in the highlands (01°17'S, 090°27'W) between the months of January and April in ten non-consecutive seasons spanning 17 years: 2004, 2005, 2006, 2008, 2010, 2012, 2013, 2014, 2016 and 2020. We collected avian vampire fly specimens from the nests of the small ground finch, small tree finch, medium tree finch, and the hybrid *Camarhynchus* tree finch (Kleindorfer et al. 2014a; Peters et al. 2017;

Loo et al. 2019a). Host species were first determined morphologically, and hybrid tree finches were retrospectively confirmed via genetic analyses (Kleindorfer et al. 2014a; Peters et al. 2017). Due to this approach, data for hybrid *Camarhynchus* finches are only available for the years 2006, 2010, 2012, 2013 and 2014. Floreana rainfall data (sum of annual rainfall; mm) were collected via satellite sourced from CPC Global Unified Precipitation Data provided by NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, downloaded from the Galápagos Vital Signs website by the Galápagos Conservancy (GalápagosConservancy 2021).

Study species

The avian vampire fly is an obligate myiasis-causing parasite of birds that feeds on the blood and tissue of developing nestlings (Fessl and Tebbich 2002). Non-parasitic adult flies feed upon decaying vegetable matter, ovipositing their eggs in active bird nests (Couri 1985; Skidmore 1985; O'Connor et al. 2010a). Upon hatching, first and early second instar larvae move to the naris and ear canals of nestlings to feed on blood and keratin (Fessl et al. 2006b). Late second and third instar larvae feed externally on nestlings at night, residing in the base of the nest during the day (Fessl et al. 2006b; O'Connor et al. 2014; Kleindorfer and Sulloway 2016). Reports of development times of larval instars vary between field and lab reared specimens, with pupation occurring after 4-10 days of feeding (Kleindorfer et al. 2014b; Lahuatte et al. 2016). Upon host fledging or death, third instar larvae pupate in the base of the nest, forming frothy cocoons and emerging as adults within 7-18 days (Kleindorfer et al. 2014b; Lahuatte et al. 2016).

Nest monitoring and P. downsi *collection*

We analysed data from 280 Darwin's finch nests on Floreana Island with all avian vampire fly specimens per nest collected and stored in ethanol following well-established field protocols (Kleindorfer et al. 2014b). Small tree finch (n = 64), hybrid tree finch (n = 34), medium tree finch (n = 55) and small ground finch (n = 127) nests were monitored for activity and brood size every 3 days during incubation and every 2 days during the nestling phase. Males of these host species build new display nests at which they sing for each new nesting event (Kleindorfer 2007b). The female either selects a display nest or selects a male and they build a new display nest together (Kleindorfer 2007b). Incubation lasts ~14 days and, if successful, nestlings fledge the nest approximately 12-14 days after hatching. Brood size was determined using a borescope to view inside the nest once nestlings had hatched. After nesting activity had finished (i.e., nest termination, either through death of the nestling or fledging), the nest was collected and dismantled within 24 hours to count the number of avian vampire fly offspring within the nest. Nestling age at death was known for a subset of nests (n = 105) from hatching date or visual aging of the nestlings via borescope. In all sampling years (except for four nests in 2004 and 2005), nestlings found dead in the nest were soaked in 70% ethanol for 24 hours to allow first instar larvae within the nestling nares or ear canals to float and be collected. We generally only collect ~8 1st and 2nd instar using this method from $\sim 8\%$ of nestlings soaked. The 1st instar larvae reside inside the nares for the first two days post-hatch and nestlings tended to survive until d7 post-hatch during 2004 and 2005 (Kleindorfer et al. 2014b). All avian vampire fly larvae, pupae, puparia and adult flies were stored in 70% ethanol within 24 hours of collection.

Larval specimens of 241 nests were assigned an age class via observation using a dissecting microscope, following instar identification protocols (Fessl et al. 2006b; Common et al. 83

2019). Parasite intensity was calculated as the total number of larvae, pupae, puparia and adult flies within a nest. Mortality in the avian vampire fly larvae was measured as the proportion of immature (first and second instar) larvae in the nest at the time of host resource termination (Nijhout and Callier 2015). This measure accounts for the possibility of third instars and pupae fully developing into adult flies following host termination (Singh and Bala 2009). This measure also provides an estimate of parasite mortality per host nest, given that first and second larval instars are unable to continue development in the absence of nutrition (Coulson and Bale 1990; Singh and Bala 2009).

Statistical analysis

All models were fitted using R version 4.0.3 (R Core Development Team 2020) with the packages lme4 (Bates et al. 2015), MASS (Venables and Ripley 2002), and car (Fox and Weisberg 2011), and were visualised with lattice (Sarkar 2008), ggplot2 (Wickham 2009) and effects (Fox 2003). Total number of avian vampire fly offspring per nest (log transformed to fulfil the assumption of normality) was analysed in relation to study year, annual rainfall and the Darwin's finch species and the interaction between year and rainfall as fixed effects with a linear regression model on the full data set (n = 280).

For the corresponding analyses considering different age classes of the parasite, we had a smaller dataset of n = 241. We repeated the analysis of total intensity in relation to year and rainfall on this subset of data to confirm the same pattern across both data sets. We analysed the total number of first and second instar larvae, third instar larvae, total larvae, pupae and puparia in relation to year and annual rainfall similar to the total number of vampire flies, but as count data with Generalized Linear Models (GLMs) with negative binomial distribution

and log link function to correct for overdispersion. Throughout we tested for linear and quadratic relationships of year and rainfall and their additive and interactive effects on the total avian vampire fly intensity, first and second instar larvae, third instar larvae, total larvae, pupae and puparia. Based on the principles of parsimony (the largest amount of variance explained with the minimum number of predictors (Burnham and Anderson 1998)), we then selected the model structure that best described our measures of parasite loads at different developmental stages.

Avian vampire fly larval mortality was modelled using the column bind ('cbind') function specifically designed to fit proportion data in logistic regression models with the number of larvae in the first and second larval instar as binomial denominator, and a quasibinomial distribution and a logit link function to correct for overdispersion. We fitted the key response variable avian vampire fly larval mortality to two different data sets: 1) considering all Darwin's finch nests on Floreana Island for which we identified larvae to age class (n = 241); and 2) considering nests where nestling age at death was known (n = 106), with nestling age at death as an additional co-variate. This second analysis accounts for changes in parasite intensities within nests according to nestling age at death (Fessl and Tebbich 2002); and highlights interspecific host differences in vampire fly larval mortality even when accounting for host age at death (Kleindorfer et al. 2014b). We fitted the study year, annual rainfall and the Darwin's finch species and the interactions (year × rainfall) as fixed effects. Initially, we also controlled for brood size, but this additional predictor did not reveal any significant result and was dropped from the final model to improve sample size (from n = 191 to n = 241in the data set without brood size). We removed non-significant interaction terms from the models to simplify the statistical approach and interpretation of the results and to ensure a valid interpretation of the remaining additive effects. The effect of host genus (Geospiza and 85

Camarhynchus), excluding hybrid tree finches to remove the effect of hybridisation (n = 213), was analysed using the full model of mortality in relation to year and annual rainfall and their interaction as fixed effects.

All quantitative variables were scaled (standardized to mean = 0 and standard deviation = 1) to bring the variables to comparable dimensions and to facilitate the correct interpretation of effect sizes for interaction terms (Grueber et al. 2011). Residual distributions of the models were inspected visually to assess model fit (diagnostic plots produced by the 'plot' function in the 'base' package: residuals versus fitted values and normal Q-Q plot displaying the theoretical quantiles versus standard Pearson residuals). Throughout, we report model effect sizes (estimates \pm SE, derived from the summary function); presented $\chi 2$ and p-values are based on an ANOVA Table of Deviance using Type III Wald $\chi 2$ tests (ANOVA function in 'car' package). No random factors were considered, as there were no repeated measurements in the data. We tested for correlations of fixed effects beforehand but did not find any indication for co-linearity in our data.

Results

Philornis downsi intensity

We found no effect of year on avian vampire fly intensity (i.e., total number of parasites per nest) across the entire study period (LM, $F_{1,275} = 0.040$, estimate 0.012 ± 0.02 , p = 0.583, Table 4.1, Figure 4.1). There was an effect of species: medium tree finches had the highest intensity of avian vampire flies per nest (57.1 \pm 5.4 vampire flies per nest compared to *C*. parvulus: 29.9 ± 2.3 ; Camarhynchus hybrids: 26.6 ± 3.3 ; *G. fuliginosa*: 36.2 ± 2.0) (LM, F_3)

 $_{275} = 3.038$, estimate 0.254 ± 0.07 , p < 0.001, Table 4.1; raw data in supplementary Table 4.S2).

Age class distribution and abundance

Analysis of avian vampire fly age classes revealed a significant quadratic relationship with year and the number of first and second instar larvae (GLM, 'year' term estimate: -0.51 \pm 0.14, p = 0.002, Table 4.2b, Figure 4.S1), with numbers of first and second instar larvae peaking in approximately 2013. The number of first and second instar larvae was consistently higher in hybrid tree finches (Table 4.S1). Furthermore, across all species, third instar larvae and the total number of larvae per nest increased until 2013 and decreased thereafter (GLM, third instar: 'year' term estimate: -0.32 \pm 0.10, p = 0.010; total larvae: 'year' term estimate: -0.38 \pm 0.10, p = 0.001, Table 4.2c,d, Figure 4.S1). Conversely, the number of pupae and puparia per nest showed the opposite pattern, decreasing until 2013 and 2014 onwards (GLM, 'year' term estimate: 0.46 \pm 0.10, p < 0.001; 'year' term estimate: 0.78 \pm 0.24, p = 0.002, respectively, Table 4.2e,f, Figure 4.S1). There was no significant effect of rainfall on the total intensity in these nests (LM, 'rainfall' term estimate: -0.04 \pm 0.03, p = 0.110, Table 4.2a, Figure 4.S1) or on any other age class considered (i.e., rainfall did not feature in any other parsimonious model, neither in the linear nor quadratic relationship).

Larval mortality

For all samples combined, there was an increase in the proportion of avian vampire fly larval mortality over time ('year' term estimate: 0.48 ± 0.19 , p =0.012, Table 4.3a) and during years with higher annual rainfall ('rainfall' term estimate: 0.40 ± 0.13 , p = 0.002, Table 4.3a). However, these additive effects should be interpreted with caution due to their involvement in

a significant interaction term (estimate 0.58 ± 0.18 , p = 0.002; Figure 4.2a). Earlier in the study period (2004-2008), when the years were drier (e.g., ~ 360 mm), vampire fly mortality was lower; while later in the study period (2010-2020), when the years were wetter (e.g., 400 - 650 mm), avian vampire fly mortality was higher (Figure 4.1a, Figure 4.3). Larval mortality did not differ between the small tree finch, medium tree finch or small ground finch, but was significantly higher in hybrid nests (least square means and post-hoc contrasts Table 4.3b and 4.3c, Figure 4.2b). Larval mortality did not differ between *Camarhynchus* and *Geospiza* host nests when excluding hybrid tree finches (estimate 'genus' term -0.15 ± 0.24 , p = 0.532; Table 4.4).

When analysing nests where the nestling age at death was known, we found a strong effect of nestling age at death on larval mortality. Larval mortality increased as nestling age at death decreased (estimate -0.34 ± 0.15 , p = 0.025, Table 4.5, Figure 4.S2c). The interaction effect of year and rainfall on mortality was marginally non-significant (estimate 0.38 ± 0.20 , p = 0.056, Table 4.5, Figure 4.S2a). Avian vampire fly mortality was significantly higher in hybrid hosts (estimate 1.36 ± 0.41 , t = 3.30, p < 0.001, Table 4.5, Figure 4.S2b). Larval mortality did not differ between the small ground, small tree, or medium tree finch (Figure 4.S2b).

Discussion

In this study, we tested patterns of larval mortality in avian vampire fly, a generalist myiasiscausing parasite of Darwin's finches, across time and host species. We did not find a significant increase in parasite mortality across time, but there were clear differences in parasite mortality across host species. Parasite mortality was lowest in nests of the medium tree finch, and highest in hybrid finch nests, even when accounting for chick age at death (Kleindorfer et al. 2014b). If host-specific selection pressures on larval mortality continue or increase, the avian vampire fly may be selected to oviposit in optimal host nests, which may result in host specialisation.

Our results provide some support for the idea that *Camarhynchus* hybridisation may be an adaptive host response to thwart a novel parasite, in line with previous findings(Peters et al. 2019). The Red Queen hypothesis is a powerful theoretical framework to predict hostparasite coevolutionary dynamics, and one expects that host-impacting change caused by the parasite is countered by the host, and vice versa (Whitlock 1996). The newly evolving Darwin's finch and avian vampire fly system is consistent with the idea of oscillating evolutionary dynamics in the wild but requires additional research into genetic and behavioural mechanisms to more fully understand these patterns. Previous research has shown that: (1) during the first part of the decade from 2004 to 2013 (Kleindorfer et al. 2014b), the average number of avian vampire flies per host nest increased and then stabilised; (2) one host species, the medium tree finch, consistently has the most avian vampire flies in the nest compared with other host species (O'Connor et al. 2010b); (3) the proportion of hybrid birds increased from 12% in 1998 to between 27% and 55% in later years, and hybrid hosts have the fewest avian vampire flies compared with other host species (Kleindorfer and Dudaniec 2020); and here we show that (4) avian vampire fly mortality was highest in hybrid finch nests and lowest in the nests of the other host species (small ground, small tree and medium tree finches), even when accounting for nestling age at death. From the perspective of the parasite, it should avoid hybrid finch nests. The mechanisms that may drive hostseeking versus host-avoidance behaviours by the parasite are unknown. However, this study uncovers two concurrent scenarios whereby both parasite intensity and parasite mortality 89

across hosts differed, especially between medium tree finch and hybrid tree finch nests during the early co-evolutionary stages of a host-parasite interaction.

In parasites that use multiple host species for different life stages, host generalism is the optimal strategy (Haaland et al. 2020). The avian vampire fly lives its parasitic life stages in a single host environment, and in this case, specialist offspring are predicted to be optimal to maximise arithmetic mean fitness (Haaland et al. 2020). The observation that different Darwin's finch host species have different average numbers of avian vampire flies per nest, even immediately after host hatching, is in line with the idea of differentiated oviposition in certain hosts (Cimadom et al. 2014; Peters et al. 2019). Despite specialisation, some specialist lineages hedge their bets by ovipositing in suboptimal hosts (Davies 2010; Haaland et al. 2020). In the case of the avian vampire fly, genetic evidence has shown oviposition by multiple females in one host nest; also, females frequently lay fewer eggs than they are able to oviposit at a time, which the supports the idea of bet hedging by ovipositing in multiple nests (Dudaniec et al. 2010). However, it is currently unknown if females oviposit preferentially in specific host nests or whether there could be host-specific lineages of avian vampire fly.

Given that high rainfall is associated with more avian vampire flies in host nests (Dudaniec et al. 2007; O'Connor et al. 2010b; McNew and Clayton 2018), we would expect to see an increase in competition between larvae during high rainfall years as more parasites compete for the same amount of resources (Begon et al. 1986; Fredensborg and Poulin 2005). Increased competition may lead to increased parasite mortality. However, in this study, we found lowest parasite mortality in nests of the host species with the most parasites, the critically endangered medium tree finch. The extreme fluctuations in rainfall within parasite 90

lifetimes and across generations on the Galápagos Islands may favour environmental generalists that maintain optimal fitness levels with rainfall fluctuation (Haaland et al. 2020). Selection pressures from introduced pathogens can lead to swamping of local environmental adaptation in favour of immune response loci (Fraik et al. 2020). Therefore, there may be a trade-off between achieving optimal parasite fitness across multiple host species and the parasite's capacity to tolerate environmental variation. In the avian vampire fly, such relationships are yet to be explored.

Host specialisation may ease the burden of parasitism in some host species yet may heighten the threat for other neighbouring species, particularly in host-limited, geographically restricted habitats, as occurring on Floreana Island (Dvorak et al. 2017). Smaller, endangered populations, such as the medium tree finch, are more likely to have low genetic diversity with a reduced capacity to evolve in response to parasites (Hedrick et al. 2001). The threat posed by the parasite is further exacerbated by the high intensity of avian vampire fly larvae found in medium tree finch nests. In comparison, the hybrid tree finch that is the result of recombination between the small and the medium tree finch may have increased genetic variation, which may offer novel genes on which selection can act to evolve resistance to parasitism (Lewontin and Birch 1966; Wolinska et al. 2008). Given the observation that female medium tree finch frequently pair with male small or hybrid tree finches rather than medium tree finch, and the potential for increased hybrid resilience (Peters et al. 2017; Peters et al. 2019), the medium tree finch population may continue to decline, eventually resulting in only a hybrid swarm (Theodosopoulos et al. 2019). Hybrid recruitment, as measured by the proportion of yearling birds in the population, has remained stable across years since 2005, whereas medium tree finch recruitment rates declined across the same period, suggesting hybrid nestlings and/or fledglings may have a selective advantage over medium tree finch 91

offspring (Kleindorfer and Dudaniec 2020). Understanding host-specific parasite fitness in this system highlights the need for directed conservation efforts to more exploited hosts or those less likely to evolve parasite resistance mechanisms. Our results suggest that such mechanisms may be evolving in the *Camarhynchus* hybrid group, but at a cost to the medium tree finch population.

The effects of host hybridisation on both host and parasite fitness have mainly been documented in plant-parasite systems, as hybridisation is common in plant species (Floate and Whitham 1993). These effects vary between systems. Host hybridisation can, for example, increase susceptibility to parasites, resulting in increased numbers of parasites and decreased hybrid fitness (Le Brun et al. 1992; Fritz et al. 1999; Theodosopoulos et al. 2019). In other cases, host hybridisation increases host resistance and tolerance, decreasing parasite loads and increasing host fitness (Moulia et al. 1995; Theodosopoulos et al. 2019). We see this latter pattern in the Darwin's finch system, where hybrid tree finches tend to have fewer parasites per nest than their parent species (Peters et al. 2019). In this study, using a subsample of nests for which we have accurate data on parasite age class, we also found a pattern of fewer parasites per nest in hybrid nests, though the difference in number of parasites across host species was not statistically significant. There is not much available data on parasite fitness in hybrid versus non-hybrid hosts, which is a research gap that requires attention. In a study on fungal pathogens infecting hybrid plant hosts, pathogens had a fitness advantage in hybrid hosts that was contingent on pathogen hybridisation (Gibson et al. 2014). In addition, the role of host hybrid fitness is expected to affect parasite fitness (Arnold and Martin 2010). If host resistance and tolerance to parasites increases as the consequence of hybridisation, then parasite fitness could be higher in hybrid hosts able to sustain the parasite, or conversely, parasite fitness could be lower in hybrid hosts that deter parasites from ovipositing. More 92

research is needed to explore different host-parasite evolutionary pathways under conditions of genetic introgression in host and/or parasite.

We don't know why avian vampire fly larval mortality differed across hosts species in this study, but it is known that blood properties of host species can vary in nutritional gain for the parasite (Harrington et al. 2001; Sarfati et al. 2005). Mortality in second and third instar avian vampire fly larvae reared on chicken blood did not differ between formulated diets of varying nutrition, however development time to pupation was fastest on the diet with the highest nutritional value (Lahuatte et al. 2016). Decreased developmental time is advantageous when resources can be terminated quickly, such as when Darwin's finch nestlings die young (Kleindorfer et al. 2014b), allowing more larvae to reach pupation faster and hence survive to adulthood in nutritionally optimal hosts. High mortality was found in first instar larvae reared on artificial diets and the possible contamination of the blood with pathogenic bacteria such as Serratia may be driving this high mortality (Lahuatte et al. 2016). Serratia, a genus with pathogenic species that affects myiasis-causing and muscid flies, was found to be uniquely associated with avian vampire flies parasitising warbler finches in a microbiome analysis of the fly (Ben-Yosef et al. 2017). Warbler finches in recent years had fewer avian vampire flies and lower host mortality compared to tree finches (Cimadom et al. 2014). Research has further shown that the avian vampire fly microbiome differs significantly across Darwin's finch host species, which is suspected to be associated with differences in finch diets within and across habitats (Ben-Yosef et al. 2017; Knutie 2018; Knutie et al. 2019; Loo et al. 2019a; Loo et al. 2019b). Overall, the findings of this and previous research suggest that larval mortality may be driven by multiple factors, including host nutritional quality, habitat, and microbiome.

We found high parasite mortality in hybrid avian hosts, which we document in a generalist and recently introduced parasite to the Galápagos archipelago. The parasite did best in nests of the Floreana Island endemic, the medium tree finch. Theory predicts that the vampire fly should be selected to oviposit preferentially in medium tree finch nests, given that it has the highest pupation success in medium tree finch nests, and avoid hybrid finch nests where most of its offspring fail to pupate. Understanding the mechanisms by which the avian vampire fly avoids or selects host nests, invests in generalist or specialist offspring, or alters its strategy to survive in prevailing environmental conditions are at the forefront of research into this rapidly evolving host-parasite interaction system on the Galápagos Islands. Our study provides evidence for differential fitness of an invasive parasite in nests of different host species.

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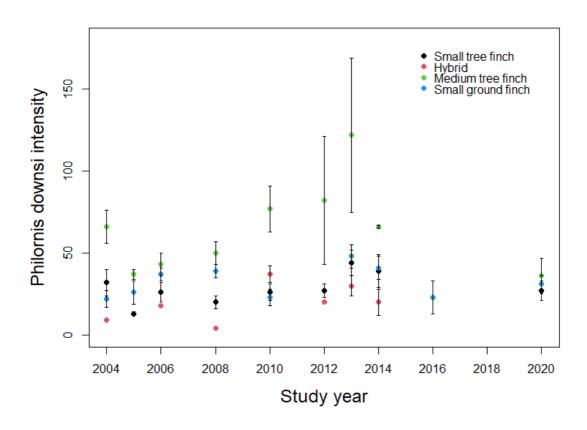


Figure 4.1 Number (mean \pm SE) of avian vampire flies (*Philornis downsi*) per nest of Darwin's finch species per year on Floreana Island. Each Darwin's finch species is denoted by a different colour and symbol.

(a) (b)

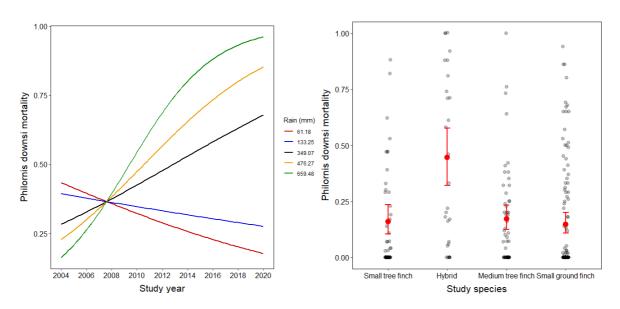


Figure 4.2

The relationship between avian vampire fly (*Philornis downsi*) in-nest larval mortality and (a) the interaction between study year and annual rainfall (sum in mm); and (b) the different Darwin's finch species. Note the interaction is plotted for min (rainfall = 61.18 mm, red line), 1st quantile (rainfall = 133.25 mm), median (rainfall = 349.07 mm), 3rd quantile (rainfall = 476.27 mm) and max (rainfall = 659.48 mm, black dashed line) values; while the additive effect is plotted as effect sizes plus 95% CIs. Model details provided in Table 4.3.

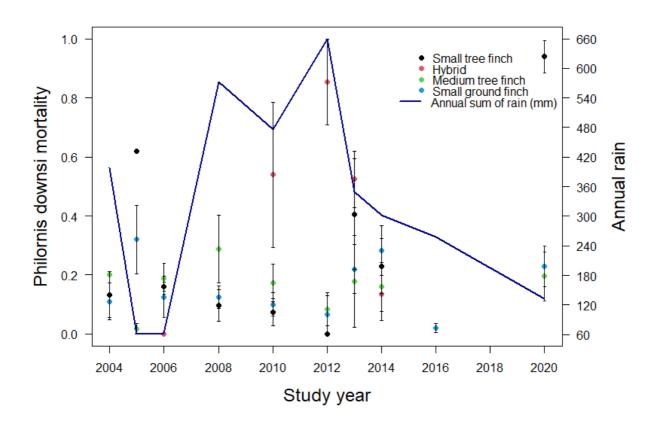


Figure 4.3

The relationship between avian vampire fly (*Philornis downsi*) in-nest mortality and year across the Darwin's finch host species, with cumulative annual rainfall on Floreana Island.

Dots represent the proportion of vampire fly larvae that died upon termination of the host and are labelled according to the four different Darwin's finch species.

Table 4.1

Linear model for avian vampire fly (*Philornis downsi*) intensity in relation to year and host species collected between 2004 and 2020 from Darwin's finch nests on Floreana. 'Rain' did not feature into the most parsimonious model. Avian vampire flies collected from Darwin's finch nests over 10 years across a 17-year period on Floreana Island.

Philornis downsi intensity (n =	T 4: 4	CE	Sum	10	D 1
280)	Estimate	SE	t-value Sq	df	P-value
Intercept	1.401	0.045	30.857		<0.001
Year	0.012	0.022	0.549 0.040	1	0.583
Hybrid [‡]	-0.086	0.078	-1.115 7		
Medium tree finch	0.254	0.067	3.796 - 3.038	3	< 0.001
Small ground finch	0.041	0.056	0.728		

For Species 'small tree finch' was used as a reference category. Note the response variable

Philornis downsi intensity was log transformed to achieve normality and all quantitative input
variables were scaled and centred.

Table 4.2

Linear Models exploring the effects of rain and year on avian vampire fly (*Philornis downsi*) intensity and age class. (a) response variable *P. downsi* infection intensity, log transformed to achieve normality; and Generalized Linear Models (negative binomial distribution) of (b) first and second larval instar; (c) third larval instar; (d) total number of larvae; (e) total number of pupae; and (f) total number of puparia for the different life stages of *P. downsi* in relation to year and rainfall (fitted in a linear or quadratic relationship). We show the most parsimonious model after considering linear and quadratic relationships of year and rainfall and their interaction. Analysis of Deviance Table (Type III tests). Avian vampire flies collected from Darwin's finch nests over 10 years across a 17-year period (2004 - 2020) on Floreana Island.

(a) P. downsi intensity (n =	Estimate	SE	t-value	Sum Sq	Дf	P-value
241; log transformed)	Estimate	SE	t-value	Sum Sq	uı	1 -value
(Intercept)	1.42	0.03	54.73			<0.001
Rain	-0.04	0.03	-1.60	0.42	1	0.110
(b) P. downsi first and						
second larval instar (n =	Estimate	SE	z-value	$LR \chi^2$	df	P-value
241)						
(Intercept)	1.72	0.14	12.43			
Year (linear)	-0.06	0.14	-0.45	12.69	2	0.002
Year (quadratic)	-0.51	0.14	-3.60	12.09	2	0.002
(c) P. downsi third larval instar (n = 241)	Estimate	SE	z-value	LR χ²	df	P-value
(Intercept)	2.47	0.10	23.76			

Year (linear)	0.01	0.10	0.12	0.12	2	0.010
Year (quadratic)	-0.32	0.10	-3.10	9.13	2	0.010
(d) P. downsi total larvae (n						
= 241)	Estimate	SE	z-value	LR χ ²	df	P-value
(Intercept)	2.86	0.10	28.49			
Year (linear)	0.00	0.10	-0.03	13.90	2	0.001
Year (quadratic)	-0.38	0.10	-3.79		۷	0.001
(e) P. downsi pupae (n =	Estimate	SE	z-value	LR χ ²	df	P-value
241)	Estimate	SE	z-varue	LKχ	uı	1 -value
(Intercept)	2.45	0.10	24.98			
Year (linear)	0.01	0.10	0.07	20.22	2	.0.001
Year (quadratic)	0.46	0.10	4.71	20.22	2	<0.001
(f) P. downsi puparia (n =		GE.		ID 1	10	
241)	Estimate	SE	z-value	LR χ ²	df	P-value
(Intercept)	0.80	0.24	3.39			
Year (linear)	-0.52	0.24	-2.22	10.40	2	0.002
Year (quadratic)	0.78	0.24	3.31	12.40	2	0.002

Note all quantitative input variables were scaled and centred. A full intercept is only displayed for Linear Models and cannot be derived with the ANOVA function for Generalized linear models.

Table 4.3

Generalized linear model for (a) avian vampire fly (*Philornis downsi*) in-nest mortality in relation to year and rainfall (interaction term) and species; (b) Ismeans (least squares means; extracted with the 'emmeans' package) and (c) post-hoc contrasts for vampire fly in-nest mortality between Darwin's finch species. Avian vampire flies collected from Darwin's finch nests in 10 sampling years across a 17-year period (2004 - 2020) on Floreana Island.

(a) P. downsi in-nest mortality	T 4: 4	CE	4 1	ID.2	16	P-
(n = 241)	Estimate	SE	t-value	LR χ ²	df	value
Intercept	-1.659	0.245	-6.758			<0.001
Year	0.477	0.188	2.533	6.660	1	0.010
Rain	0.402	0.128	3.128	9.997	1	0.002
Hybrid [†]	1.436	0.366	3.924			
Medium tree finch	0.084	0.316	0.267	23.483	3	<0.001
Small ground finch	-0.093	0.304	-0.305			
Year × Rainfall	0.577	0.182	3.170	10.496	1	0.001
(b) Ismeans between species			Probability	SE	LCL	UCL
Small tree finch			0.16	0.03	0.11	0.24
Hybrid			0.45	0.07	0.32	0.58
Medium tree finch			0.17	0.03	0.12	0.23
Small ground finch			0.15	0.02	0.11	0.20
(c) Post-hoc contrast		odds ratio	SE	Z-	P-	
(c) I ost-noc conti ast			vuus I auv	SE	ratio	value
Small tree finch / Hybrid			0.24	0.09	-	0.001
zman acc mich i 11 joint				J	3.92	3.001

Small tree finch / Medium tree	0.92	0.29	-	0.993	
finch	0.92	0.29	0.27	0.773	
Small tree finch / Small ground	1.10	0.33	0.31	0.990	
finch	1.10	0.55	0.31	0.550	
Hybrid / Medium tree finch	3.87	1.27	4.12	0.000	
Hybrid / Small ground finch	4.62	1.54	4.59	<.0001	
Medium tree finch / Small	1.19	0.33	0.65	0.915	
ground finch	1.17	0.33	0.03	0.913	

[†] Species 'small tree finch' was used as a reference category. × indicates an interaction term Dispersion Parameter for quasibinomial family taken to be 12.381. Ismeans intervals are back-transformed from the logit scale and post-hoc contracts were performed on the log-odds ratio scale following the tukey method.

Table 4.4

Generalized linear model for avian vampire fly (*Philornis downsi*) in-nest mortality in relation to year and rainfall (interaction term); and genus (*Camarhynchus* sp. n = 108; *Geospiza* sp. n = 115), excluding *Camarhynchus* hybrids. Avian vampire flies collected from Darwin's finch nests in 10 sampling years over a 17-year period (2004 - 2020) on Floreana Island.

P. downsi in-nest mortality (n =	Estimate	SE	t-value	LR	df	P-value
213)	Esumate	SE	t-value	χ^2	aı	r-value
Intercept	-1.605	0.151	-10.614			<0.001
Year	0.462	0.192	2.405	6.007	1	0.014
Rain	0.377	0.130	2.894	8.467	1	0.004
Ground finch (Geospiza fuliginosa)	-0.150	0.240	-0.625	0.392	1	0.531
Year × Rain	0.562	0.184	3.059	9.763	1	0.002

[†] Genus *Camarhynchus* sp. 'tree finch' was used as a reference category. × indicates an interaction term

Note all quantitative input variables were scaled and centred. Dispersion Parameter for quasibinomial family taken to be 12.421.

Table 4.5

Generalized linear model for avian vampire fly (*Philornis downsi*) in-nest mortality in relation to year and rainfall (interaction term) and species, including the co-variate 'nestling age at death, ranging from 1-14 days). Including this co-variate reduces our data set to n = 106. Avian vampire flies collected from Darwin's finch nests in 10 sampling years over a 17-year period (2000 - 2020) on Floreana Island. Significant estimates indicated in bold.

P. downsi in-nest mortality	Estimate	SE	t-value	LR χ²	df	P-value
Intercept	-1.128	0.283	-3.985			<0.001
Year	0.116	0.228	0.508	0.390	1	0.609
Rain	0.194	0.168	1.152	0.915	1	0.248
Hybrid [‡]	1.361	0.413	3.296			
Medium tree finch	-0.189	0.383	-0.494	21.040	3	<0.001
Small ground finch	-0.063	0.369	-0.170			
Nestling age at death	-0.344	0.152	-2.270	5.500	1	0.018
Year × Rainfall	0.378	0.201	1.877	4.112	1	0.056

^{*} Species 'small tree finch' was used as a reference category. × indicates an interaction term

Note all quantitative input variables were scaled and centred. Dispersion Parameter for quasibinomial family taken to be 12.273.

Chapter 5 – Temporal and spatial variation in sex-specific

abundance of the Avian Vampire Fly (Philornis downsi)

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Abstract

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Understanding the range and behaviour of an invasive species is critical to identify key habitat areas to focus control efforts. Patterns of range use in parasites can differ temporally, across life stages and between sexes. The invasive avian vampire fly, *Philornis downsi*, spends the larval stage of its life within bird nests, feeding on developing nestlings and causing high levels of mortality and deformation. However, little is known of the ecology and behaviour of the non-parasitic adult fly life stage. Here, we document sex-specific temporal and spatial patterns of abundance of adult avian vampire flies during a single Darwin's finch breeding season. We analyse fly trapping data collected across seven weeks in the highlands (N = 405 flies, 32 traps) and lowlands (N = 12 flies, 32 traps) of Floreana Island (Galápagos). Lowland catches occurred later in the season, which supports the hypothesis that flies may migrate from the food-rich highlands to the food-poor lowlands once host breeding has commenced. Fly abundance was not correlated with host nesting density (oviposition site) but was correlated with distance to the agricultural zone (feeding site). We consistently caught more males closer to the agricultural zone and more females further away from the agricultural zone. These sex differences suggest that males may be defending or lekking at

feeding sites in the agricultural zone for mating. This temporal and sex-specific habitat use of the avian vampire fly is relevant for developing targeted control methods and provides insight into the behavioural ecology of this introduced parasite on the Galápagos Archipelago.

Introduction

In an era of increasing human and animal global mobility, the proportion of invasive species is rapidly increasing, exacerbated by the effects of climate change, over-exploitation, pollution and habitat fragmentation (Pelletier and Coltman 2018). Invasive parasites that pose risks to public health (González et al. 2017; Ruberanziza et al. 2019) or that negatively impact host species of conservation concern (Olson et al. 2013) warrant monitoring for informing control strategies. A single species may occupy and utilise different areas within its range across seasons, life stages and between sexes (Bierzychudek and Eckhart 1988; Ruckstuhl and Neuhaus 2006; Maxwell et al. 2019). Understanding the distribution and behaviour of an introduced species is useful to identify seasonally or geospatially restricted habitat areas to focus control and management efforts (Woodworth et al. 2005; Escobar et al. 2019; Raghavan et al. 2019; Mathieu-Bégné et al. 2020).

In parasitic arthropods, studies have found selective spatial and temporal habitat use between the sexes (Warburg and Yuval 1997; Papadopoulos et al. 2003; Sciarretta et al. 2018; Wong and Jim 2018). In general, sexual conflict and sexual dimorphism have been shown to drive sex-specific distributions in arthropods (Foster and Soluk 2006; Romey and Wallace 2007; Stanley et al. 2018). Patterns of male and female abundance can differ due to sex-biased dispersal, with extremes where one sex disperses while the other is sedentary, which is especially relevant during range expansions or invasions (Beirinckx et al. 2006; Miller and

Inouye 2013; Dudaniec et al. 2021). In other cases, females may aggregate together, away from areas of high male density, to avoid harassment from males, which is seen in systems with high costs to females from multiple mating (Stone 1995; Warburg and Yuval 1997; Stanley et al. 2018; Roswell et al. 2019). Understanding patterns of sex-specific distribution, location of oviposition and feeding sites in invasive parasite populations is useful to control mating behaviours and frequencies, and to maximise the impact of targeted control interventions (Dunn and Hatcher 2015).

In resource-based mating, which is common in many insects, including parasitic insects (Dodson 1997; Warburg and Yuval 1997; Preston-Mafham 2001; Wilkinson and Johns 2005), males compete to guard a resource, such as a food or oviposition site, and mate with females that are attracted to the resource (Parker 1978; Choe and Crespi 1997). Resources must be predictable and defendable but uncommon enough to attract females. When resources are scattered or ubiquitous, swarm-based mating or mate searching systems, where males actively search for receptive mates, tend to prevail (Emlen and Oring 1977; Wilkinson and Johns 2005). Defended resources differ across species and are commonly food or oviposition sites (Preston-Mafham 2001; Wilkinson and Johns 2005). Males may lek near resources (Hendrichs et al. 1991; Warburg and Yuval 1997; Yuval 2005) or defend non-resource-based territories (Yeates and Dodson 1990) to attract and mate with females. Integrating information on the spatial and temporal distribution of invasive species, their mating systems, and monitoring of female population densities is therefore critical for the success of large-scale eradication programs (Yamagishi et al. 1993; Enkerlin et al. 2017).

The avian vampire fly (*Philornis downsi*, Dodge and Aitken, 1968) (Diptera: Muscidae) is a generalist invasive ectoparasite whose larvae consume the blood and tissue of developing 107

birds across a range of host species (Dudaniec and Kleindorfer 2006; McNew and Clayton 2018). Introduced to the Galápagos Islands during the 1960s, avian vampire fly larvae were first discovered in Darwin's finch nests in 1997 (Fessl et al. 2001; Causton et al. 2006). Since then, the fly has been detected on 14 islands across the archipelago (Wiedenfeld et al. 2007; Fessl et al. 2018). Adult avian vampire flies are non-parasitic and feed on fruit, nectar and decaying vegetable matter (Fessl et al. 2018). However, their larvae are obligate parasites of nestlings, feeding both internally and externally on the host (Fessl et al. 2006b; O'Connor et al. 2010a). In its invasive range, the avian vampire fly is highly virulent, causing severe innest mortality or alternatively, naris deformations in nestlings that persist into adulthood and thus affect song and foraging technique (Kleindorfer and Dudaniec 2016; Kleindorfer et al. in review; Kleindorfer et al. 2019a). The effects of avian vampire fly parasitism, such as lowered body condition, naris deformation and mortality, are of particular concern for declining populations of critically endangered Darwin's finch species (Fessl et al. 2010; O'Connor et al. 2010b; Lawson et al. 2017).

Our understanding of adult avian vampire fly behaviour comes from genetic sources where multiple mating behaviour was established via larval sib-ship reconstructions (Dudaniec et al. 2010), or from video recordings at host nests that showed adult flies entering and leaving nests (O'Connor et al. 2010a; Lincango et al. 2015). Male and female avian vampire flies differ in their minimum longevity determined under laboratory rearing conditions (males ~188 days; females ~265 days) (Causton et al. 2019). The height at which adult flies were caught differed between the sexes on Floreana Island – females were more commonly caught at lowest and highest heights (2m and 7m) where there were fewer males (Kleindorfer et al. 2016). Wild avian vampire fly adults collected from Santa Cruz Island also have sex-specific microbiomes, which suggests the sexes may differ in diet and therefore foraging behaviour, 108

perhaps due to different nutritional needs between sexes (Jose et al. 2021). Despite increasing knowledge on the avian vampire fly and its effects on hosts, we know little about where adult flies feed or mate. Mating behaviour has only been studied in a laboratory setting and has yet to be fully understood in the wild (Causton and Lahuatte pers. observation). In the laboratory, flies mate after being fed an enriched papaya diet and as well as a range of native and introduced plant species that have been offered to them, including the invasive blackberry (Causton and Lahuatte, pers. observation). As such, the agricultural zones of the inhabited islands may be an important area for avian vampire fly feeding because of the abundance of fruiting trees.

As the avian vampire fly is the greatest threat to the survival of all Galápagos land birds, it has been targeted for management and control (Causton et al. 2013). Therefore, it is critical to understand the various drivers of adult abundance and distribution. Host species nesting abundance may drive local avian vampire fly abundances, as adults may be attracted to nests for oviposition, and emerge from nests following development and pupation (Fessl et al. 2018). Although both larval and adult avian vampire fly populations occur ubiquitously across habitats (Dudaniec et al. 2007; Kleindorfer and Dudaniec 2016; Causton et al. 2019), intensity does differ across years and when accounting for host species (Kleindorfer and Dudaniec 2016).

It has been suggested that adult avian vampire fly populations may persist in highland refugia outside the host breeding season, where they can access agricultural crops and experience higher rainfall, and then disperse to lower, drier elevations once the host breeding season commences (Wiedenfeld et al. 2007; Causton et al. 2013; Kleindorfer and Dudaniec 2016). On Santa Cruz Island, catch rates of males decreased significantly across the non-breeding 109

season in line with a shorter lifespan, while catch rates in females remained comparatively stable across the year (Causton et al. 2019). High rainfall was also shown to suppress daily catch rates of both male and female adult flies, likely due to decreased flight activity (Causton et al. 2019). Seasonal movements, key habitats, catch rates, and sex differences in habitat use in male and female avian vampire flies across islands are currently poorly understood. Due to the fly's geographically widespread occurrence, identifying key sites and times of peak reproductive and dispersal activity within a Darwin's finch breeding season is of special interest for the development of targeted control techniques, such as for mass release of biological control agents or sterile males.

In this study, we are interested in whether male and female avian vampire flies have different temporal or spatial distribution patterns that could be associated with different resource types (i.e., food resource = fruit from agricultural zone vs. reproductive resource = host nests) across two habitat types: humid, dense highlands and dry lowlands. We quantify the number of male and female avian vampire flies captured in traps on Floreana Island, Galápagos, during the Darwin's finch breeding season in 2020 and examine patterns of avian vampire fly abundance in relation to: a) date of trapping, b) distance to the agricultural zone, and c) the nesting density of Darwin's finches within the highland and lowland study areas. We predict: a) female-biased sex ratio at the onset of breeding due to sex differences in minimum longevity and catch rates documented on Santa Cruz (Causton et al. 2019); b) increased avian vampire fly abundance closer to the agricultural zone at the start of the breeding season, c) increased avian vampire fly abundance, particularly female abundance as host nesting density increases; and d) increased male abundance closer to the agricultural zone near higher densities of fruiting trees.

Materials and methods

Study site and species

We collected adult avian vampire flies from traps on Floreana Island, Galápagos Archipelago, between January 19th – March 6th, 2020, during the Darwin's finch breeding season. Trapping occurred in both the highlands and lowlands (Figure 1). The highland site receives between 600-2300 mm of rain per year (Ben-Yosef et al. 2017; Charles Darwin Researcher Solanda Rea at Bella Vista; Galapagos; GalápagosConservancy 2021). The highland site is a humid Scalesia forest at an elevation of 300-400 m asl located at the base of Cerro Pajas volcano (01°17'S, 090°27'W), and is adjacent to the agricultural zone (01°18'S, 090°26'W). The lowland site (01°16'S, 90°29'W) receives between 100-700 mm of rain per year (Charles Darwin Foundation Researcher Heinke Jäger at Puerto Ayora). The lowland is dominated by Palo Santo (Bursera graveolens) and Acacia (Parkinsonia aculeata and Scutia spicata) (Dvorak et al. 2017) with elevation of 0-150 m asl and is adjacent to the town of Puerto Velasco Ibarra (Figure 1). On Floreana Island, human food production for the population (~110 people) occurs in the highland agricultural zone with only scattered fruiting trees near homes of individual families in the lowlands. Daily highland rainfall data were collected via satellite from CPC Global Unified Precipitation Data provided by NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, downloaded from the Galápagos Vital Signs website (GalápagosConservancy 2021).

The avian vampire fly is a myiasis-causing parasite whose free-living semi-hematophagous larvae feed on the developing nestlings of altricial birds (Fessl and Tebbich 2002; Dudaniec and Kleindorfer 2006). Avian vampire fly eggs are laid inside the host nests (O'Connor et al. 2010a) and once hatched, 1st instar larvae move to the nares and ear canals of newly hatched

nestlings to feed on blood and tissue (Fessl et al. 2006b). Second and third instar larvae generally reside in the base of the nest during the day, feeding internally (in nares) and externally on the nestlings at night (Fessl et al. 2006b; O'Connor et al. 2010a). After 4-10 days of feeding, larvae pupate in a frothy cocoon in the base of the nest and emerge as adults after 7-18 days (Kleindorfer et al. 2014b; Lahuatte et al. 2016). Adult flies feed on decaying vegetable matter including fruits and flowers (Skidmore 1985; Fessl et al. 2018), and can be attracted to baited traps using fruit juice lures (Lincango and Causton 2009).

Philornis downsi trapping

Adult vampire flies were collected using baited McPhail traps hung in trees (Lincango and Causton 2009; Causton et al. 2019). Traps were baited with 150 mL of liquid lure comprised of 600 g ripe Hawaiian papaya, 75 g sugar and 4 L of water, blended and fermented in the sun three days prior to use. Trapping occurred in the highland and lowland site. At each site, traps were placed within four study plots, each containing 12 traps separated by 50 m in a three by four trap lattice (Figure 1). In addition, four traps were placed in two more study plots along a single transect each separated by 50 m (N = 32 traps per site, total N = 62 traps; Figure 1). Traps were placed alternatively at four and seven metres high to capture potential sex ratio differences of flight height found previously by Kleindorfer et al. (2016). Bait lure was replaced and all specimens collected every five days. This was repeated nine times from January 19th to March 5th for a total of 563 trapping events. Collected flies were stored in 70% ethanol, identified and sexed under a stereomicroscope following morphology described in Kleindorfer et al. (2016).

GPS coordinates were collected for each trap as they were deployed. Distance of each trap to the agricultural zone boundary was calculated using coordinate data. The host nesting density, i.e. the number of active Darwin's finch nests per 200 m x 100 m study plot, was collected from our long-term nest monitoring protocol (see Kleindorfer et al. 2014b), which occurred concurrently with trapping. Search effort for active nests within study plots was equal across highland and lowland sites. The host species monitored were the small ground finch (*Geospiza fuliginosa*), cactus finch (*Geospiza scandens*), small tree finch (*C. pauper*) and the hybrid tree finch (*C. parvulus* × *C. pauper* as well as introgressed individuals). Each monitored host nest that was with eggs (incubation phase) or nestlings (feeding phase) within each study plot (100 m x 200 m) during each trapping period (5 days) was counted as an active nest, giving a nesting density of Darwin's finch nests per plot for each trapping event.

Mapping

Map figures were prepared using ArcMap 10.8.1 (ESRI 2011), with UTM 15S projection. Primary data obtained via ESRI Web Map included Ecosistemas Galápagos 2016, and Vías (roads) layers (ESRI 2017). The shoreline was obtained from NOAA Shoreline World Vector Shoreline (Wessel and Smith 1996; NOAA 2016) and the Digital Elevation Model (Souris 2018) was used to create elevation vectors at 50m intervals for display purposes.

Statistical analysis

All models were fitted in R version 4.0.0 (R Core Development Team 2020) using the packages 'lme4' (Bates et al. 2015), 'car' (Fox and Weisberg 2011) and 'effects' (Fox 2003). Results are presented as estimate ± standard error, unless otherwise stated. Total number of

avian vampire flies (N = 417) across habitats (lowlands: N = 12; highlands: N = 405) was analysed using a Generalised Linear Mixed Model (GLMM) with negative binomial distribution to account for the non-normal distribution and over-dispersion of the count data. To incorporate the dependency among observations of the same trap, we used 'trap ID' as random intercept. To test for the effects of rainfall in highland avian vampire fly abundance, average daily rainfall was calculated per trapping event (5 days) for both sites. However, due to the strong correlation between average daily rainfall and Julian date (Pearson's correlation test: rho = -0.71), rainfall was excluded from further analysis; patterns of rainfall for highlands are instead described in the results. The number of highland avian vampire flies caught in traps was analysed using negative binomial GLMM in relation to Julian date, distance to agricultural zone and host nest, the interaction term Julian date × distance to agricultural zone, with trap ID as a random intercept (N = 417) and a log link function. Corresponding analysis for male (N = 199) and female (N = 205) highland abundance in relation to Julian date, distance to agricultural zone, host nesting density and number of the opposite sex caught in the same trap were analysed using GLMMs with negative binomial distribution, log link function and trap ID as a random intercept. The proportion of male avian vampire flies, representing the sex ratio, was analysed separately for the highlands in relation to Julian date, distance to agricultural zone and host nesting density using a GLM with the command bind ('cbind') function specifically designed to fit ratio data within the binomial family. The number of male avian vampire flies was the binomial denominator, quasibinomial (correct for overdispersion) distribution and a logit link function. All quantitative variables were scaled (mean = 0 and standard deviation = 1) to bring variables into comparable scales and allow interpretation of the magnitude of all main effects (Grueber et al. 2011). Here, we report model effect sizes as estimate \pm SE (summary function in 'lme4'; Bates et al. 2015); $\chi 2$ and p-values from the ANOVA Table of Deviance using Type III $\chi 2$ tests (ANOVA function in the package 'car'; Fox and Weisberg 2011).

Results

The number of avian vampire flies was significantly higher in the highlands compared to the lowlands (GLMM, 3.49 ± 0.33 , p < 0.001, Table 1a; Figure 2; Supplementary Figure 1). Catch rates increased across the breeding season (0.57 ± 0.07 , p < 0.001, Table 1a). There was no effect of host nesting density on the number of flies caught (0.03 ± 0.07 , p = 0.715, Table 1a) and distance to agricultural zone was not included in the most parsimonious model. Trap ID accounted for 0.145 ± 0.38 of the variance in fly abundance. We caught a total of 12 avian vampire flies in the lowlands (0.003 males and 0.005 females per trap per day) and 405 in the highlands (0.135 males and 0.140 females per trap per day). At the onset of trapping (January 19th), which occurred before the onset of Darwin's finch egg laying and nesting (approximately January 25^{th} in highlands, January 31^{st} in lowlands), we caught 13 males and 15 females from 59 traps in January in the highlands and no males or females from 62 traps in the lowlands. Average daily rainfall decreased across the study period (t = -17.037, t = 282, t = -0.71, t = 0.001). During the first trapping period in the highlands (January t = 282, t = -0.71, t = 0.001). During the first trapping period (February t = 282), there was t = 31.9 mm of rain per day. At the end of the study period (February t = 290).

Lowlands

Flies were not collected in the lowlands until the fourth replicate of trapping (February 5th – February 10th; Supplementary Figure 1), despite equal trapping effort across the season and across habitats. On the contrary, in the highlands, flies were collected in the first replicate of

trapping (January 20^{th} – January 25^{th} ; Figure 2). Due to the small sample size of flies collected in the lowlands (N = 12) and low statistical power, we are unable to analyse the effects of host nesting density on adult avian vampire fly abundance or change in sex ratio across the season in the lowlands.

Highlands

Highland avian vampire fly abundance (N = 405) increased across the breeding season (GLMM, 0.57 ± 0.07 , p < 0.001, Table 2a, Figure 2). There was no effect of distance to the agricultural zone (0.09 ± 0.01 , p = 0.370, Table 2a) or host nesting density (0.04 ± 0.08 , p = 0.607, Table 2a) on the overall abundance in the highlands. There was also no interaction effect between trapping date and distance to the agricultural zone (-0.08 ± 0.07 , p = 0.273). Trap ID accounted for 0.16 ± 0.40 of the variance in highland abundance. The sex ratio did not change significantly across the breeding season (GLM, 0.21 ± 0.12 , p = 0.072, Table 1b). There was no effect of host nesting density (N = 16 total active nests in highland study plots across breeding season, Figure 3) on sex ratio when accounting for date of capture (-0.05 ± 0.12 , p = 0.679, Table 1b; Figure 3). The sex ratio was highly skewed towards males closer to the agricultural zone (-0.51 ± 0.11 , p < 0.001, Table 1b) and skewed towards females further from the agricultural zone.

Examining abundance patterns in each sex in the highlands separately, male avian vampire fly abundance (N = 199) increased across the breeding season (GLMM, 0.54 ± 0.10 , p < 0.001, Table 2b), and increased closer to the agricultural zone (- 0.27 ± 0.10 , p = 0.008, Table 3b), with no effect of host nesting density (0.04 ± 0.10 , p = 0.653, Table 2b). There was a positive association between the number of male flies and female flies collected in the same

trap (0.37 \pm 0.07, p < 0.001, Table 2b), as the number of female flies increased, so did the number of male flies. Trap ID accounted for 0.06 ± 0.25 of the variance in male fly abundance. Female avian vampire fly abundance (N = 205) increased across the breeding season (GLMM, 0.28 ± 0.09 , p < 0.001, Table 2c), decreased closer to the agricultural zone (0.38 \pm 0.08, p < 0.001, Table 2c), and did not increase in relation to host nesting density (0.04 \pm 0.08, p = 0.643, Table 2c; Figure 3). Trap ID only accounted for $3.4 \times 10^{-9} \pm 5.8 \times 10^{-5}$ of the variance in female abundance.

Discussion

Understanding sex-specific range use, key resources, and habitat use by populations is critical for developing effective control techniques for invasive species. We found significant differences in temporal patterns of abundance of the invasive avian vampire fly across the Darwin's finch breeding season and two habitat types on Floreana Island. The number of flies caught in traps increased significantly across the breeding season (January – March), which might be due to increasing numbers of adult flies emerging from host nests towards the end of the host breeding season. The prevalence of *P. downsi* in monitored nests with nestlings was 100%, however if the nests failed during incubation, no avian vampire fly larvae were found. Contrary to our prediction, there was no effect of host nesting density on overall abundance or sex-specific abundance. Our data suggest that avian vampire flies may use the highland *Scalesia* and nearby agricultural zone as a refugium during the non-breeding season. Adult flies were collected in the highlands during the first trapping event but were not collected in the lowlands until 20 days into trapping. Avian vampire flies may have dispersed from the humid highlands to the arid lowlands as host breeding increased, as there is evidence that adult flies are capable of dispersing large distances (Fessl et al. 2018). We did not find

support for our predictions of a female-biased sex-ratio or increased abundance near the agricultural zone at the onset of the breeding season. This contrasts with previous research that found males have a shorter lifespan and are unlikely to survive to the next year's breeding season, resulting in a female-biased sex ratio (Causton et al. 2019). Although the sex ratio remained stable across habitats (highlands vs lowlands) and time, we caught more male avian vampire flies closer to the agricultural zone, with females showing the opposite pattern. Our results highlight the importance of the highland habitat, on Floreana Island, as a key location to plan control measures for the avian vampire fly.

The sex differences in catch numbers close to the agricultural zone raise questions about the mating behaviour of the avian vampire fly. It is possible, though untested, that males near the agricultural zone may be guarding food resources or lekking near fruits to attract females. In many species that display resource-based mating, males defend oviposition sites, with both mating and oviposition occurring at the guarded site (Warburg and Yuval 1997; Preston-Mafham 2001; Wilkinson and Johns 2005). This may not be a preferred option for male avian vampire flies, due to the presence of the incubating or brooding female at the nest (Kleindorfer et al. in review). There is video evidence that avian vampire flies wait outside the host nest, only entering once the nest is unattended, and leaving once the female returns (O'Connor et al. 2010a; Lincango et al. 2015). There may be a risk of predation by the insectivorous bird if a fly enters or mates near an occupied nest, making the host nest a less attractive 'mate attraction' resource, especially when considering that the length of mating reported under laboratory conditions is often between 5 and 7 minutes (Causton and Lahuatte et al, pers. comms.). Sex-differences in dispersal or climatic tolerance (Miller and Inouye 2013; Lyons et al. 2014; Enriquez and Colinet 2017) are other explanations for the possible differences in spatial patterns we observed. The proximity to the agricultural zone stands out 118

as a key element that warrants further study, and points to possible differences in nutritional needs between the sexes or mating behaviour. A key factor known to drive sex-specific ranges is male harassment, which may operate in conjunction with or independently of the mating system (Stone 1995; Stanley et al. 2018). Male harassment, and its associated costs to females, has previously been suggested as one possible explanation for sex-specific microhabitat use in the avian vampire fly (Kleindorfer et al. 2016), though it is still untested. Due to the limitations of this study, we are unable to determine the cause of the sex-specific distribution in avian vampire fly on Floreana Island during 2020. Future research could test ideas to disentangle the potential role of mating system and foraging ecology in avian vampire flies in relation to proximity to fruiting trees in the agricultural zone.

Although we did not find a correlation between host nesting density and number of avian vampire flies, there may be a time lag between nest termination and fly emergence, and therefore an increase in fly abundance. Due to the restricted sampling period of this study, we do not know how the abundance of the avian vampire fly changes after host breeding has finished on Floreana. On Santa Cruz, female catch rates remained approximately stable across the non-breeding season, with male catch rates decreasing between breeding seasons (Causton et al. 2019). It is possible that the fly population decreases slowly after the breeding season as adult flies die off. This is in contrast to the rapid drop-off in nesting density seen in this study, which may be why we did not find a relationship between fly abundance and host density; however this remains to be tested. Other host-parasite systems display a parallel pattern of density in host and parasite populations (Byers et al. 2008; Oorebeek and Kleindorfer 2008; Young et al. 2015), however this pattern is not universal (Cardon et al. 2011). Extending the trapping duration outside of the host breeding season can further

explore the sex-specific population dynamics on Floreana Island in relation to host nesting density.

The marked habitat differences in number of flies caught between highlands (N = 405) and lowlands (N = 12) could be explained by rainfall, host density, or number of fruiting trees, whereby our patterns stand in marked contrast to those found on Santa Cruz Island (Causton et al. 2019). On Santa Cruz Island, catch rates of avian vampire fly adults, in particular female flies, was higher in lowland sites compared to highland sites (Causton et al. 2019). Differences in catch rates between the sexes in these two studies -0.08 females vs 0.08 males per trap per day in this study compared to 0.25 females vs 0.08 males per trap per day in Causton et al. (2019) – are likely due to the differences in sampling duration (6 weeks versus one year). It is also notable that the Santa Cruz lowland sites were sampled near a dense urban area with higher human population and potential access to fruiting trees, fresh water and human food (INEC 2015). Causton et al. (2019) trapped adults across the entire year and found lower catch rates for male avian vampire flies during the host non-breeding season. In contrast to Causton et al. (2019), we did not catch fewer males at the start of the host breeding season. The number of birds is generally lower in the lowlands than in the highlands on both Santa Cruz and Floreana Islands (Dvorak et al. 2012; Dvorak et al. 2017). In terms of nesting, across the same time period, monitoring effort and area, we encountered five lowland nests with eggs and 16 highland nests with eggs (Kleindorfer et al. unpublished). While the summary data suggest that host nesting density could explain the different trapping success in lowlands versus highlands, the host nesting density did not predict the number of adult flies caught in highland traps, and so we reject this explanation. We suggest that rainfall, human population and fruiting trees may be more important factors for avian vampire fly abundance.

Although our results are based on one season of data, the clear patterns combined with conservation urgency call for future research and targeted intervention. For example, the Sterile Insect Technique (Hendrichs et al. 2002), whereby large numbers of sterile insects that produce no offspring are released, could be targeted to high male density areas. Utilising mating or attractant pheromones to disrupt mating (Carde and Minks 1995) can be deployed where mating most commonly occurs. Future research should extend trapping into the nonbreeding season and within the agricultural zone at particular fruiting trees to determine patterns of migration across habitat types (Midgarden et al. 2014). Sex-specific population control could be effective in managing and eradicating invasive populations, especially in populations that display sex segregation (Papathanos et al. 2014). If the avian vampire fly is restricted to the highlands and agricultural zone during the non-breeding season, with males primarily distributed in the agricultural zone, this could be a critical area for male population suppression (Hendrichs et al. 2005). Male annihilation techniques, such as those used to decrease fruit fly populations, could be used during the non-breeding season to decrease male density, which can be used alone or in conjunction with sterile insect release (Vargas et al. 2014). With promising developments on survival rates for reared avian vampire fly larvae in a laboratory setting (Lahuatte et al. 2016), the potential for sterile insect breeding and release is increasing, although more research is needed.

Invasive parasites often pose challenges to biodiversity, but they also represent a chance to learn about novel host-parasite systems under new evolutionary selection regimes (Sakai et al. 2001; Allendorf and Lundquist 2003). In an emerging host-parasite system on Floreana that was likely established after 1960 (Kleindorfer and Sulloway 2016), and has been studied since 2004 (Kleindorfer et al. 2014b), we find differences between habitats in numbers of adult flies and sex ratio in relation to proximity to the agricultural zone and across the 121

breeding season. In neither sex did host nesting density predict number of flies caught, but we caught more males close to the agricultural zone at the onset of the breeding season. Intriguingly, the patterns we found are markedly different from those on Santa Cruz Island (Causton et al. 2019), where the number of flies caught was higher in the lowlands than in the highlands and a female-biased sex ratio at the onset of the host breeding season occurred. If the avian vampire fly populations are inhabiting different areas on different islands, this could pose additional challenges for biological control. Future work should extend trapping into the non-breeding season and assess long-term abundance changes in host and parasite abundances across years. Understanding of the movements and populations of both sexes of the avian vampire fly, such as the findings of this study, can inform island-specific targeted control, particularly relevant to control techniques that manipulate breeding behaviour such as the Sterile Insect Technique and pheromone-based mating disruption. This information, as well as the possible occurrence of lag effects of parasite abundance, would further inform the optimal timing and distribution of deploying control efforts. Further research into the spatial and temporal behaviour and ecology of the avian vampire fly in relation to island is critical to untangle the various drivers of adult populations in its invasive range.

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Table 5.1

(a) Highland and lowland avian vampire flies (*Philornis downsi*) from McPhail traps collected during the Darwin's finch breeding season. Generalized linear mixed model with negative binomial distribution for total number of adult avian vampire flies caught in relation to habitat, Julian date and host nesting density. Trap ID was used as a random factor (variance = 0.145 ± 0.38). (b) Generalized linear model with quasibinomial distribution for avian vampire fly sex ratio in the highlands in relation to Julian date, distance to agricultural zone and host nesting density. Sex ratio calculated as the proportion of male flies in relation to female flies. Avian vampire flies collected from McPhail traps on Floreana Island during the 2020 Darwin's finch breeding season.

(a) Total number of <i>P. downsi</i>	Estimate	SE	z-value	LR χ²	df	P-value
(N = 417)	Estimate	SE	z-value	LK	uı	1 -value
Intercept	-3.384	0.31	-10.938			<0.001
II.Liaa				111.9		
Habitat	3.492	0.33	10.582	9	1	<0.001
Julian date	0.586	0.07	8.122	65.96	1	< 0.001
Host nesting density	0.027	0.07	0.365	0.13	1	0.089
(b) Highland <i>P. downsi</i> sex ratio (N = 405)	Estimate	SE	t-value	LR χ ²	df	P-value
Intercept	-0.130	0.12	-1.077			0.283
Julian date	0.207	0.11	1.810	3.318	1	0.072
				23.68		
Distance to agricultural zone	-0.516	0.11	-4.731	6	1	< 0.001
Host nesting density	-0.051	0.12	-0.415	0.173	1	0.678

Dispersion parameter for negative binomial model (a) taken to be 1.981 for quasibinomial model (b) taken to be 0.938.

Table 5.2

Highland avian vampire flies (*Philornis downsi*) from McPhail traps collected in 2020 during the Darwin's finch breeding season from Jan – Mar. Generalized linear mixed model for a) total number of adult avian vampire flies in relation to Julian date, distance to agricultural zone, elevation and host nesting density (random factor trap ID variance = 0.16 ± 0.40); b) number of male avian vampire flies in relation to Julian date, distance to agricultural zone, host nesting density and number of females (trap ID variance = 0.06 ± 0.25); c) number of female avian vampire flies in relation to Julian date, distance to agricultural zone, host nesting density and number of males (trap ID variance = $3.4 \times 10^{-9} \pm 5.8 \times 10^{-5}$). N is the raw number of flies caught.

a) Total number of <i>P. downsi</i> (N							
= 405)	Estimate	SE	z-value	LR χ ²	df	P-value	
Intercept	0.106	0.11	1.005			0.315	
Julian date	0.562	0.07	7.627 58.167		1	<0.001	
Distance to agricultural zone	0.065	0.10	0.648 0.420		1	0.517	
Host nesting density	0.042	0.08	0.076	0.300	1	0.584	
Julian date × Distance to							
agricultural zone	-0.081	0.07	-1.096	1.201	1	0.273	
b) Male <i>P. downsi</i> (N = 199)	Estimate	SE	z-value	LR χ ²	df	P-value	
Intercept	-0.735	0.12	-6.260			<0.001	
Julian date	0.544	0.10	5.420	29.373	1	< 0.001	
Distance to agricultural zone	-0.267	0.10	-2.645	6.994	1	0.008	
Host nesting density	0.045	0.10	0.449	0.202	1	0.653	
Number of females	0.373	0.07	5.238	27.440	1	<0.001	

c) Female <i>P. downsi</i> (N = 205)	Estimate SE		z-value	LR χ ²	df	P-value
Intercept	-0.548	0.09	-6.244			<0.001
Julian date	0.283	0.09	3.269	10.687	1	< 0.001
Distance to agricultural zone	0.380	0.08	4.676	21.862	1	< 0.001
Host nesting density	0.039	0.08	0.464	0.215	1	0.643
Number of males	0.372	0.06	6.582	43.329	1	<0.001

Dispersion parameter for negative binomial model (a) taken to be 1.925; (b) taken to be 2.455; (c) taken to be 5.966.

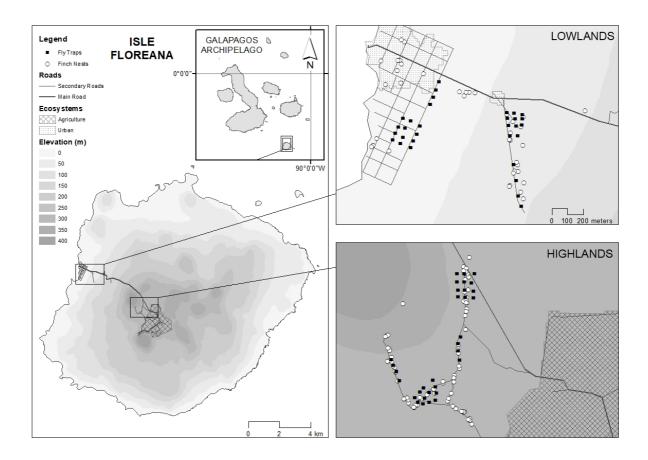


Figure 5.1

Map of Floreana Island, Galápagos Archipelago. McPhail trap locations are marked with black squares, location of Darwin's finch nests monitored across the 2020 breeding season (small tree finch, medium tree finch, small ground finch and cactus finch) are marked with white dots.

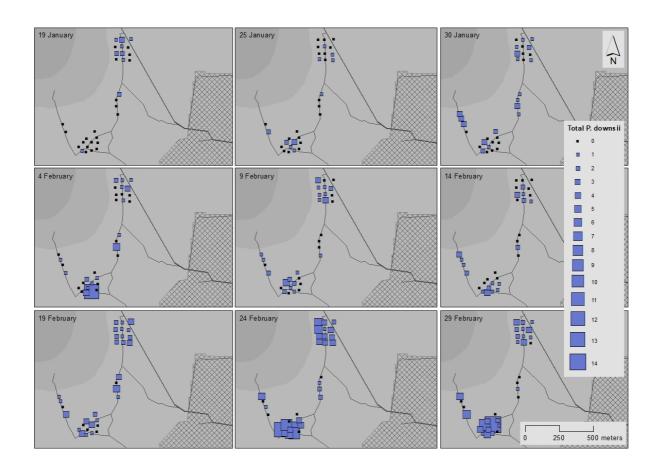


Figure 5.2Avian vampire fly abundance per trapping event (top left of each frame indicates date of trap deployment, trapping events last 5 days) in the highlands of Floreana Island during the 2020

Darwin's Finch breeding season (January 19th to March 5th).

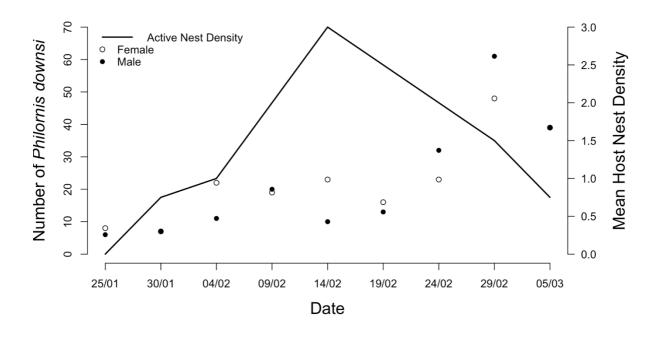


Figure 5.3

Highland number of male and female avian vampire flies (*Philornis downsi*) across date of collection (trapping replicate duration 5 days) with mean host nesting density per study plot (200 m x 100 m). Open circles represent the number of female avian vampire flies caught in the highlands during a trapping event, closed circles represent the number of male avian vampire flies caught across the same time period. Avian vampire flies collected from McPhail traps in the highlands of Floreana Island in 2020 during the Darwin's finch breeding season (January 19th to March 5th).

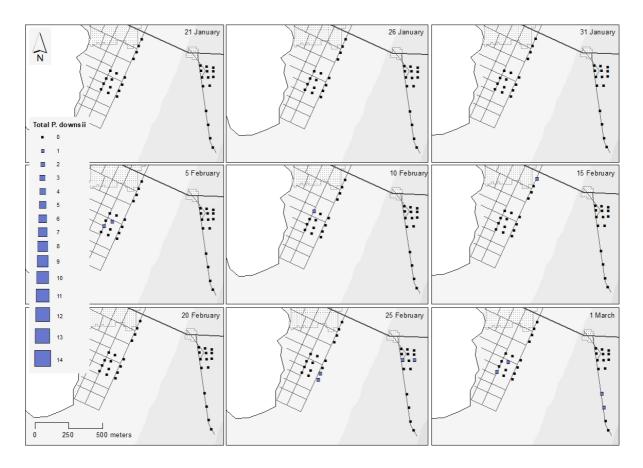


Figure S5.1

Avian vampire fly abundance per trapping event (top right of each frame indicates date of trap deployment, trapping events last 5 days) in the lowlands of Floreana Island during the 2020 Darwin's Finch breeding season (January 19th to March 5th).

Chapter 6 – Genetics reveals shifts in reproductive behaviour of

the invasive bird parasite Philornis downsi collected from

Darwin's finch nests

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Biological Invasions (2022): 1-19.

Abstract

Due to novel or dynamic fluctuations in environmental conditions and resources, host and

parasite relationships can be subject to diverse selection pressures that may lead to significant

changes during and after invasion of a parasite. Genomic analyses are useful for elucidating

evolutionary processes in invasive parasites following their arrival to a new area and host.

Philornis downsi (Diptera: Muscidae), the avian vampire fly, was introduced to the

Galápagos Islands by 1964 and has since spread across the archipelago, feeding on the blood

of developing nestlings of endemic land birds. Since its discovery, there have been significant

changes to the dynamics of P. downsi and its novel hosts, such as shifting mortality rates and

changing oviposition behaviour, however no temporal genetic studies have been conducted.

We collected P. downsi from nests and traps from a single island population over a 14-year

period, and genotyped flies at 469 single nucleotide polymorphisms (SNPs) using restriction-

site associated DNA sequencing (RADSeq). Despite significant genetic differentiation (F_{ST})

between years, there was no evidence for genetic clustering within or across four sampling

years between 2006-2020, suggesting a lack of population isolation. Sibship reconstructions

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from *P. downsi* collected from 10 Darwin's finch nests sampled in 2020 showed evidence for shifts in reproductive behaviour compared to a similar genetic analysis conducted in 2004-2006. Compared with this previous study, females mated with fewer males, individual females oviposited fewer offspring per nest, but more unique females oviposited per nest. These findings are important to consider within reproductive control techniques, and have fitness implications for both parasite evolution and host fitness.

Introduction

Biological invasions by pathogenic and parasitic species are an increasing threat to the health of humans, wildlife, and ecosystems (Fisher et al. 2012; Early et al. 2016; Ricciardi et al. 2017; Jactel et al. 2020). Island populations are especially vulnerable to the impacts of invasive pathogens and parasites, given their isolation, susceptibility to disease and lack of evolved resistance (Wikelski et al. 2004; Russell et al. 2017; Brettell et al. 2021). Invasions can exert extreme selection pressures on both introduced and native species, as well as parasites and hosts (Sakai et al. 2001; Keller and Taylor 2008; Whitney and Gabler 2008; Le Roux 2021), which can influence both the success of invasions and the effectiveness of control methods (Leger and Espeland 2010; Chown et al. 2016; Mayer et al. 2021). This is particularly important for invasive parasites, which are affected by both novel environmental processes and novel hosts. Environmental variables within a parasite's invasive range may impact its growth and life cycle, and thereby influence how parasites affect their novel hosts (e.g., Cline et al. 2014; Liu et al. 2021). Novel hosts exert unique selection pressures on invasive parasites that may lead to altered host-parasite dynamics (Telfer and Bown 2012; McIntire and Juliano 2021) via shifts in parasite strategy and population genetic structure (Brown et al. 2009; Emde et al. 2014; Beaurepaire et al. 2019). Long-term studies of parasite

invasions are critical for identifying evolutionary changes within introduced populations, which may be consequential for the conservation and survival of vulnerable host species.

Long-term studies of invasion dynamics are rare, particularly for invasive species with complex life cycles, such as pathogens and parasites (Miura et al. 2006; Feis et al. 2016). Genetic analyses are useful for elucidating the invasion history, contemporary dispersal, and evolutionary shifts within introduced parasite populations (Lawson Handley et al. 2011; Cristescu 2015; Kamenova et al. 2017). Understanding these genetic processes within invasive species can inform management by identifying dispersal routes between populations, the risk of subsequent invasions, or the evolution of resistance to control measures (Sakai et al. 2001; Gaskin et al. 2011). The efficacy of biological control methods often requires rigorous and contemporary information on mating behaviour, selection patterns and demography of the target population (Roderick and Navajas 2003; Lance and McInnis 2005). For example, the Sterile Insect Technique (SIT) is a method used to suppress and eradicate insect pests (e.g., Diptera: Enkerlin et al. 2017; Lepidoptera: Dyck et al. 2021; Gato et al. 2021; Coleoptera: Himuro et al. 2022). SIT involves the release of large numbers of sterile adult males, and relies on low female remating frequency (i.e., polyandry) and low dispersal (Hendrichs et al. 2005; Lance and McInnis 2005). Dispersal rates may increase significantly during insect invasions and range expansions as selection can favour individuals with greater dispersal capacity (Travis and Dytham 2002; Lombaert et al. 2014; Dudaniec et al. 2021). Rates of female remating have been found to increase during range expansion in insects (Laugier et al. 2013; Crowther et al. 2019), potentially as a strategy to increase female fecundity and genetic diversity at colonised sites. Therefore, changes to parasite dispersal and reproductive behaviours can affect the feasibility of costly and time-consuming control measures. Changes to parasite reproductive behaviour may also affect parasite relatedness, 134

and therefore host fitness (Buckling and Brockhurst 2008; Gleichsner et al. 2018). Kin selection predicts that high parasite relatedness leads to decreased competition between parasites and more prudent exploitation of the host, therefore higher host fitness and lower host mortality (Frank 1992; Buckling and Brockhurst 2008). Thus, understanding these changes in invasive systems is critical for both effective management, and measuring impacts on affected native host species.

The avian vampire fly, *Philornis downsi* (Diptera: Muscidae) (Dodge and Aitken, 1968), was discovered in the nests of Darwin's finches on the Galápagos Islands in 1997 (Fessl et al. 2001). It was accidentally introduced to the Galápagos archipelago *circa* 1964 from its native distribution on the South American mainland (Fessl et al. 2001; Causton et al. 2006; Fessl et al. 2018). The free-living larvae of *P. downsi* reside in nests of many land bird species, feeding on the blood and tissue of developing nestlings (Fessl et al. 2006b). This novel parasitism has significant effects on avian hosts on the Galápagos, causing anaemia, mortality, and permanent physical deformations (Fessl et al. 2006a; Kleindorfer and Dudaniec 2016; Katsis et al. 2021). *Philornis downsi* has been detected on 15 of the 17 islands across the archipelago, infesting nearly all studied passerine species (Wiedenfeld et al. 2007; Fessl et al. 2018; McNew and Clayton 2018). Due to its severe mortality effects on hosts, particularly critically endangered Darwin's finch species (Lawson et al. 2017; Kleindorfer et al. 2021b), control and eradication of *P. downsi* on the Galápagos is a high priority.

Previous studies have found that *Philornis downsi* shows little evidence for genetic differentiation across five islands in the Galápagos archipelago (Santa Cruz, Floreana, Isabela, Santiago and San Cristobal) and across the lowland and highland habitats (Dudaniec 135

et al. 2008a; Koop et al. 2020), indicative of moderate to high genetic dispersal. However, given strong selection pressures associated with invasion (Le Roux 2021), genetic drift in small founding populations (Polechová 2018), and interactions with multiple novel hosts (Telfer and Bown 2012; McIntire and Juliano 2021), P. downsi is expected to show evolutionary shifts since its introduction. Further, previous research detected morphological changes in P. downsi, with an ~11% decrease in female body length and a ~26% decrease in female abdomen size between 2004-2016, the latter trait being strongly correlated with fecundity (Common et al. 2020). Similarly, host mortality rates and parasite intensity (number of parasites per nest) shifted between 2000-2014, increasing in some species while decreasing in others (Dudaniec et al. 2007; Cimadom et al. 2014; Kleindorfer and Dudaniec 2016). The oviposition behaviour of P. downsi also appears to be changing over recent decades. Previously, females oviposited primarily during late incubation, so their eggs hatched simultaneously with eggs of their host (O'Connor et al. 2010a). In later years, larvae were detected in incubating nests of several host species, suggesting females are ovipositing earlier in the nesting cycle (Common et al. 2019). These changes in body size, intensity, and oviposition behaviour lend support that shifts in dispersal and reproductive behaviour may be occurring across time and could be measurable at the genetic level.

The mating and oviposition behaviour of *Philornis downsi* has not been measured genetically since Dudaniec et al. (2010), which used microsatellite data to explore *P. downsi* remating frequency and oviposition behaviour with data from 2004-2006. Via microsatellite-based sibship reconstructions (i.e., inference of full- and half-sibling relationships between individuals within a nest) and genetic relatedness, Dudaniec et al. (2010) found that one to six females infested a single nest, each contributing an average of ~5 offspring per nest. Multiple mating was common, with each female *P. downsi* mating with an average of 1.9 males 136

(Dudaniec et al. 2010). In this study, we analyse *P. downsi* collected from traps and the nests of the small tree finch (Camarhynchus parvulus), medium tree finch (C. pauper) and small ground finch (Geospiza fuliginosa) on Floreana Island (Galápagos) with four sampling years that span a 14-year period. This time period represents 64% of the period in which the fly has been documented in finch nests (Fessl et al. 2001). With genomic dataset with higher resolution than the previous microsatellite study, derived from restriction site associated DNA sequencing (RADSeq), we aim to examine temporal shifts in genetic structure and reproductive behaviour in *P. downsi*. Specifically, we examine inter-annual variation in i) genetic divergence, ii) effective population size, and iii) evidence for population bottlenecks. Within infra-populations (i.e., the parasites present within each host nest), we use sibship reconstructions to examine for temporal shifts in iv) genetic relatedness, v) the number of female flies ovipositing per nest, vi) the number of offspring assigned per female, vii) the number of male flies contributing to the offspring in each nest, and viii) the number of males assigned to the offspring of each female, (i.e., an estimate of female remating frequency). We anticipate that this information will offer an updated and temporal insight into P. downsi evolution within its invasive Galápagos range, with implications for host fitness, and future control programs.

Materials and methods

Study species

Philornis downsi is a Dipteran ectoparasite that feeds on developing nestlings (Fessl and Tebbich 2002; Kleindorfer and Dudaniec 2016; Common et al. 2019). Adult *P. downsi* are vegetarian and non-parasitic, feeding on decaying vegetable matter and fruit (Fessl et al. 2018). Adult females lay their eggs in incubating or brooding nests of 150 known host bird

species across 10 Orders in their native distribution across mainland South America, and their invasive range in the Galápagos Islands (Fessl et al. 2018; McNew and Clayton 2018). The eggs of *P. downsi* usually hatch concurrently with host species hatching, and first instar larvae move to the nares of the nestlings where they feed on blood and tissues of the developing birds (Fessl et al. 2006b). Second and third instar larvae also feed within the nares, but commonly move to the base of the nest during the day, feeding both internally, within the nares and ear canals, and externally, piercing the skin of the nestling, at night (Fessl et al. 2006b; O'Connor et al. 2010a). Larvae pupate in the bottom of the nest after 4-7 days of feeding, emerging as adults after 7-14 days (Kleindorfer et al. 2014b; Lahuatte et al. 2016; Bulgarella et al. 2017).

Field sample collection

Philornis downsi adults, larvae and pupae were collected during the 2006, 2008, 2014 and 2020 Darwin's finch (Passeriformes: Thraupidae) breeding seasons (January-April) from nests of small ground finches (*G. fuliginosa*), small tree finches (*C. parvulus*) and medium tree finches (*C. pauper*) on Floreana Island. The study site was located in the highlands on Floreana (01°17'S, 090°27'W, 300-400 m asl), a humid *Scalesia* forest at the base of Cerro Pajas volcano. Nests were located and monitored from incubation to nest termination (fledging or nestling death) following well-established field protocols (Kleindorfer et al. 2014b). Nests were monitored every three days during the egg phase and every two days during the feeding phase, with nest activity determined using a borescope. Within 24 hours of nest termination, nests were dismantled to collect all *P. downsi* specimens residing within the base of the nest. All collected specimens were identified to age class (i.e., first – third instar larvae, pupae, adult, Common et al. 2019) under a Leica MS5 dissecting microscope,

preserved in 90% ethanol and stored in a -20 °C freezer. The number of nests per year and the number of specimens collected from each nest are presented in Table S1.

To sample adult *P. downsi*, McPhail traps (BioQuip Products, California, USA) were deployed in two of the four sampling years, 2014 (February 18th – April 15th) and 2020 (January 19th – March 5th). McPhail traps were baited with 150 mL of fermented papaya sugar mixture (600 g ripe papaya, 75 g white sugar, 4 L water, blended and fermented for three days; Lincango and Causton 2009). In 2014, 28 traps were placed every 15 m along four 90 m transects at two to seven metres high (m above ground). Bait lure was replaced, and specimens were collected every seven days, for a total of seven trapping events. In 2020, 32 traps were placed every 50 metres in a 200 m × 100 m lattice in two study plots, and along two 200 metre transects in two separate plots (Common et al. 2022b). Traps were hung at four and seven metres high, to capture both male and female *P. downsi*, as previous research found a difference in capture height between the sexes (Kleindorfer et al. 2016). Bait lure was replaced, and specimens were collected every five days, for a total of nine trapping events

DNA extraction, sequencing and filtering

DNA extraction was undertaken using whole specimens of 285 P. downsi individuals (larvae, pupae and adults) by Eurofins BioDiagnostics Inc. (Wisconsin, USA). Sample sizes for each sampling year were: 2006 = 27; 2008 = 40; 2014 = 43; 2020 = 175 (Table S2). The whole specimen was extracted for larvae (n = 26) and pupae (n = 199). For adult specimens, the head, thorax, and several legs were used for extraction (n = 60). Extracted DNA concentrations were standardised to $10 \text{ng/}\mu\text{l}$ and prepared in to paired-end Restriction-site Associated DNA sequencing (RADSeq) libraries with the *Sbf I* restriction enzyme, similar to

the method of Baird et al. (2008) and following a protocol performed by Floragenex, Inc. (Oregon, USA), as described in Text S1. Samples were sequenced on the Illumina HiSeq4000 platform.

A bioinformatics pipeline implemented by Floragenex Inc. (Oregon, USA) was used to process the raw sequencing data, call variants and filtering, as described in Text S1. A locus was retained if it was present in a minimum of 60% of individuals and had a minor allele frequency (MAF) of 0.02 and a minimum read depth >8. Individual missingness (i.e., the percentage of missing data per individual specimen) was calculated in vcftools (Danecek et al. 2011). Due to poor DNA quality, missing data was of concern, therefore we subsampled and analysed the data for 10% and 30% missingness (Figure S1). These values for missing data were chosen to explore the effects of low and high missing data on our results, and to examine these patterns with a stringent (10%) data set and a dataset with more individuals (30%). Further filtering was conducted in Plink 1.9 (Chang et al. 2015) for each dataset to identify and remove loci deviating from Hardy-Weinberg equilibrium (HWE) at P < 0.01. To minimise the effects of linkage disequilibrium (LD), we excluded one marker from each pair with R² > 0.5 using the window-based method.

Genetic variation and structure

To assess changes in genetic structure across the sampling period, all specimens collected from nests and traps were analysed 1) for all years pooled, and 2) separately per year (2006, 2008, 2014, 2020), and for each of the 10% and 30% missingness datasets. Samples from a given year are independent generations as previous studies found that *P. downsi* only survives

to the next year's breeding season, but not subsequent breeding seasons, and our sampling periods are 2-6 years apart (Causton et al. 2019; Bulgarella et al. 2022).

To avoid potential bias in allele frequencies due to highly related individuals within nests, we calculated pairwise relatedness in vcftools (Danecek et al., 2011), which calculates relatedness using the method developed by Manichaikul et al. (2010). We subsequently removed one individual from each pair that had a relatedness value > 0.25 (i.e., full siblings) from the dataset. Trapping data represents a random sample of the adult fly population and therefore all trapping samples were included in the analysis, together with those collected from nests. The sample sizes for analysing genetic structure per year were: 10% missingness: 2006 = 15, 2008 = 14, 2014 = 19, 2020 = 73; total N = 121; 30% missingness: 2006 = 20, 2008 = 22, 2014 = 28, 2020 = 101; total N = 171.

Genetic diversity parameters were calculated per year using the R package *hierfstat* (Goudet 2005) including observed heterozygosity (H_o), expected heterozygosity (H_e), and the inbreeding coefficient (F_{IS}). Allelic richness statistics were calculated per year using the R package *PopGenReport* (Adamack and Gruber 2014). Pairwise F_{ST} between years was calculated using the method developed by Weir, Cockerham (1984) implemented in *hierfstat*, with bootstrapped 95% confidence intervals and 10,000 permutations to determine significance. An analysis of molecular variance (AMOVA) was calculated to determine the variance between years in GenoDive 3.05 (Meirmans 2020).

We examined for genetic structure using two approaches: 1) pooling all samples across the four years, and 2) separating the samples by year to determine within-year substructure. Two methods to detect genetic structure were applied. We used discriminant analysis of principal 141

components (DAPC; Jombart et al. 2010) implemented in the package adegenet (Jombart 2008). DAPC is a model-free approach that transforms genotypes into principal components (PC), applies a discriminant analysis to the number of PCs retained to optimize among-group variation and minimize within-group variation, calculating the optimum number of clusters using Bayesian Information Criterion. Finally, we used the software STRUCTURE 2.3.4 (Pritchard et al. 2000), which utilises an iterative Bayesian clustering method, to calculate allele frequencies and individual assignments to genetic clusters. In STRUCTURE, 10 runs with 20,000 Markov Chain Monte Carlo (MCMC) iterations after a 10,000-iteration burn-in period was conducted for each K value (all years combined: K = 1 - 10; per year analysis: K = 1 - 5). The number of distinct genetic clusters, K, was selected using STRUCTURESELECTOR (Li and Liu 2018), which implements two methods for K selection. The change in K (Δ K) approach compares the rate of change of the log probability to predict the optimal K (Evanno et al. 2005). STRUCTURESELECTOR also reports four estimators MEDMEDK, MEDMEAK, MAXMEDK, and MAXMEAK to determine the optimal K (Puechmaille 2016). These methods assign subpopulations to a cluster if the mean or median individual membership coefficient was above the threshold of 0.5.

Effective population size

The effective population size (N_e) of *P. downsi* on Floreana Island was estimated using the program COLONY 2.0.6.7 (Jones and Wang 2010). COLONY uses a maximum likelihood method on the frequency of full- and half-sibling assignments within pairs of randomly selected individuals to determine effective population size (Wang 2009). This method was selected as it is more flexible than other N_e estimation methods, and therefore more robust in handling violations of assumptions, such as non-random mating (Wang 2009). As COLONY

assumes all individuals are from the same population within a single generation, the samples were split per year and analysed separately. Highly related individuals (r > 0.25) were removed from the dataset for the purpose of N_e estimation, and the 10% and 30% missingness datasets were further analysed separately. To explore the effect of genotyping error rate on N_e estimates (e.g., allelic dropouts and missing data), all datasets were analysed using three error rates: 1%, 5% and 10% (Wang 2019; Guppy et al. 2020).

Bottleneck detection

We used the program BOTTLENECK 1.2.02 (Piry et al. 1999) to assess whether a recent population bottleneck has occurred. BOTTLENECK tests for heterozygosity excess compared to expectations under mutation-drift equilibrium (Cornuet and Luikart 1996) by comparing expected and observed heterozygosity and allele frequency per locus to determine if a locus is in heterozygosity excess or deficit, and whether this difference is significant.

Data were analysed for each year (and for each of the 10% and 30% datasets) under two mutation models, the infinite allele model (IAM) and the stepwise mutation model (SMM) as these models are most suitable for biallelic SNP data (Cornuet and Luikart 1996; Kogura et al. 2011). The sign test with 1,000 permutations was used to determine the significance of heterozygosity excess across loci. To detect a mode-shift in allele frequencies that may be indicative of a population bottleneck (Luikart et al. 1998), we examined the allele frequency distribution. An approximately L-shaped distribution is expected under mutation-drift equilibrium (Luikart et al. 1998).

Within-nest relatedness and sibship reconstruction

To estimate genetic relatedness and reconstruct sibships within each sampled nest, P. downsi were analysed from seven small ground finch (G. fuliginosa) and three small tree finch (C. parvulus) nests collected in 2020. Parasite intensity in sampled nests was 41.7 ± 7.6 (N = 10). Due to the small body size of first and second instar larvae, only third instar, pupae and adult specimens collected from nests were sequenced. Dudaniec et al. (2010) found relatedness did not vary with the number of individuals sampled from a nest and concluded a subsample of 10% of the infra-population was deemed sufficient to estimate in-nest relatedness. Thus, at least 10% of each infrapopulation was analysed (average 24%, range 13.3% - 36.0%) for a total of 98 specimens sampled from 10 nests.

Mean pairwise genetic relatedness was calculated between individuals within each nest in vcftools using the method of Manichaikul et al. (2010). The program COLONY 2.0.6.7 (Jones and Wang 2010) was used to reconstruct sibship and parentage within each infrapopulation. COLONY uses a maximum likelihood method to infer parentage and sibship structure from multi-locus genotype data (Jones and Wang 2010). We ran COLONY across the two datasets: 10% and 30% missingness. Each nest was run using an error rate of 1%, 5% and 10% to explore the effect of genotyping error on number of putative maternal and paternal genotypes identified in each nest (Wang 2019; Guppy et al. 2020). Polyandry was selected for females, as studies on other Dipterans (Arnqvist and Nilsson 2000; Dunn et al. 2005), and on *P. downsi* specifically (Dudaniec et al. 2010), found evidence for multiple mating. As in Dudaniec et al. (2010), monogamy was selected for males because it is highly unlikely that females that mated with the same male are ovipositing in the same nest due to random mating and a large population size.

We explored the effects of individual missingness (10% and 30%) and error rate on the number of putative maternal and paternal genotypes inferred from COLONY using ANOVA in R 4.1.0 (R Core Development Team 2020). Furthermore, to test the assumption that 10% of the infrapopulation sampled is sufficient to assess relatedness, we used multiple linear regression in R to explore the effect of the percentage of the total infrapopulation genotyped and total infrapopulation size (parasite intensity) on the number of putative female and male genotypes, and on mean pairwise individual relatedness within nests.

Results

Data filtering

A total of 127,871 SNP variants were obtained from RAD sequencing. After removing SNPs due to low quality or missing data, 7021 SNPs remained. The total number of reads and RAD clusters per dataset are presented in Table S3. After removing SNPs with a MAF < 0.02, those in linkage disequilibrium and deviating from HWE, a total of 469 SNPs remained in the 10% missingness dataset (N = 138) and 462 SNPs remained in the 30% missingness dataset (N = 188) (Table S4). For analyses that required only unrelated individuals (i.e., genetic structure), individuals with pairwise relatedness greater than 0.25 were removed, giving final sample sizes of: 10% = 121, 30% = 171.

Genetic diversity and structure

Across years combined, observed heterozygosity was lower than the expected heterozygosity $(H_o=0.290,\,H_e=0.383,\,Table\,1;\,Table\,S5$ for results at 30% missingness) and the inbreeding coefficient (F_{IS}) was = 0. 243 (Table 1). Within years, H_o ranged from 0.255 – 0.323 and F_{IS}

from 0.112-0.352 (Table 1). Mean allelic richness increased consistently from 2006 (1.91) to 2020 (2.00, Table 1). The study year 2020 was the only year with private alleles (i.e., unique alleles found only within this sampling year), with four alleles being unique to the 2020 specimens (Table 1). Pairwise F_{ST} between years ranged from 0.003 to 0.010. All pairwise F_{ST} values between years were significant to P < 0.05, suggesting some genetic differentiation between study years (Table S6a). However, analysis of molecular variance (AMOVA) comparing years showed that only 0.7% of the molecular variance was explained by year (mean $F_{ST} = 0.007 \pm 0.001$ standard deviation (SD), P < 0.001), with 12.2% of the variation explained among individuals (mean $F_{IS} = 0.123 \pm 0.017$ SD, P < 0.001), and 87.1% explained within individuals (mean $F_{IT} = 0.129 \pm 0.017$).

For the analysis of genetic structure pooling all years using DAPC, the lowest BIC value suggested an optimal K of 1 at 10% missingness, and K = 2 at 30% missingness (Figure S2). The result of K = 2 was not explained by year, sex or age and therefore is not biologically meaningful. The most likely number of clusters of all years pooled determined by the maximum likelihood method of STRUCTURE was K = 2 for 10% missingness and K = 1 for 30% missingness, and the Δ K method of cluster selection suggested K = 3 for both datasets. However, the assignment probabilities of each individual to the second or third clusters were low (e.g., 10%: cluster 1 = 0.46 ± 0.009, cluster 2 = 0.46 ± 0.009; cluster 3 = 0.08 ± 0.01), suggesting little to no support for genetic clustering across years. DAPC analysis on data separated by year suggested an optimal K of 1 for each year, with the exception of 2020 using the 30% missingness dataset, which had an optimal K of 2 (Figure S3). The mean percentage of individual missingness was high in individuals assigned to cluster 2 compared to cluster 1 (e.g., 2020; cluster 1: 3.78% ± 0.40, N = 82; cluster 2: 21.15% ± 1.14, N = 18), which may

partially explain the detection of two genetic clusters. Furthermore, the clusters did not follow any pattern of clustering by age, sex or host species. When analysing each year separately using STRUCTURE, K = 1 was also deduced with the MedK method for each year. Across all analyses, the individuals assigned to the second or third clusters were not supported by assignment probabilities and did not show a pattern across year, host species, age or sex. Therefore, the *P. downsi* population on Floreana shows little to no evidence for restricted gene flow or genetic divergence across the sampling period.

Effective population size and bottlenecks

Estimates of the effective population size of $P.\ downsi$ on Floreana Island were low and varied across years (Table S7). Estimated N_e using COLONY was highest in 2006 (10% missingness: $N_e = 420,95\%$ jack-knifed $CI = 115 - \infty$, Table S7) and 2014 (10% missingness: $N_e = 342,95\%$ jack-knifed $CI = 133 - \infty$, Table S7). Estimated N_e was lowest in 2020 (10% missingness: $N_e = 64,95\%$ jack-knifed CI = 45 - 93, Table S7). However, many of the upper 95% jack-knifed confidence intervals were undefined, and so we interpret the N_e values as relative estimates across years and with significant caution. Evidence for population bottlenecks was supported by a mode-shift distortion of allele frequencies from the expected L-shaped distribution in all study years (Figure S4), detected by BOTTLENECK. There was a significant deviation from expected equilibrium heterozygosity using both the IAM and the SMM (Table S8, sign test: P < 0.001) in all study years.

Infrapopulation genetic relatedness and sib-ship reconstruction

Mean individual pairwise relatedness varied between *P. downsi* infrapopulations, ranging from -0.089 to 0.205 (10%: 0.079 \pm 0.016; 30%: 0.027 \pm 0.030). Relatedness did not vary

with the percentage of the infrapopulation genotyped (Table S9, 10% range: 13.3% - 36.0% genotyped per nest; 0.001 ± 0.003 , P = 0.709) or infrapopulation size in either dataset (Table S9, 10%: -0.001 ± 0.001 , P = 0.344). Therefore, our genetic relatedness estimates were not affected by genotyping effort per infrapopulation.

The percentage of individual missingness (i.e., 10% or 30% dataset) did not significantly affect the number of putative parental genotypes identified in each nest (Table S10, putative maternal genotypes: $F_{1,50} = 1.63$, P = 0.405; putative paternal genotypes: $F_{1,50} = 10.21$, P = 0.182). There was also no effect of error rate on number of parental genotypes (Table S10, putative maternal genotypes: $F_{1,50} = 2.11$, P = 0.636; putative males: $F_{1,50} = 13.78$, P = 0.299). The number of putative maternal or paternal genotypes did not differ with percentage of the infrapopulation genotyped or infrapopulation size (Table S11- S12). Thus, our genotyping effort was sufficient to characterise the sibship relationships within infrapopulations.

All results below are presented as mean \pm standard error for the 10% missingness dataset at 5% error rate (Table 2, see Table S13 for results of 30% missingness dataset, which gave similar results). Multiple female infestations per nest were common, with an estimate of 4.88 \pm 0.48 (range = 3 – 7) maternal genotypes estimated from each nest. Each of these maternal genotypes were associated with 14.3 - 33.3% of the infrapopulation. The number of P. downsi offspring assigned to each maternal genotype ranged between 1.00 and 3.00 (Table 2, mean = 1.89 \pm 0.28). The number of paternal genotypes contributing to each infrapopulation was 6.13 \pm 0.61 (range = 3 – 7). There was evidence for female multiple mating, with a mean of 1.27 \pm 0.09 (range = 1.00 – 1.75) paternal genotypes per maternal genotype.

Discussion

Understanding how demographic and reproductive processes change throughout parasite invasion is critical for tracking novel host-parasite interactions and the success of control techniques, especially when native host species are at risk. We found evidence that female reproductive behaviour has shifted since the 2004-2006 genetic study conducted by Dudaniec et al. (2010). Compared to 2004-2006, the number of female infestations per nest increased by ~70% from 2004 to 2020 (Figure 1). The number of paternal genotypes per nest remained stable (5.44 \pm 0.45 in Dudaniec et al. 2010, compared to 6.13 \pm 0.61, Figure 1), but the number of reported males per female (i.e., rate of multiple mating) decreased ~35%. Concordantly, the genetically assigned females in 2020 had fewer offspring assigned to each of them per nest, with a smaller percentage of the infrapopulation assigned to each maternal genotype comparing time periods (2004-2006: $43.6\% \pm 4.38$, from Dudaniec et al. 2010; 2020: 22.0% \pm 2.22). Female P. downsi are investing fewer offspring per nest, but more females are ovipositing in each nest. These changed have occurred with no significant change to parasite intensity within nests over the sampling period (2004-2006: 30.8 ± 16.5 , Dudaniec et al. 2010; 2020: 43.4 ± 8.3 ; Common et al. 2021). Our study did not detect any genetic clustering of P. downsi on Floreana Island across our four sampling years between 2006 and 2020, however F_{ST} indicated some genetic differentiation between years. Although there was no compelling evidence for neutral genetic structure, the changes in reproductive behaviour we report suggest that P. downsi may be under selection pressures that could lead to adaptation to its novel environment and hosts.

Shifts in reproductive behaviour

Sibship reconstructions of *P. downsi* offspring collected from nests revealed temporal shifts in female reproductive behaviour over a 14-year period. Between 2004 and 2006, Dudaniec et al. (2010) found that on average, approximately three females oviposited in a single nest, each mating with an approximate average of two males and contributing an average of five offspring per nest. Compared to the previous microsatellite data that used the same analytical approach (Dudaniec et al. 2010), we found evidence that P. downsi females are mating with fewer males on average, more females are ovipositing per nest, and each female is contributing fewer offspring per nest (Figure 1). Although the number of loci used in this study was low (469 loci at 10% missingness and 462 loci at 30% missingness), it is sufficient for reconstructing family structure as ~10 SNPs is equivalent to one microsatellite (Wang and Santure 2009). Thus, this genetic dataset has much higher resolution than the previous 2010 study (Dudaniec et al. 2010), which used eight microsatellites (Dudaniec et al. 2008b). The two different datasets may have differences in data resolution and therefore this may account for some of the differences in the results. However, studies evaluating the performance of SNPs versus microsatellites found high power to resolve relatedness and parentage when using 100-500 SNPs, and results between the two types of data were highly congruent (Flanagan and Jones 2019; Premachandra et al. 2019; Weng et al. 2021). Despite the congruence of the two datasets, we note that there may be inherent variation in their results due to the differences in genetic approach, thus care should be taken when comparing them. The maximum likelihood method implemented in COLONY (Jones and Wang 2010) is robust even with high inbreeding coefficients (~0.4; Wang and Santure 2009). It is also important to note that we were unable to genotype smaller first and second larvae collected from the nests, and therefore we may have missed offspring from females ovipositing later in the nesting cycle. This methodology was also used by Dudaniec et al. (2010), with only a 150

small proportion (1%) of genotyped specimens being second instar. Hence, our estimates of the number of females per nest may be underestimated but are comparable to Dudaniec et al. (2010).

Mating of females to multiple males (i.e., polyandry) is common in insects and generally beneficial to female fecundity (Arnqvist and Nilsson 2000; Dunn et al. 2005; Abraham et al. 2011). However, increased multiple mating can decrease female longevity in insects, due to the increased cost of both mating and egg production (Arnqvist and Nilsson 2000; Kawagoe et al. 2001; Gotoh and Tsuchiya 2008). Given that females must survive during the arid, non-breeding period on the Galápagos (Causton et al. 2019; Bulgarella et al. 2022), selection for increased female longevity may be driving the observed decrease in multiple mating. The costs and benefits of polyandry in *P. downsi* are currently not understood. Research into the fitness of invasive species under different rates of multiple mating may further our understanding of the functional significance of changes to mating behaviour.

There are several potential explanations for, and fitness consequences of, changing oviposition behaviour in *P. downsi*. Female body size, in particular abdomen size, has decreased on Floreana Island between 2004 and 2016 (Common et al. 2020). Female body and abdomen size are correlated with fecundity (i.e., number of eggs laid: Pincheira-Donoso and Hunt 2017; Common et al. 2020). Smaller-bodied females may have fewer eggs to lay, and therefore may lay fewer eggs overall or per nest. Because of this, more females may be able to oviposit per nest, without increasing intensity. Female insects and parasites have been known to adjust their clutch size depending on conspecific density, competition, or host quality (Van Alphen and Visser 1990; Damman 1991; Visser and Rosenheim 1998; Díaz-Fleischer and Aluja 2003; Aluja et al. 2019). A decrease in the number of eggs laid per nest 151

may also be due to smaller host size (Díaz-Fleischer and Aluja 2003; Dudaniec et al. 2007). Nestlings are dying younger, and younger nestlings are smaller than older nestlings (*G. fuliginosa* and *C. parvulus* nestling body mass increases approximately 1 g per day: Kleindorfer et al. unpublished data) meaning *P. downsi* larvae are feeding on smaller hosts than in earlier years (Kleindorfer et al. 2014b). Earlier age of host death also creates an unreliable resource, as host death, often due to *P. downsi* parasitism, could occur before larvae are ready to pupate. The unreliability of host resources could contribute to declining parasite clutch sizes, as females oviposit fewer eggs in more nests as a form of spatial bet hedging (Hopper 1999; McLaughlin and Wasserberg 2021). Due to small sample sizes of our two host species, we were unable to analyse *P. downsi* reproductive behaviour between species, which is important for continued monitoring of host-parasite dynamics. Exploration of *P. downsi* clutch size on larger-bodied hosts, such as the Galápagos Mockingbird (*Mimus parvulus*), which is better able to tolerate *P. downsi* parasitism (Knutie et al. 2016), may reveal differences in oviposition behaviour between different host species.

The changes in oviposition behaviour we report may also have implications for avian host fitness, as their nests are forced to sustain increasingly unrelated cohorts of parasites in their nest. When the most optimal parasite strategy requires prudent exploitation of the host (i.e., when parasite transmission requires a living host or requires host resources to reach maturity), increasing parasite relatedness is predicted to decrease virulence (i.e., damage to the host, Buckling and Brockhurst 2008). This is because cooperation between highly related individuals results in indirect fitness benefits for the parasite, i.e., lowered virulence ensures the host survives, allowing for parasites to reach maturity at greater rates, therefore maximising inclusive fitness (Buckling and Brockhurst 2008). Alternatively, where cohorts are highly unrelated, more virulent parasites have a competitive advantage, selecting for 152

increased host exploitation to increase individual parasite development and fitness (Frank 1992; Buckling and Brockhurst 2008). Increased parasite relatedness has further been found to increase parasite transmission, decrease host reproduction and survival (Davies et al. 2002; Gleichsner et al. 2018). Darwin's finches may therefore be facing increased exploitation by *P. downsi* as infrapopulation relatedness decreases, potentially increasing nestling mortality and driving decreasing host age at death (Kleindorfer et al. 2014b).

Genetic diversity and bottlenecks

Genetic drift or bottleneck events after species' introductions can decrease genetic variation, with a strong negative effect on allelic richness, particularly shortly after the event occurs (Dlugosch and Parker 2008; Santos et al. 2012). Allelic richness increased in *P. downsi* across years from 2.06 – 2.38. Allelic richness and heterozygosity in invasive populations increase and stabilize over large time scales, with high gene flow, and with multiple introductions (Dlugosch and Parker 2008; Greenbaum et al. 2014). Observed heterozygosity *in P. downsi* was lower compared to Dudaniec et al. (2008) from 2004 – 2006, but consistent with Koop et al. (2020) from 2015 – 2017. The decrease in heterozygosity we detect across time, particularly in earlier years (2006 – 2014) may be due to genetic drift after introduction or bottleneck effects. Multiple introductions of *P. downsi* to the archipelago from the mainland have not been investigated, so historical introductions may have occurred. As no *P. downsi* have been detected on the Galápagos by 1964, there may have been enough generations after invasion to recover from founder effects.

We found evidence for a recent bottleneck in all years, consistent with past studies (Dudaniec et al. 2008a). With low rainfall and hence a paucity of active hosts, coupled with shorter male

survivorship (188 days) compared with females (265 days; Causton et al. 2019), the annual bottleneck on Floreana Island may be associated with high male (and likely female) mortality at the end of the year, prior to the onset of the next fly breeding season. Therefore, large variation in annual *P. downsi* populations are expected, not only because of the initial colonisation bottleneck, but also given annual reductions in population size after the host breeding season ends (Causton et al. 2019). Only relatively small numbers of adults survive to the next breeding season (Causton et al. 2019; Bulgarella et al. 2022), and therefore only a small proportion of the census population contributes to the next generation.

We report a high inbreeding coefficient (F_{IS}) in *P. downsi* across years of 0.243. Studies have found that F_{IS} can be correlated with the proportion of missing data, and when data were not missing at random (Marandel et al. 2020). Therefore, our results may be biased towards a higher F_{IS} ; although values of our 10% dataset are in line with those found on Floreana by Koop et al. (2020; $F_{IS} = 0.19$). Although high, our reported F_{IS} was also consistent with estimates for *P. downsi* on other islands, on the South American mainland (Koop et al. 2020), and with other invasive insect populations (Karsten et al. 2013; Kirk et al. 2013; Andersen and Mills 2018; Do et al. 2022).

Lack of genetic structure

Our study found no evidence for genetic clustering in the P. downsi Floreana Island population within and across years, suggesting significant gene flow that is consistent across time, and thus, suggests the island is not genetically isolated. Genetic differentiation (F_{ST}) was lower in this study than previously found in P. downsi populations (Dudaniec et al. 2008a; Koop et al. 2020). However, this is not unexpected as previous studies compared

genetic differentiation across islands and habitats, whereas this study compared within a single island and habitat across time. Despite low F_{ST} values, pairwise comparisons between years were all significant, indicating subtle genetic differentiation across the years examined. This differentiation may be affected by the annual population bottlenecks we detected and as suggested by other studies of *P. downsi* (Dudaniec et al. 2008a; Causton et al. 2019). *Philornis downsi* shows high dispersal capability between habitats and islands (Dudaniec et al. 2008a; Fessl et al. 2018; Koop et al. 2020), and combined with frequent tourist movement (Toral-Granda et al. 2017) and strong air currents (Peck 1994) between islands, it is likely that the island populations of *P. downsi* are well-connected. The lack of genetic structure detected in this study could support this, however more study is required to fully understand *P. downsi* movement and gene flow across the entire archipelago, and how these drive or restrict genetic differentiation of separate island populations.

High gene flow may swamp selection and therefore local adaptation to specific islands or host species (Tigano and Friesen 2016; Jacob et al. 2017), but testing for selection was beyond the scope of our data resolution. It is possible that *P. downsi* may still be adapting to the habitat and hosts of the Galápagos Islands, given high genetic differentiation and low gene flow reported between the archipelago and mainland source populations in Ecuador (Koop et al. 2020). Evidence for gene flow between islands in more recent studies (Koop et al. 2020), and the lack of genetic structure we report on Floreana suggests that *P. downsi* on this island is no longer showing signs of genetic isolation from other islands, as concluded by Dudaniec et al. (2008a) who examined gene flow between three islands.

Effective population size

It is important to note the infinite values and upper bounds in our estimates of effective population size, which indicate that values of N_e could be much higher than reported (Wang 2009; Do et al. 2014). We emphasize that these results should be interpreted with caution. The small N_e values may be an artefact of our sampling design or poor data resolution. Particularly in the 2020 study year, a higher proportion of specimens were collected from nests. Although highly related individuals were removed before analysis, it is possible that this sampling design artificially inflated sibship frequencies and hence underestimated the effective population size. Larger numbers of individuals collected from traps with increased number of loci analysed could refine the estimation of effective population size.

The 'relative' effective population size of $P.\ downsi$ on Floreana Island showed considerable variation between years. N_c was highest in 2006 and 2014 (but values for 2008 could not be obtained for the 10% missingness dataset), and lowest in 2020. Notably, this temporal decrease matches a decrease in mean in-nest $P.\ downsi$ intensities (Common et al. 2021) and daily catch rates of adult $P.\ downsi$ across the sampling years (2014: 0.35 ± 0.03 ; Kleindorfer et al. unpublished data; 2020: 0.28 ± 0.02 ; Common et al. 2022b). The estimated values for effective population size were significantly smaller than expected, given the widespread distribution and high in-nest intensity of $P.\ downsi$. However, there are many reasons why N_c may be lower than expected. Positive selection (Charlesworth 2009), individual variation in reproductive success (Hedrick 2005), and temporal variation in population size (Hedrick 2009) can all result in smaller N_c in relation to census size, with evidence for all these factors present in $P.\ downsi$.

Missing data

Due to high rates of missing data in our dataset, we considered the effects of individual missingness on our results. To maximise the number of individuals included in the dataset, we analysed the data with a maximum of 30% missingness, and to maximise the confidence in our data, we analysed the data with a maximum of 10% missingness. Overall, we found results between the 10% and 30% individual missingness data sets were congruent. As stated previously, the inbreeding coefficient, F_{IS}, was also higher in the 30% missingness dataset, likely because F_{IS} is correlated with missingness where missing data is non-random (Marandel et al. 2020). Although there were differences in cluster assignments between the two datasets, the number of genetic clusters remained at one for both rates of missingness. We anticipate that missingness in the data likely drove the detection of K = 2 or 3, and therefore it is important to understand how missingness is distributed across individuals within each cluster, as this may bias results and lead to falsely identifying genetic clusters (Yi and Latch 2022). For the in-nest relatedness and sib-ship reconstruction analyses, we did not find an effect of missingness on the number of putative maternal or paternal genotypes. Therefore, although 30% missingness was sufficient in our study to explore genetic structure and relatedness, researchers must be aware of the biases associated with high missingness datasets. Where missing data is a problem, we recommend a more conservative value of 10% individual missingness to ensure high confidence of results.

Management implications

The lack of genetic structure and divergence in *P. downsi* between years suggests a lack of isolation and frequent gene flow from other islands to Floreana, and possibly throughout the archipelago. Therefore, a targeted, island-specific control method for *P. downsi* may not be

effective in the face of ongoing colonisations. Archipelago-wide strategies, such as increasing quarantine restrictions on boats moving between islands, may be more effective in the longer term. More information on gene flow and migration between human inhabited and uninhabited islands, and Galápagos and the mainland, is needed to fully determine the efficacy of targeted control methods. Changing reproductive behaviours may affect some control methods as female remating is a key factor in the success of SIT. The decrease in remating frequency reported in this study may decrease the risk of females mating with a viable male, and therefore increase SIT success (Hendrichs et al. 2005; Lance and McInnis 2005). Another potential control technique is the utilisation of synthetic gene drives; selfish transgenic elements that bias heredity with the aim to suppress or eradicate populations, such as pests or invasive species (Sinkins and Gould 2006; Bunting et al. 2022). Determining the efficacy of synthetic gene drives requires detailed information of both the genetic structure and life history of target populations (James et al. 2020). The lack of population isolation on Floreana suggests that use of a self-sustaining gene drive on this island could spread throughout the archipelago. Our study, and other recent genetic and ecological studies on P. downsi provide the knowledge base upon which potential control methods can be explored.

Conclusions

While some previous studies have examined spatial genetic variation in *P. downsi* (Dudaniec et al. 2008a; Koop et al. 2020), here we investigated temporal changes in genetic variation and reproductive behaviours and provided insight into the evolutionary dynamics of this invasive host-parasite system. For the Floreana Island population of *P. downsi* sampled between 2006 and 2020, we characterised genetic processes and changes approximately 25 years after the fly's discovery in Darwin's finch nests. There was evidence for subtle genetic

differentiation between years, however there was limited to no evidence for genetic clustering. If gene flow was restricted to Floreana and the P. downsi population was isolated, we would expect to see genetic differentiation across the 14-year sampling period, particularly given the population bottlenecks we observe, the low estimated effective population size and previous studies indicating host-parasite co-evolution over this period. Changes to reproductive behaviour are concordant with previous morphological shifts in P. downsi, and may suggest host-parasite co-evolutionary interactions or environment adaptations that require further study. The inclusion of multiple locations and comparison to the mainland for exploring signatures of selection would provide a more comprehensive idea of how P. downsi is responding to novel habitats and hosts during its invasion. Decreased relatedness among parasites in nests could be leading to increased host exploitation, and therefore increasing rates of mortality in threatened Darwin's finch populations. Historical and contemporary estimates of genetic migration rates could further resolve the parasite's colonisation history and gene flow across the archipelago. This information could then be used to predict the success of targeted management strategies and how control will affect gene flow and adaptation. Continued research into temporal genetic changes in parasitic populations is imperative for planning adaptive and effective control strategies, and hence the conservation of vulnerable host species.

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Observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), allelic diversity and richness calculated for all years combined and separated by year on the 10% missingness dataset. Mean AR = Mean Allelic Richness.

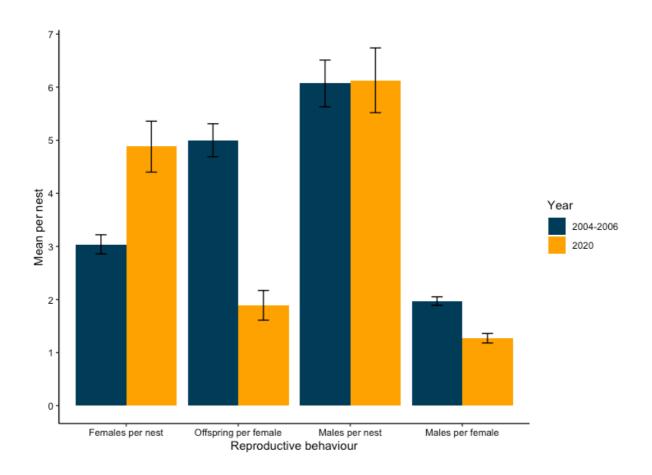
	N	H _o	H _e	F _{IS}	# of alleles	Mean AR	# Private Alleles
All	121	0.290	0.383	0.243			
2006	15	0.323	0.363	0.112	1.913	2.06	0
2008	14	0.255	0.393	0.352	1.934	2.30	0
2014	19	0.278	0.394	0.294	1.966	2.35	0
2020	73	0.290	0.383	0.206	2.000	2.38	4

Table 6.1

Table 6.2

Results of sibship reconstruction analysis for all 2020 data with 10% individual missingness (N = 10 nests). Data are presented as mean \pm SE; range. Value descriptions are as follows: # female infestations: mean number of reconstructed female genotypes per infrapopulation; # paternal genotypes: mean number of reconstructed male genotypes per infrapopulation; # males per female: mean number of males assigned to the offspring of each female per nest; # offspring per female: mean number of offspring assigned to each maternal genotype; % total offspring per female: mean percentage of the total offspring contributed by each female per infrapopulation.

Error	# Female	# Paternal	# Males	# Offspring	% Total offspring
rate	infestations	genotypes	per female	per female	per female
1%	$5.13 \pm 0.52;$	$6.63 \pm 0.71;$	1.30 ±	$1.80 \pm 0.26;$	$21.07 \pm 2.29;$
	3 – 7	3 – 8	0.12;	1.00 - 3.00	14.29 – 33.33
			1.00 - 2.00		
5%	$4.88 \pm 0.48;$	6.13 ± 0.61 ;	1.27 ±	$1.89 \pm 0.28;$	$22.00 \pm 2.22;$
	3 – 7	3 – 8	0.09;	1.00 - 3.00	14.29 – 33.33
			1.00 - 1.75		
10%	$4.75 \pm 0.53;$	$5.63 \pm 0.60;$	1.21 ±	$2.01 \pm 0.34;$	$23.04 \pm 2.63;$
	3 – 7	3 – 7	0.10;	1.00 - 3.20	16.67 – 33.33
			1.00 – 1.75		



Summary of COLONY results, comparing the results found by Dudaniec et al. (2010) from *P. downsi* collected in Darwin's finch nests between 2004-2006 (microsatellite data, with typing errors (5%); N nests = 57; N individuals = 1020), to *P. downsi* collected from Darwin's finch nests in 2020 (SNP data, 10% missingness, 5% error dataset; N nests = 10; N individuals = 77). Data are presented as mean ± standard error.

Figure 6.1

Text S6.1

Library preparation, alignment and filtering.

Genomic DNA was digested with the restriction endonuclease *Sbf I* and processed into RAD libraries similar to the method of Baird et al. (2008). Genomic DNA was digested for 60 min at 37 °C in a 15 μ L reaction with 20 units (U) of *Sbf I* (New England Biolabs, Inc., Ipswich, Massachusetts, USA). After digestion, samples were heat-inactivated for 20 min at 80 °C followed by addition of 2.0 μ L of 1 nM P1 Adapter(s), a modified Solexa adapter (Illumina, Inc.). P1 adapters each contained a unique multiplex sequence index (barcode), which is read during the first ten nucleotides of the Illumina sequence read. 1 uM P1 adaptors were added to each sample along with 10x T4 DNA Ligase Buffer (Enzymatics, Inc), high concentration T4 DNA Ligase (Enzymatics, Inc), and 0.8 μ L H₂O which was then incubated at room temperature for 20 min.

Samples were heat-inactivated again at 65 °C for 20 minutes, pooled, and randomly sheared with a Bioruptor (Diagenode) to an average size of 500 bp. Samples were then run out on a 1.5% agarose, 0.5X TBE gel, and DNA 250 bp to 800 bp was isolated using a MinElute Gel Extraction Kit (Qiagen). End Repair / dA-Tailing module (NEB) was used to polish the ends of the DNA. After subsequent purification, 1 μL of 1 μM P2 adapter, a divergent modified Solexa adapter (Illumina, Inc.), was ligated to the obtained DNA fragments at RT. Samples were again purified and eluted in 15 μL . The eluate was quantified using a Qubit Fluorometer with the dsDNA HS assay kit (Invitrogen) and ~200 ng of this product was used in 40 50 μL PCR amplifications with 25 μL 2x Phusion Master Mix (NEB), 2.5 μL of 10 μM modified Solexa Amplification primer mix (Illumina, Inc.) and up to 22.5 μL H₂O. 200 μL of the amplification product was cleaned and run on a 1.5% agarose (Sigma), 0.5X TBE gel and DNA 350 bp to 900 bp was excised and purified as before. Each library was quantified with a Qubit fluorometer and run on an Agilent Bioanalyzer with the High Sensitivity kit to determine size distribution. 2x150pb paired end sequencing was performed on the HiSeq 4000.

Sequences were aligned to an assembled *de novo* reference from a single high performing sample. Sequences were filtered through Floragenex bioinformatic pipeline using the programs BOWTIE version 1.1.1 (Langmead et al. 2009), BWA version 0.6.1 (Li and Durbin 2009), SAMTOOLS version 0.1.16 (Li et al. 2009) and VELVET version 1.2.10 (Zerbino and Birney 2008). SNPs with a Phred score below 20 were filtered for a using vcftools 0.1.16 (Danecek et al. 2011) and a minimum read depth filter of >8x was applied.

The number of raw reads initially obtained was 344,184,316 (mean per sample = 1,199,248.5 3,215,032.5). After initial filtering, the total number of reads was 5,964,599 (Table S2 for mean number of reads per dataset). The mean number of RAD clusters across individuals per dataset after filtering are presented in Table S2.

Distribution of specimens within sampled nests across years. Species indicates the host species of the nest: STF = small tree finch (*Camarhynchus parvulus*), MTF = medium tree finch (*Camarhynchus pauper*), and SGF = small ground finch (*Geospiza fuliginosa*).

Year	arhynchus pauper Species	Nest ID	Larvae	Pupae	Adults
2006	STF	STF08	0	1 upae	0
2006	STF	STFX09	0	2	0
2006	STF	STF10b	0	2	0
2006	STF	STFX17	0	2	0
2006	STF	STFX21	0	2	0
2006	STF	STFX22	0	1	0
2006	STF	STFX28	0	1	0
2006	STF	STF30	0	1	0
2006	MTF	Y9	0	1	0
2006	MTF	Y19	0	1	0
2006	SGF	SGF02	0	1	0
2006	SGF	SGF06	0	1	0
2006	SGF	SGF07	0	1	0
2006	SGF	SGF09	0	1	0
2006	SGF	SGF16	0	2	0
2008	MTF	8LTF2	0	2	0
2008	MTF	8MTF1	0	1	0
2008	MTF	8MTF18	0	2	0
2008	MTF	8MTF21	0	1	0
2008	MTF	8MTF31	0	2	0
2008	SGF	8SGF16	0	1	0
2008	SGF	8SGF17	0	1	0
2008	SGF	8SGF2	0	2	0
2008	SGF	8SGF21	0	2	0
2008	SGF	8SGF22	0	0	1
2008	SGF	8SGF27	0	1	0
2008	SGF	8SGF37	0	2	0
2008	SGF	8SGF39	0	1	0
2008	STF	8STF16	0	1	0
2008	STF	8STF20	0	1	0
2008	STF	8STF21	0	1	0
2020	SGF	20SGF08	9	2	0
2020	SGF	20SGF10	5	10	0
2020	SGF	20SGF11	0	2	0
2020	SGF	20SGF16	2	19	0
2020	SGF	20SGF17	0	2	0
2020	SGF	20SGF40	1	3	0
2020	SGF	20SGF59	0	10	0
2020	SGF	20SGF06	0	0	2
2020	SGF	20SGF78	0	1	0
2020	SGF	20SGF79	5	7	0
2020	SGF	20SGF83	0	3	0

Table S6.1

2020	SGF	20SGF85	0	5	2	
2020	STF	20STF52	0	7	0	
2020	STF	20STF55	1	9	0	
2020	STF	20STF60	0	6	0	

Table S6.2

Distribution of *P. downsi* individuals sequenced per study year and life stage.

	N Individuals					
Year	Larvae	Pupae	Adult	Total		
2006	0	27	0	27		
2008	1	38	1	40		
2014	0	0	43	43		
2020	25	134	16	175		
All	26	199	60	285		

Table S6.3

Mean \pm SE number of reads and RAD clusters for each dataset analysed.

Dataset	Reads	RAD Clusters
Relatedness: <10% missingness	869680.32 ± 50057.35	17848.29 ± 999.92
Relatedness: <30% missingness	827768.24 ± 45654.77	17579.80 ± 875.96
Structure: <10% missingness	1115280.76 ± 95601.88	17816.94 ± 772.59
Structure: <30% missingness	1015889.70 ± 74205.75	17260.50 ± 657.58

Table S6.4

Number of loci remaining after each step of filtering per data set.

Filtering step	Min Coverage 8, Minimum Genotyped 60%				
Initial N	127871				
Floragenex Bioinformatics pipeline	7021				
Low probability variants removed	3305				
	10% missingness	30% missingness			
MAF > 0.02	669	674			
HWE P < 0.01	639	624			
$LD R^2 > 0.5$	469	462			

Table S6.5

Observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficient (F_{IS}) , allelic diversity and richness calculated for all years combined and separated by year, at 30% missingness.

	H _o	H _e	F _{IS}	# of alleles	Mean Allelic Richness	# Private Alleles
All	0.240	0.423	0.434			
2006	0.265	0.411	0.356	1.955	2.56	0
2008	0.215	0.433	0.505	1.955	2.71	0
2014	0.227	0.430	0.474	1.974	2.72	0
2020	0.253	0.419	0.395	2.000	2.78	2

Table S6.6

Pairwise F_{ST} with 95% Confidence Intervals comparing years, using the a) 10% missingness and b) 30% missingness dataset. Pairwise F_{ST} calculated using Weir, Cockerham (1984) method in hierfstat. * where value is significantly different from zero as determined by bootstrapped confidence intervals at P < 0.05.

a)	2006	2008	2014
2008	0.006*		
	95% (0.001–0.012)		
2014	0.006*	0.010*	
	95% (0.002 – 0.011)	95% (0.004–0.016)	
2020	0.003*	0.009 *	0.010 *
	95% (0.001–0.007)	95% (0.005 – 0.012)	95% (0.006 – 0.013)
b)	2006	2008	2014
2008	0.002		
	95% (-0.001 – 0.006)		
2014	95% (-0.001 – 0.006) 0.003	0.005*	
2014		0.005* 95% (0.001 – 0.009)	
2014	0.003		0.009*

Table S6.7

Effective population size with 95% confidence intervals estimated using COLONY (Jones and Wang 2010), from *P. downsi* collected in nests and traps in a) 2006, b) 2008, c) 2014 and d) 2020. Effective population size was estimated across two datasets: 10% and 30% individual missingness, and three error rates: 1%, 5% and 10%.

				ing rando		ting		ing non-r	andom	mating
			Alpha	Ne	LCI	UCI	Alpha	Ne	LCI	UCI
a)	10%	1%	0.00	Infinite	1	Infinite	0.02	Infinite	1	Infinite
2006		5%	0.00	420	115	Infinite	0.02	396	117	Infinite
		10%	0.00	210	87	Infinite	0.02	198	79	Infinite
	30%	1%	0.00	760	219	Infinite	0.14	535	169	Infinite
		5%	0.00	380	155	Infinite	0.14	268	106	Infinite
		10%	0.00	253	116	Infinite	0.14	178	78	Infinite
b)	10%	1%	0.00	Infinite	1	Infinite	0.18	Infinite	1	Infinite
2008		5%	0.00	Infinite	1	Infinite	0.18	Infinite	1	Infinite
		10%	0.00	364	100	Infinite	0.18	236	64	Infinite
	30%	1%	0.00	231	119	2754	0.27	128	62	5924
		5%	0.00	231	114	7727	0.27	128	62	1506
		10%	0.00	231	113	5110	0.27	128	63	1807
c)	10%	1%	0.00	342	137	Infinite	0.14	241	96	Infinite
2014		5%	0.00	342	133	Infinite	0.14	241	94	Infinite
		10%	0.00	342	122	Infinite	0.14	241	82	Infinite
	30%	1%	0.00	756	275	Infinite	0.24	440	172	Infinite
		5%	0.00	504	219	Infinite	0.24	293	127	Infinite
		10%	0.00	302	153	Infinite	0.24	176	84	7196
d)	10%	1%	0.00	78	57	109	0.11	59	40	88
2020		5%	0.00	64	45	93	0.11	48	32	73
		10%	0.00	54	37	81	0.11	41	27	64
	30%	1%	0.00	107	80	144	0.20	67	48	99
		5%	0.00	87	64	120	0.20	55	38	80
		10%	0.00	78	55	109	0.20	49	33	74

Table S6.8

Equilibrium heterozygosity (H_{exp}), number of loci with deficiency of heterozygosity (H deficiency) and excess of heterozygosity (H excess) as determined by BOTTLENECK (Piry et al. 1999) using the 1) the infinite allele model (IAM) and 2) the stepwise mutation model (SMM) for a) 2006, b) 2008, c) 2014 and d) 2020 at 30% missingness. Significance as determined by sign test with 1,000 permutations, all comparisons were significant at P < 0.0001.

	$\mathbf{H}_{\mathrm{exp}}$	H deficiency	H excess	
1) 10%				
i) IAM				
a) 2006	184.97	93	335	

b) 2008	202.21	113	325	
c) 2014	201.59	108	345	
d) 2020	182.54	73	396	
ii) SMM				
a) 2006	215.27	104	324	
b) 2008	224.21	137	301	
c) 2014	224.39	138	315	
d) 2020	209.24	102	367	
2) 30%				
i) IAM				
a) 2006	192.55	106	335	
b) 2008	189.87	116	325	
c) 2014	190.57	125	325	
d) 2020	176.16	79	383	
ii) SMM				
a) 2006	215.38	127	314	
b) 2008	215.03	141	300	
c) 2014	215.80	148	302	
d) 2020	202.36	106	356	
·	·			

Table S6.9

Linear regression of the effect of percentage of the infrapopulation genotyped (% genotyped) and total infrapopulation size (intensity) on mean individual pairwise relatedness (Manichaikul et al. 2010) across two datasets.

a) 10% missingness	Estimate	SE	t-value	P-value
$(\mathbf{R}^2 = -0.1441, \mathbf{N} = 8)$				
Intercept	0.138	0.08	1.789	0.134
% genotyped	-0.001	0.003	-0.395	0.709
Intensity	-0.001	0.001	-1.045	0.344
b) 30% missingness	Estimate	SE	t-value	P-value
$(R^2 = -0.01121; N = 10)$				
$\frac{(R^2 = -0.01121; N = 10)}{\text{Intercept}}$	0.209	0.14	1.538	0.168
	0.209 -0.006	0.14 0.004	1.538 -1.266	0.168 0.246

Table S6.10

ANOVA of the effect of percentage missingness and error rate on number of putative females and number of putative males (<= 30% missingness and <= 10% error).

a) Number of putative females	df	Sum Sq	Mean Sq	F Value	P-Value
Residuals	50	115.59	2.312		
Missingness	1	1.63	1.633	0.707	0.405
Error rate	1	2.11	1.056	0.457	0.636
b) Number of putative males	df	Sum Sq	Mean Sq	F Value	P-Value
Residuals	50	278.85	5.577		
Missingness	1	10.21	10.208	1.830	0.182
Error rate	1	13.78	6.889	1.235	0.299

Table S6.11

Linear regression (N = 8) of the effect of percentage of the infrapopulation genotyped (% genotyped) and total infrapopulation size (intensity) on a) number of putative female genotypes and b) number of putative male genotypes, 10% missingness.

a) Number of putative female genotypes						
i) 5% error rate ($R^2 = 0.1369$)	Estimate	SE	t-value	P-value		
Intercept	3.325	2.02	1.643	0.161		
% genotyped	0.0001	0.07	0.001	0.999		
Intensity	0.036	0.02	1.718	0.147		
ii) 10% error rate (R ² 0.0005)	Estimate	SE	t-value	P-value		
Intercept	4.123	2.09	1.971	0.106		
% genotyped	-0.029	0.07	-0.399	0.706		
Intensity	0.026	0.02	1.232	0.273		
b) Number of putative male geno	otypes					
i) 5% error rate ($R^2 = 0.473$)	Estimate	SE	t-value	P-value		
Intercept	1.764	2.32	0.762	0.480		
% genotyped	0.097	0.08	1.218	0.278		
Intensity	0.050	0.02	2.118	0.088		
ii) 10% error rate ($R^2 = 0.3512$)	Estimate	SE	t-value	P-value		
Intercept	2.648	2.27	1.167	0.296		
% genotyped	0.030	0.08	0.387	0.714		
Intensity	0.056	0.02	2.400	0.062		

Table S6.12

Linear regression (N = 8) of the effect of percentage of the infrapopulation genotyped (% genotyped) and total infrapopulation size (intensity) on a) number of putative female genotypes and b) number of putative male genotypes, 30% missingness.

a) Number of putative female genotypes						
i) 5% error rate ($R^2 = 0.5699$)	Estimate	SE	t-value	P-value		
Intercept	3.164	1.48	2.133	0.070		
% genotyped	-0.012	0.05	-0.251	0.809		
Intensity	0.054	0.01	3.585	0.009		
ii) 10% error rate ($R^2 = 0.5699$)	Estimate	SE	t-value	P-value		
Intercept	3.164	1.48	2.133	0.070		
% genotyped	-0.012	0.05	-0.251	0.809		
Intensity	0.054	0.01	3.585	0.009		
b) Number of putative male geno	types					
i) 5% error rate ($R^2 = 0.7665$)	Estimate	SE	t-value	P-value		
Intercept	0.0317	1.82	0.017	0.987		
% genotyped	0.103	0.06	1.723	0.129		
Intensity	0.102	0.02	5.589	< 0.001		
ii) 10% error rate ($R^2 = 0.7981$)	Estimate	SE	t-value	P-value		
Intercept	0.921	1.51	0.611	0.560		
% genotyped	0.066	0.05	1.334	0.224		
Intensity	0.093	0.02	6.130	< 0.001		

Table S6.13

Results of sib-ship reconstruction analysis for all data with 30% individual missingness (N = 9). Data are presented as mean \pm SE; range. Value descriptions are as follows: # female infestations: mean number of reconstructed female genotypes per infrapopulation; # paternal genotypes: mean number of reconstructed male genotypes per infrapopulation; # males per female: mean number of males assigned to the offspring of each female per nest; # offspring per female: mean number of offspring assigned to each maternal genotype; % total offspring per female: mean percentage of the total offspring contributed by each female per

Error	# Female	# Paternal	# Males per	# Offspring	% Total offspring per female
rate	infestations	genotypes	female	per female	
1%	$5.60 \pm 0.54;$ 2-7	$7.80 \pm 1.03;$ 2-13	$1.37 \pm 0.12;$ $1.00 - 2.00$	$1.71 \pm 0.25;$ 1.00 - 3.00	$20.72 \pm 3.51;$ 14.29 - 50.00
5%	$5.10 \pm 0.50;$	$6.80 \pm 0.84;$	$1.32 \pm 0.09;$	$1.87 \pm 0.26;$	$22.36 \pm 3.36;$
	2-7	2 – 11	1.00 - 1.75	1.00 - 3.00	14.29 - 50.00
10%	$5.10 \pm 0.50;$ 2-7	$6.40 \pm 0.75;$ 2 - 10	$1.24 \pm 0.08;$ $1.00 - 1.75$	$1.87 \pm 0.26;$ 1.00 - 3.00	22.36 ± 3.36 ; 14.29 - 50.00

infrapopulation.

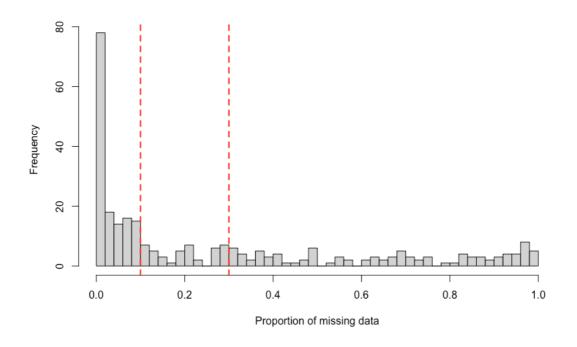
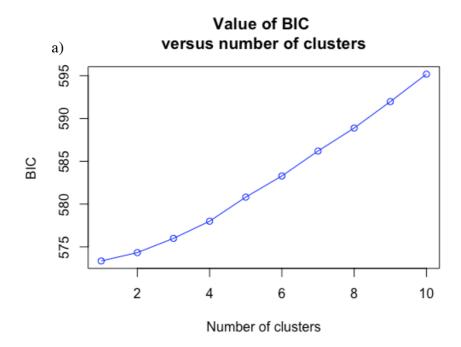


Figure S6.1

Distribution of the proportion of missing data across all sequenced specimens before filtering (N=285). Red dotted lines indicate the cut off points for individual missingness across our two datasets, 10% and 30%.



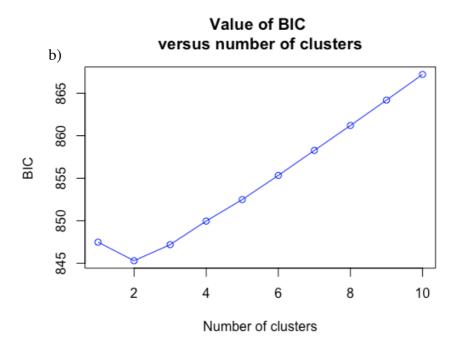
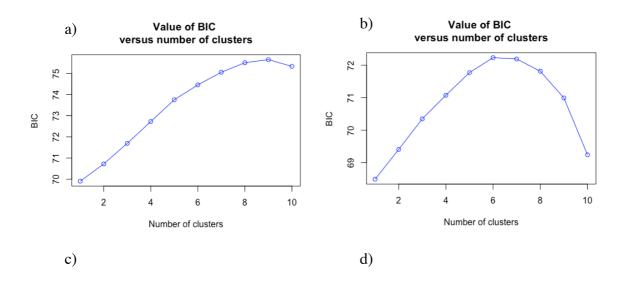


Figure S6. 2 BIC values for number of clusters calculated using DAPC conducted in adegenet on all years (2006, 2008, 2014 and 2020) pooled for a) 10% missingness (N = 121), and b) 30% missingness (N = 171).



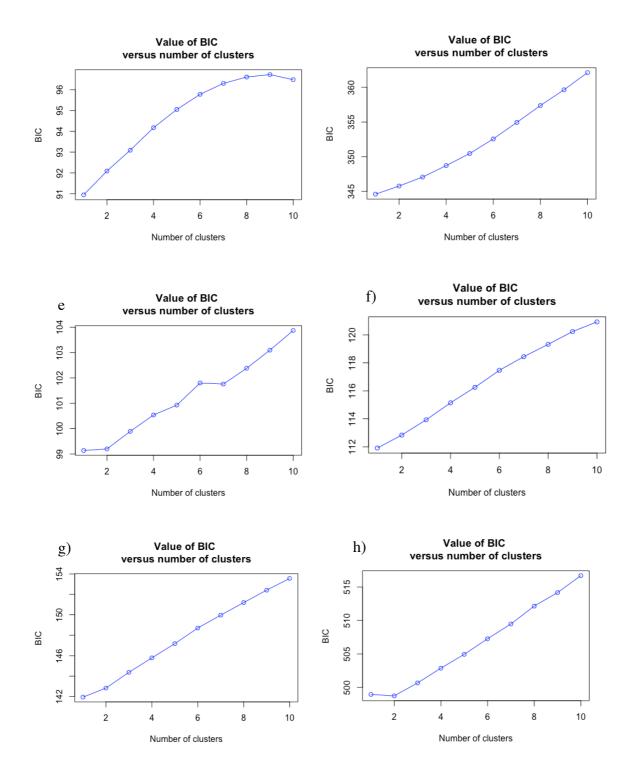
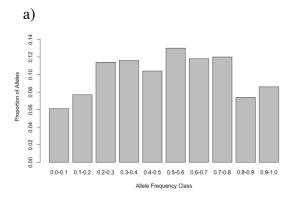
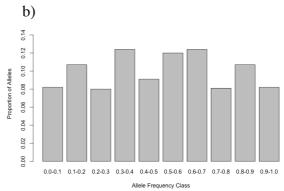
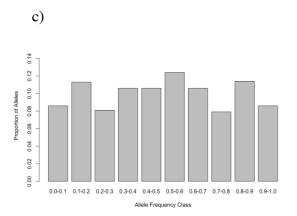


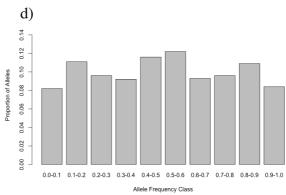
Figure S6.3

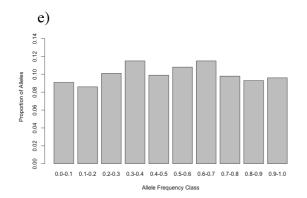
BIC values for number of clusters in the 10% missingness dataset a) 2006 (N = 15), b) 2008 (N = 14), c) 2014 (N = 19) and d) 2020 (N = 73); and the 30% missingness dataset e) 2006 (N = 20), f) 2008 (N = 22), g) 2014 (N = 28), h) 2020 (N = 101). DAPC conducted using adegenet.

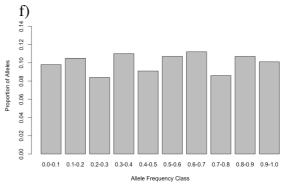


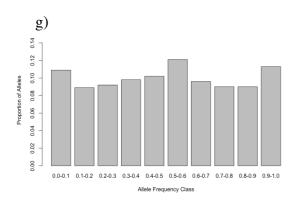












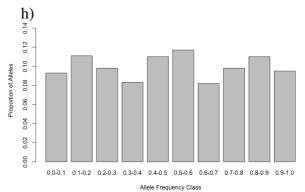


Figure S6.4

Distribution of allele frequencies with 10% missingness in a) 2006 (N = 15), b) 2008 (N = 14), c) 2014 (N = 19) and d) 2020 (N = 73); and the 30% missingness dataset e) 2006 (N = 20), f) 2008 (N = 22), g) 2014 (N = 28), h) 2020 (N = 101). Bars represent the proportion of alleles found in each allele frequency class.

Chapter 7 – Discussion

Synthesis of findings

Since its introduction to the Galápagos Islands and discovery in the nests of Darwin's finches, there has been increasing evidence of shifts in the host-parasite relationship between the avian vampire fly, P. downsi, and its novel avian hosts. My thesis provides a rare look into the real-time evolution of host-parasite dynamics and invasive species in the wild. High host mortality, driven by both P. downsi parasitism (Kleindorfer and Dudaniec 2016), habitat change (Fessl et al. 2010; Cimadom et al. 2014) and nest predation (Kleindorfer et al. 2021b) exerts extreme selective pressures on P. downsi larvae that require nutrients from the host to reach adulthood. As predicted, under such selective pressures, as well as selection arising from the invasive process (i.e., genetic bottlenecks, genetic drift and novel environments; Le Roux 2021), P. downsi has changed across time. Specifically, P. downsi morphology (Chapter 3; Common et al. 2020), and mating and oviposition behaviour (Chapter 2; Common et al. 2019; Chapter 6; Common et al. 2022a) has shifted to smaller female abdomen size and more unrelated P. downsi larvae per nest in recent years. At a landscape scale, P. downsi males and females differed in their spatial and temporal distributions (Chapter 5; Common et al. 2022b). The finding of host-specific intensity and fitness in P. downsi larvae raises questions about the effect of host hybridisation on parasite fitness, and the evolution of host-specificity (Chapter 4; Common et al. 2021). Philornis downsi intensity is highest in the critically endangered medium tree finch, and larvae have the highest mortality in hybrid nests between the small and medium tree finches (Chapter 4; Common et al. 2021). These findings highlight the dynamic and rapidly fluctuating relationship between invasive species, particularly invasive parasites, and their novel hosts and environment. Documenting and understanding these fluctuations and changes is critical for effective

management, particularly when reproductive behaviours, the basis for many control techniques, are affected.

In contrast to natural and sexual selection, fecundity selection is a poorly studied form of selection acting upon traits associated with increased or decreased fecundity (Roff 2001; Pincheira-Donoso and Hunt 2017). In insects, body size is correlated with fecundity, and therefore selection acting upon this trait affects long-term adult fecundity (Sivinski and Dodson 1992; Andersen 1994; Teder and Tammaru 2005; Hurlbutt 2008). I found a significant decrease in pupae and adult body size, and notably a significant decrease in female abdomen size (Chapter 3; Common et al. 2020), a trait functionally linked with fecundity (i.e., number of eggs laid; Chapter 3; Preziosi et al. 1996; Olsson et al. 2002; Parker et al. 2011; Winkler et al. 2012). Therefore, female fecundity (number of eggs they have available to lay) has decreased on Floreana Island. This decrease could potentially lead to decreasing numbers of parasites in the nest, and may also have implications for female P. downsi mating behaviour, longevity and fitness (Pincheira-Donoso and Hunt 2017). Sibship of *P. downsi* collected from nests in 2020 was reconstructed, and compared to an earlier study in 2004 – 2006 utilising similar molecular genetic methods (Dudaniec et al. 2010). From this, I found changes in mating and oviposition behaviour. Compared to earlier years, females contributed fewer offspring to each nest, in both number and proportion (Chapter 6; Common et al. 2022a). Given the high mortality rates, and the decreasing age at which the nestling hosts die, P. downsi may also hedge their bets by laying fewer eggs in multiple host nests (Hopper 1999; McLaughlin and Wasserberg 2021). Despite this decrease in both fecundity and number of offspring contributed to nests, in-nest intensity has remained stable (Chapter 4; Common et al. 2021), likely due to increasing *P. downsi* population densities, which has yet to be explored.

Although each female is contributing fewer offspring to nests, total intensity of *P. downsi* has remained stable across 16 years (Chapter 4; Common et al. 2021). I found that more females are ovipositing in each nest than in earlier years, so although each female is ovipositing fewer eggs, the intensity has remained similar (Chapter 4; Common et al. 2021; Chapter 6; Common et al. 2022a). Given the increase in number of females contributing to each nest, the parasites within each nest are becoming increasingly unrelated (Chapter 6; Common et al. 2022a). Under the kin selection hypothesis, unrelated individuals are more likely to increase their virulence (i.e., damage to the host) to increase the developmental rate and increase their survival to adulthood (Buckling and Brockhurst 2008; Gleichsner et al. 2018). Therefore, although the parasites are smaller (Chapter 3; Common et al. 2020), and numbers in-nest have stabilised (Chapter 4; Common et al. 2021), the decreasing relatedness could still result in increasing mortality in the hosts. This effect could potentially worsen over time, as increasing host mortality drives decreasing parasite size and fecundity, leading to further bethedging and decreasing parasite relatedness. Decreased parasite relatedness can lead to increasing virulence, as prudent exploitation of the host benefits other parasites in the nest (Frank 1992; Buckling and Brockhurst 2008). When these parasites are unrelated, and therefore their survival does not confer an indirect benefit, exploitation rate and intensity increases, leading to increasingly severe damage to the host (Frank 1992; Buckling and Brockhurst 2008). The potential for increasing virulence highlights the need for timely and effective management to ensure the survival of all Darwin's finch species.

When comparing rates of *P. downsi* intensity in the nests of three host species and one hybrid group, I found that intensity was the highest in critically endangered medium tree finch nests (Chapter 4; Common et al. 2021). Larval mortality of *P. downsi* was low in medium tree 179

finch nests, suggesting the medium tree finch can sustain more larvae to adulthood than other host species. Philornis downsi parasitism is one of the greatest threats to the survival of the medium tree finch, causing 45% mortality in a species already under threat from introduced predators (Kleindorfer et al. 2021b). Conversely, the hybrid tree finch, resulting from hybridisation of the small and medium tree finches, had the highest larval mortality rate, and low intensity (Chapter 4; Common et al. 2021). Therefore, the parent species, the medium tree finch, is likely to be a preferred host over the hybrid. Changing dynamics of intensity and host mortality between the small tree finch and the warbler finch (Certhidea olivacea) have been found on Santa Cruz (Dudaniec et al. 2007; Kleindorfer and Dudaniec 2009; Cimadom et al. 2014), however the fitness of P. downsi larvae in these host nests is currently not known. The patterns of differing intensity and larval mortality found in this thesis may also be the first step towards host specialisation in the extremely generalist *P. downsi*. As fitness differs between host groups, parasites are predicted to preferentially infest hosts that maximise their fitness (Kawecki 1998; Egas et al. 2004), potentially represented as the increased intensity in medium tree finch nests found within this thesis. However, more research is needed, particularly over larger time scales, to understand the host-specific interactions of *P. downsi* on the Galápagos.

The lack of substantial genetic differentiation over time (Chapter 6; Common et al. 2022a) suggests that gene flow between Floreana Island *P. downsi* population and other island populations is not restricted as previously suggested (Dudaniec et al. 2008a). This is supported by recent genetic studies across five islands that found little genetic differentiation between islands, suggesting high gene flow (Koop et al. 2020). Despite this lack of separation across islands, there is evidence that the Floreana Island *P. downsi* population is restricted on

a temporal scale. Adult flies are restricted to the humid highlands across the non-breeding season, moving to the arid lowlands as breeding commences (Chapter 5; Common et al. 2022b). Adult male P. downsi are restricted further and found primarily in the agricultural zone, an area rich with fruiting trees. During the non-breeding season, the abundance of adult flies, particularly males, decreases significantly (Causton et al. 2019; Chapter 5; Common et al. 2022b). Therefore, the highlands, specifically the agricultural zone, in the months preceding the breeding season are a key location for control and management of P. downsi on Floreana. Specifically, this information informs control techniques that rely on the annihilation of fertile males before the release of infertile males (e.g., the Sterile Insect Technique; Vargas et al. 2014; Dyck et al. 2021; Himuro et al. 2022). Therefore, trapping of male flies can be targeted to the agricultural zone before the breeding season, allowing the infertile males to be released at sufficient densities before breeding commences. Another developing genetic technology to assist with invasive species management is gene drive, which involves the release of selfish genetic elements that display super Mendelian inheritance. These genetic elements can be targeted to disrupt genes necessary for reproduction and survival, leading to rapid population suppression (Raban et al. 2020). Ecological dynamics, and population size and density fluctuations are important factors in the success in gene drives, and modelling suggests that population suppression is more effective when the release occurs at the start of the target species' breeding season (North et al. 2019; Bier 2022).

Implications of this research for other species

As *P. downsi* is widespread and exhibits high generalism, its effects of each host species, and how they differ between hosts, is a key area of research on the Galápagos. The finding of

differences in larval fitness between host species could have implications for many species (Chapter 4; Common et al. 2021). For example, the Galápagos mockingbird (*Mimus parvulus*) is a larger bodied bird that is able to compensate for, and therefore tolerate, the effects of *P. downsi* (Knutie et al. 2016). Although the fitness of *P. downsi* larvae reared in the nests of the Galápagos mockingbird are currently unknown, it is expected to be higher than hosts that are unable to tolerate parasitism and therefore die before the parasite reaches maturity. The Galápagos mockingbird may therefore be acting as a reservoir species for *P. downsi*, due to their larger body size facilitating increased parasite fitness (Knutie et al. 2016). However, the apparent tolerance of the Galápagos mockingbird is not consistent across years, in particular when conditions are dry (McNew et al. 2019), and the impacts of *P. downsi* can affect the success of future nesting attempts (McNew et al. 2020). Despite this, targeted efforts to control *P. downsi* numbers in mockingbird nests may significantly decrease the general *P. downsi* population.

Conversely, other host species are particularly negatively affected by *P. downsi* parasitism. The mangrove finch (*Camarhynchus heliobates*) of Isabela Island is critically endangered and negatively affected by invasive species. Mangrove finch nests suffer both predation by introduced black rats and parasitism by high numbers of *P. downsi*. This is mirrored on Floreana Island, where critically endangered medium tree finch nesting success is low due to both rats and *P. downsi*. Although *P. downsi* continues to cause mortality in medium tree finch nests, the probability of failure during the nestling phase has decreased across time (Kleindorfer et al. 2021b), with more birds surviving to fledging despite high intensities in recent years (Kleindorfer et al. pers. comms.). The causative factors for the differences in response from these two similar, but contrasting host species, should be investigated. Increasing success during the nestling phase may be due to the decreasing body size of *P*. 182

downsi found in this thesis (Chapter 3; Common et al. 2020), which, on Floreana, may be driven by the earlier average age at host death, or smaller average host body size given the absence of many larger-bodied host species present on other islands. Unlike Isabela and Santa Cruz Islands, the Galápagos mockingbird is extinct on mainland Floreana Island and hence cannot act as a potential reservoir species. Alternatively, hybridisation and introgression of the medium tree finch with the small tree finch may confer benefits against *P. downsi*, and hybridisation has not been documented in the mangrove finch population. Future research exploring fecundity and fitness in *P. downsi* parasitising the mangrove finch may uncover key differences leading to success, or failure, of a host species to survive a novel parasite.

Recent research into the effects of *P. downsi* in the endemic and vulnerable little vermilion flycatcher (*Pyrocephalus nanus*) on Isabela Island found *P. downsi* significantly decreased breeding success (Leuba et al. 2020; Mosquera et al. 2022). Notably, both studies found *P. downsi* larvae present in nests that failed during incubation (Leuba et al. 2020; Mosquera et al. 2022). Earlier oviposition and hatching of *P. downsi* has been found in recent years on Santa Cruz Island (Cimadom et al. 2016), and is reviewed in this thesis (Chapter 2; Common et al. 2019). Despite this documented change in *P. downsi* on two islands, there is no evidence of earlier onset of breeding on Floreana Island (Kleindorfer et al. pers. comms.). The reason for this divergence in behaviour between *P. downsi* populations is not currently known. Comparing island specific studies highlights key differences that warrant investigation. Although gene flow is evidently not restricted between the currently studied islands, different host sizes, host assemblages, habitat and weather are likely to affect *P. downsi* populations and therefore may alter their evolutionary trajectory.

Current developments and future directions

Increasing research documenting and understanding the effects of P. downsi across many host species has contributed significantly to our understanding of the interactions with invasive parasites. The literature spans many teams focusing on different islands and different host species, such as tolerance in Galápagos mockingbirds on Santa Cruz (McNew group), the mangrove finch project on Isabela island (Cunninghame group), parasitism and urban ecology on San Cristobal (Knutie group), early oviposition and mating behaviour (Tebbich group), effects on the little vermillion flycatcher (Galápagos Land Bird group), genetic diversity of *P. downsi* in its native and invasive range, informing potential invasion pathways (Koop group), and *P. downsi* on mainland South America (Bulgarella and Quiroga's group, also with Heimpel's group). Huge strides have been made rearing P. downsi in captivity (Lahuatte et al. 2016; Lahuatte et al. pers. comms.), leading to advancements in our understanding of *P. downsi* ecology and development (Lahuatte et al.; Yuval et al., pers. comms). In particular, in preparation for potential biological control options there has been recent successful importation of parasitoids from the native range of P. downsi for testing under controlled conditions (Bulgarella et al. 2017; Ramirez et al. 2022; Heimpel et al., pers. comms). The publication of the *P. downsi* genome by Romine et al. (2021) will allow for genomic studies of increasing complexity, further developing our genetic understanding of P. downsi and invasive parasites. As evidenced by the findings of this thesis, changes in P. downsi are occurring rapidly, highlighting the importance of long-term field research.

One critical next step will be to assess genomic patterns with increased resolution to explore gene flow, genetic differentiation and selection across the entire archipelago and potential mainland Ecuadorian source populations. This information will inform island-specific control techniques. For example, if gene flow is high between two islands, then simultaneous control 184

is recommended to decrease the risk of repopulation. Detecting signatures of selection and identifying the function of genes under selection will give insight into the evolutionary pressures P. downsi is experiencing and how the invasive population is changing in its novel environment. Focus should be given to exploring the potential genetic basis of decreasing body size in P. downsi through signatures of selection, and laboratory rearing of larvae to explore body size across time and under controlled nutrition conditions. Despite significant and continued study, little is still known about the behaviour and ecology of free-flying adult P. downsi. Pike et al. (2021) documented four occasions of P. downsi mating at Galápagos Flycatcher nests after fledging had occurred. Although this thesis did not find an association between nest density and the abundance of adult flies (Common et al. 2022b), only active nests were considered. Future research should investigate nests in the post-fledging phase as mating resources that males defend. Research should investigate evidence for different mating systems in P. downsi, such as a lekking based system. Determining the mating system, or relevant defended resource, will aid in the development of targeted control methods. Alongside research focusing on the fly, continuing research into responses to P. downsi parasitism across host species is imperative. For example, exploring adaptations such as hybridisation and introgression, and P. downsi-specific antibodies in the medium ground finch (G. fortis) (Huber et al. 2010) that may confer an advantage for some host species. Compared to Darwin's finches, other host species on both the Galapagos and mainland South America are relatively understudied and their relationship with *P. downsi* is poorly understood. Continuing efforts to build a basis of up-to-date, host- and island-specific ecological information is imperative and will allow for development and deployment of successful control and eradication techniques.

The *P. downsi* Action Group led by Charlotte Causton, in collaboration with the Galápagos Landbird Group led by Birgit Fessl and cooperation with Galápagos National Park is a collaborative effort comprised of 103 researchers across 36 institutions. Multi-dimensional, multi-scale problems require large, multi-disciplinary collaborative networks to continue to gain insights to effectively target weak links. This thesis contributes to the goals of the *P. downsi* action plan, highlighting the importance of monitoring the fluctuating dynamics of both hosts and parasites across longer temporal scale. Long-term studies, such as this thesis, allow us to explore novel evolutionary changes that affect host-parasite dynamics in the context of parasite invasion, and inform management of invasive pathogens in a changing landscape.

Appendix

Appendix 1

Chapter

Taxonomic Shifts in *Philornis*Larval Behaviour and Rapid Changes in *Philornis downsi* Dodge & Aitken (Diptera: Muscidae): An Invasive Avian Parasite on the Galápagos Islands

Lauren K. Common, Rachael Y. Dudaniec, Diane Colombelli-Négrel and Sonia Kleindorfer

Abstract

The parasitic larvae of Philornis downsi Dodge & Aitken (Diptera: Muscidae) were first discovered in Darwin's finch nests on the Galápagos Islands in 1997. Larvae of P. downsi consume the blood and tissue of developing birds, causing high in-nest mortality in their Galápagos hosts. The fly has been spreading across the archipelago and is considered the biggest threat to the survival of Galápagos land birds. Here, we review (1) Philornis systematics and taxonomy, (2) discuss shifts in feeding habits across *Philornis* species comparing basal to more recently evolved groups, (3) report on differences in the ontogeny of wild and captive P. downsi larvae, (4) describe what is known about adult P. downsi behaviour, and (5) discuss changes in P. downsi behaviour since its discovery on the Galápagos Islands. From 1997 to 2010, P. downsi larvae have been rarely detected in Darwin's finch nests with eggs. Since 2012, P. downsi larvae have regularly been found in the nests of incubating Darwin's finches. Exploring P. downsi ontogeny and behaviour in the larger context of taxonomic relationships provides clues about the breadth of behavioural flexibility that may facilitate successful colonisation.

Keywords: Protocalliphora, Passeromyia, Philornis, nest larvae, hematophagous, subcutaneous, Darwin's finches, Passeriformes

1. Introduction

Three genera of flies within the order Diptera have larvae that parasitise avian hosts: *Protocalliphora* Hough (Calliphoridae), as well as *Passeromyia* Rodhain & Villeneuve (Muscidae) and *Philornis* Meinert (Muscidae). The adult flies in these genera are free-living and do not parasitise birds, but their larvae develop in the nests of altricial birds, feed on their avian hosts, and exhibit feeding behaviours

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from hematophagy to coprophagy [1, 2]. Most larval infestations have been documented in host nests of the order Passeriformes, but larvae have also been found in nests of Accipitriformes, Apodiformes, Strigiformes and other avian taxa (*Protocalliphora*: [3]; *Passeromyia*: [4]; *Philornis*: [5, 6]). The effect of these parasitic fly larvae on host survival can be severe to mild, depending on many factors including host population size, body size, nesting density and the presence of behavioural or immunological defence mechanisms [6–8].

Protocalliphora is widely distributed throughout the Holarctic and contains 40+ species with obligate avian parasitic larvae [3]. Within Muscidae, only Passeromyia and *Philornis* larvae parasitise birds [4, 9, 10]. Both *Passeromyia* and *Philornis* are members of the subfamily Cyrtoneurininae, however their complete evolutionary relationships have yet to be resolved [11, 12]. Due to the similarities between Passeromyia and Philornis, many workers regarded the two genera as close relatives, including Skidmore [9], who stated that their similarities could not be based on convergent evolution alone. The five *Passeromyia* species include *P. steini* (Pont), P. heterochaeta (Villeneuve), P. indecora (Walker), P. longicornis (Macquart) and P. veitchi (Bezzi), and are distributed throughout Europe, Africa, Asia and Australasia [4, 13]. Passeromyia species differ in their larval habits. For example, P. steini larvae scavenge nests for organic matter and *P. indecora* larvae consume host resources as subcutaneous parasites. The 52 Philornis species are distributed primarily in Neotropical South America and southern North America [1, 2, 10]. Philornis species also show a wide range of feeding habits, including free-living coprophagous larvae, free-living semi-hematophagous larvae, and subcutaneous hematophagous larvae (**Table** 1). One species, *P. downsi*, is a recently discovered invasive species on the Galápagos Islands [14, 15]. Its semi-hematophagous larvae cause significant in-nest host mortality in their novel Galápagos land bird hosts [16]. Cladistics and molecular phylogenetic analyses suggest that the parasitic larval habits of Passeromyia and Philornis evolved independently [10, 12] despite the similarities between both genera including cocoon-wrapped puparia, life history, and clade.

The Galápagos Islands have been listed as a World Heritage site in 1978. Given a suite of threats, including introduced species, the archipelago was added to the 'List of the World Heritage in Danger' in 2007 and then removed from this list in 2010 because of actions by the Government of Ecuador to reduce invasions [17, 18]. Biological invasion is considered the greatest threat to biodiversity in the Galápagos Islands [19]. Currently, 543 terrestrial species have been introduced, of which 55 are considered harmful or potentially harmful to native biodiversity [17].

In this chapter, we consider changes in the development and behaviour of the accidentally introduced fly P. downsi Dodge and Aitken (Diptera: Muscidae), that is now considered the biggest threat to the survival of Galápagos land birds [20]. The first P. downsi larvae were collected from Galápagos land bird nests on Santa Cruz Island in 1997 [21]. From examination of museum specimens collected in 1899 (during the Stanford University Expedition led by Robert Snodgrass and Edmund Heller), in 1905-1906 and 1932 (during expeditions sponsored by the California Academy of Sciences), and in 1962 (by Robert Bowman) on Floreana Island, there is no current evidence to suggest P. downsi was present or abundant on the Galápagos Islands prior to 1964, though this is possible [22, 23]. By collating information from a range of researchers investigating *Philornis* in general and *P. downsi* in particular, we aim to improve our understanding of the ontogeny and behaviour of an invasive Philornis species within the larger context of Dipteran parasites of birds. We review Philornis systematics and taxonomy, discuss feeding habits across Philornis species, report on differences in the ontogeny of wild and captive P. downsi larvae, report on adult P. downsi behaviour, and describe changes in P. downsi behaviour since its discovery on the Galápagos Islands.

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Aitkeni group	Larval habits	Falsificus group	Larval habits	Angustifrons group	Larva habits
P. fasciventris [37]	FLC	P. fumicosta		P. downsi [30]	FLSH
P. schildi		P. univittatus		P. niger [1, 30]	SubH
P. amazonensis		P. grandis		P. porteri ¹ [43]	SubH
P. lopesi		P. sabroskyi		P. mimicola ² [40]	SubH
P. aikteni [30]	FLC	P. falsificus [1, 30]	FLSH	P. sperophilus [1]	SubH
P. zeteki				P. carinatus [47]	SubH
P. rufoscutellaris [36]	FLC			P. deceptiva [48, 49]	SubH
P. rettenmeyeri				P. trinitensis [30]	SubH
P. setinervis				P. glaucinis [30]	SubF
				P. pici [24]	SubF
				P. vespidicola ³ [2]	SubF
				P. medianus [33]	SubF
				P. vulgaris [1]	SubF
				P. masoni	SubF
				P. diminutus [1]	SubF
				P. querulus [30]	SubF
				P. albuquerquei	
				P. frontalis [1]	SubF
				P. gagnei [33]	SubF
				P. insularis [33]	SubF
				P. obscurinervis	
				P. petersoni	
				P. torquans [1]	SubF
				P. angustifrons [30]	SubF
				P. bellus [2]	SubF
				P. sanguinis [30]	SubH

¹Some P. porteri larvae found in ear canals and nares of nestlings; some later instars found feeding externally on abdomen and wings [41, 43].

Table 1

Philornis species ordered according to taxonomy, from the most basal 'aitkeni-group' to the most recently evolved 'angustifrons-group' (groups from [33]). Larval feeding habits are shown where known and abbreviated as follows: free-living coprophagous larvae (FLC), free-living semi-hematophagous larvae (FLSH), subcutaneous hematophagous larvae (SubH). The following nine species are not included in the list as they are currently not assigned to a taxonomic group [33] given insufficient information: P. molesta, P. nielseni, P. blanchardi, P. cinnamomina, P. convexus, P. mima, P. obscurus, P. steini, P. umanani.

2. Philornis systematics and taxonomy

Macquart [24] provided the first description of *Philornis* larvae when he described *Aricia pici*; a subcutaneous larval parasite found on an adult Hispaniolan woodpecker (*Melanerpes striatus*; previously *Picus striatus*) Muller (Piciformes: Picidae). Meinert [25] erected the genus *Philornis* for the single species, *P. molesta*,

²P. mimicola larvae found in the nasal cavity of owls, mainly subcutaneous on body [40].

³Only known specimens of P. vespidicola collected from nests of the wasp Paracharitopus frontalis (Hymenoptera: Vespidae) [2, 29].

⁴P. nielseni proposed synonym of P. seguyi [34].

based on larvae with distinctive posterior spiracles found parasitising nestlings. At this time, *Philornis* was suggested to be a synonym for *Protocalliphora* and assigned to the family Calliphoridae [26]. In 1921, Malloch [27] proposed the genus *Neomusca* based on adult specimens, whereas the genus *Philornis* was based on larval characters. Aldrich [28] revised this group and synonymized *Neomusca* with *Philornis* as independent genera, assigning both within the family Muscidae (Anthomyiidae at the time). This revision was supported by further work on *Philornis* species, as new and previously described species were transferred from other genera including *Hylemyia*, *Mesembrina*, *Neomusca* and *Mydaea* [9, 28–31]. *Philornis* adults are distinguished from other muscid genera by the presence of hair on the anepimeron and the postalar wall [1, 32].

Using morphological and ecological data, *Philornis* can be divided into three phylogenetic groups: the 'aitkeni-group', the 'falsificus-group' and the 'angustifrons-group' [33]. Male characters (given few female specimens) generally define the most basal lineage of *Philornis*, the 'aitkeni-group', including enlarged upper eye facets in holotypic males [29, 33]. The members of this group display adult character states that are considered primitive among muscids (i.e., enlarged upper eye facets and presence of cilia on the surface of the wing vein R_{4+5}) [33]. This group includes P. aitkeni (Dodge), P. rufoscutellaris (Couri), and P. fasciventris (Wulp). The phylogeny of the aitkeni-group is not completely resolved because of missing information about life history and morphology, as female and larval specimens are not available for many species. The second group, the falsificus-group, is defined primarily by P. falsificus (Dodge and Aitken), whose larvae are free-living [9]. Common morphological characters include five scutellar marginal setae that also place P. funicosta (Dodge), P. univittatus (Dodge), P. grandis (Couri) and P. sabroskyi (Albuquerque) within this group [33]; however, data on the ecology of these species are missing. More information on larval life history is necessary to confirm whether species other than P. falsificus belong in this lineage. Despite a similar life history to P. falsificus, P. downsi is not within the falsificus-group [1, 9, 33], but forms a sister-group to all species within the angustifrons-group for which larval habits have mostly been documented (Table 1). The angustifrons-group is the most recently evolved and largest of the three Philornis lineages and contains species with subcutaneous hematophagous larvae as well as P. downsi with semi-hematophagous larvae.

Comparative taxonomic analyses of *Philornis* species have been hampered by a lack of specimens and information [9]. For several species of *Philornis*, their morphological descriptions are based solely on one sex, generally males. In others, the holotype is missing or destroyed, and so other traits and ecological information are missing. Philornis blanchardi (Garcia) has been originally identified and described in Argentina from a single female specimen, which has since been lost [34]. This specimen may belong to a previously described species as it has not been captured and identified since, however the original description is considered sufficiently unique that it may be a separate species [34]. The single male holotype used to describe P. umanani (Garcia) has also been lost and due to the lack of detail provided in the original description, this species is deemed unrecognisable and is now considered a nomen dubium [34]. Evidence of a *Philornis* species complex within specimens of *P. seguyi* (Garcia) and *P.* torquans (Nielsen) in Argentina throws further doubt on the original taxonomic characterisation of many Philornis species [35]. These issues highlight the need for more extensive molecular and morphological analysis of currently recognised *Philornis* species to confirm species classifications and their evolutionary relationships.

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3. Larval feeding habits across Philornis species

3.1 Philornis larval behaviour

Philornis species differ in their larval feeding habits, which include coprophagous and hematophagous diets (**Table** 1). Larval habits have been documented for 30 out of 52 described species (**Table** 1). The most basal group in the Philornis phylogeny (aikteni) have free-living coprophagous larvae [33]. These larvae parasitise cavity nesting host species that do not remove waste, such as the rufous-tailed jacamar (Galbula ruficauda) Cuvier (Piciformes: Galbulidae) and appear to be specific to this type of nest [2, 5, 30, 36, 37]. Free-living saprophagous larvae in the nest are regarded as the ancestral trait, evolving into coprophagous larvae, semi-hematophagous larvae and then subcutaneous larvae [9, 33]. This transition is also supported in Passeromyia where species show a similar order of descent [4, 10, 33]. Two documented species, P. downsi (angustifrons-group) and P. falsificus (falsificus-group), have free-living and semi-hematophagous larvae, although other undescribed species within the falsificus-group may also have free-living larvae [1, 30, 33].

Most *Philornis* species (83%) have larvae with subcutaneous hematophagous feeding habits, which is also the primary larval strategy in the angustrifrons-group. Within this group, only *P. downsi* has non-subcutaneous larvae. The semi-hematophagous P. downsi larvae may be similar to P. falsificus (falsificus-group), which is also suspected of having free-living semi-hematophagous larvae [33]—but not enough is known about the biology of the falsificus-group. While P. falsificus is considered a free-living ectoparasite [30], this assessment is limited by the observations to date of later instars and puparia [38, 39]. On the other hand, in two species with subcutaneous feeding habits in the angustifrons-group, a few Philornis larvae have been also observed in avian nares. Specifically, *P. mimicola* larvae have been found in the nares of ferruginous pygmy-owl nestlings (*Glaucidium brasilianum*) Gmelin (Strigiformes: Strigidae), but most larvae occurred subcutaneously [40]. Larvae of *P. porteri* (Dodge) have been found in the nares and ear canals of some nestlings [41, 42], and 3rd instar larvae observed to feed externally on the abdomen and wings of their hosts [41, 43]. In the semi-hematophagous P. downsi larvae, 1st instars regularly reside within the avian nares [44-46] and later instars move to the base of the nest where they emerge at dusk and dawn to feed externally on the blood and tissue of the developing birds [45, 46]. Lineages with free-living larvae have been far less studied than lineages with subcutaneous larvae (Table 1). Free-living larvae move freely within the host nest, detach from the host at various times and reside in the nest base during the day, making them less conspicuous to human observers [45, 46]. In contrast, subcutaneous larvae reside under the skin of the host and hence can be detected when nestlings are examined.

4. Philornis downsi larval development in the wild and in the laboratory

4.1 Philornis downsi larval instars

Philornis downsi larval development is split into three instar development stages. 1st instar larvae generally reside in the naris and ear canals of developing nestlings, but some have also been found moving freely within the nesting material [21, 52, 53]. First instars are commonly collected from 2 to 3 day old nestlings [43]. Late 2nd and 3rd instar larvae are generally free-living, residing within the base of the nest and feeding externally on nestlings at night [14, 45, 46]. These later instar larvae feed

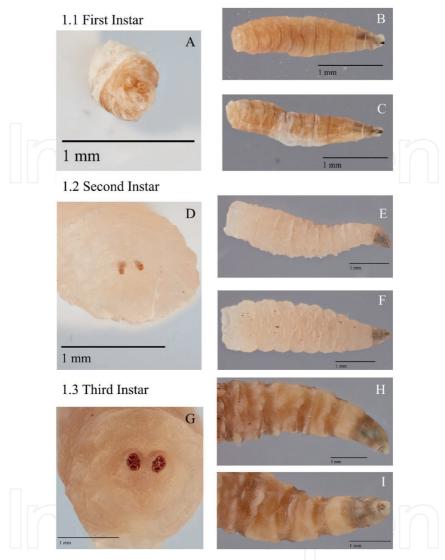


Figure 1.

Three larval stages of Philornis downsi. (1) First instar: (A) posterior spiracles, (B) lateral view, (C) ventral view, (2) second instar: (D) posterior spiracles, (E) lateral view, (F) ventral view, (3) third instar: (G) posterior spiracles, (H) lateral view, (I) ventral view. Obtained by the authors from larvae collected on Floreana Island, Galápagos, Ecuador between 2010 and 2014. The photographs were taken using a visionary digital LK imaging system (dun, Inc) with a canon EOS 5DsR camera and capture one pro 11.3.1, phase one (Flinders University). Images were produced using Zerene stacker 1.04, Zerene systems LLC, software, and cropped and resized in Photoshop CS5.

on the blood and fluids of their host by penetrating the skin of the nestlings [2, 30]. Larval instar morphological descriptions are given by Fessl et al. [44]. The most distinct character between the instars is the posterior spiracles, which change in colour, shape and number of spiracular slits present throughout larval development [44].

Figure 1(1A) shows the posterior spiracles of a 1st instar *P. downsi* larva, characterised by their light pigmentation and two oval slits present [44]. The spiracles of a

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1st instar larva are separated by slightly more than their diameter. First instar larvae lack anterior spiracles (**Figure 1(1B)**). The posterior spiracles of a 2nd instar larva are similarly round with two oval slits; however, the distance between them is two to three times of their diameter (**Figure 1(2D)**; [44]). Anterior spiracles are present during the 2nd instar, and semicircular in shape, lightly pigmented and visible in **Figure 1(2E)**). 3rd instar posterior spiracular plates are darkly pigmented and round in shape, distinct C-shaped spiracular slits radiate from median ecdysial scar (**Figure 1(3G)**). Pigmentation of the median ecdysial scar is light in early 3rd instar larvae and becoming darkly pigmented later in the stage. Semi-circular anterior spiracles are retained in 3rd instar larvae (**Figure 1(3H)**). Cephaloskeleton morphology differs between instars as outlined in Fessl et al. [44]. Recent studies report a decrease in *P. downsi* puparia size across 2004–2014 [54]. Common et al. (unpublished data), and hence body size is certainly not a useful method to classify instars. In general, it is recommended to use a suite of morphological characters, including anterior and posterior spiracular morphology, to determine the larval instar.

4.2 Larval development

The developmental period of *Philornis* larvae is associated with the species' larval feeding habit. For example, time to pupation in coprophagous species takes up to 29 days, but only 4–8 days in subcutaneous species [2, 55]. Larval development periods in free-living species such as *P. downsi* are difficult to determine in the wild as the host nest needs to be dismantled to observe the larvae. Early studies of abandoned or failed nests found 1st instar larvae in nests with 1–3 day old nestlings, 2nd instars in nests with 3–6 day old nestlings and 3rd instars in nests with 3–10 day old nestlings [44]. Larval collections following the cessation of activity at host nests suggest that the minimum time for pupation in *P. downsi* is 4–7 days [54].

Compared with larval development times in the wild, larval development times under laboratory conditions are longer. First attempts to rear *P. downsi* larvae in the absence of a living host had a low success rate, with only three larvae out of 477 reaching the adult stage after a 36 day development time (mean 18 day larval development, 12 day pupation) [56]. As the diet for rearing larvae in captivity was refined, the success rate increased to 10% and larval development time decreased [57]. Development time in the laboratory ranged from 9 to 10 days from larva to pupa [57] with even faster development times occurring as the rearing conditions have improved [pers. comm. P. Lahuatte]. Egg hatch rates in captivity have been high (96%), with most mortality in 1st instar larvae (77%) [57]. Laboratory-based diets that have been developed in the absence of a bird host are primarily based on chicken blood, with more successful diets including hydrolysed protein and vitamin fortification [57]. The lack of keratin in the diet may be causing elevated 1st instar mortality, as 1st instars consume the keratin of the beak in which they reside [44], however the true cause is unknown.

5. Philornis downsi adult behaviour

The behaviour of adult *P. downsi* is much less understood than that of the larvae. The adult fly is vegetarian, feeding on decaying fruits and flowers, including the invasive blackberry (*Rubus niveus*) Thunb (Rosales: Rosaceae) [9, 15, 31]. *Philornis downsi* is commonly attracted to a mix of blended papaya and sugar, which is used to trap adult flies (developed by P. Lincango and C. Causton; used by [58], Causton et al. in review). This mix is particularly attractive to adult flies due to the presence of yeast and fermentation products such as ethanol and acetic acid [59].

A one-year study on Floreana Island found that male and female *P. downsi* display dimorphic flight patterns, with females more likely to be caught in high and low traps (2 m, most common at 6–7 m), and males more likely to be caught in traps of intermediate height (4–5 m) [58]. As the pattern of male and female abundance are quadratic opposites, this has tentatively been suggested to be an advantage for females to avoid male flies, as frequent mating in other Dipterans has been found to decrease female reproductive success and lifespan [60, 61]. This flight pattern may also explain why certain host species experience higher parasite intensities, such as the medium tree finch (*Camarhynchus pauper*) Ridgway (Passeriformes: Thraupidae) that has an average nest height of 6 m, thus making it more susceptible to being encountered by female *P. downsi* [58, 62, 63]. However, the factors that cause bird species to experience differing intensities of *P. downsi* are complicated and vary between years. Comparison of flight height in *P. downsi* on different islands is needed to test the generality of this pattern, which may be influenced by average tree height and/or other ecological variables.

5.1 Mating behaviour

The mating behaviour of *Philornis* in general is not well understood, though there are some insights into *P. downsi* mating patterns. While mating has not been observed at or inside the nest, multiple *P. downsi* flies have been video recorded to enter host nests concurrently [45, 64]. Analysis of offspring genetic relatedness has provided estimates of the re-mating frequency of *P. downsi* [65]. Evidence for multiple mating by females has been frequently detected, and each larval infrapopulation (i.e., within nests) is sired by 1–5 males (average ~1.9 males per female) [65]. How *P. downsi* adults find each other to initiate mating is unknown. Pheromones for attraction and aggregation in muscid flies have been identified and studied [66–68]. Cuticular compounds show promise for determining if *P. downsi* produces pheromones, as females and mature males showed distinct cuticular profiles and females respond to chemicals produced by males [69–71]. Cuticular profiles could be developed as an attractant to capture flies in the field [20, 72].

5.2 Oviposition behaviour

Studies into oviposition in the genus *Philornis* have revealed that species spanning diverse larval feeding habits are oviparous [1, 9, 31, 73, 74]. This current view has previously been hotly debated, in part because the majority of species remain unstudied. Laboratory rearing and field observation have confirmed that P. downsi is oviparous [45, 56, 57, 75]. Philornis flies enter and oviposit in nests regardless of nesting phase or nestling age but have not been observed to enter nests abandoned by the parent birds during the incubation phase [45, 47]. From in-nest video recordings, P. downsi flies have been observed entering nests throughout the day, but generally during dusk between 1500 and 1800, with visiting rates peaking around 1700 [45, 64]. Visit length averaged 1.3-1.5 min and occurred most commonly when the adult host is away from the nest and completed once the adult host returned [45, 64]. Eggs have been generally deposited on nesting material and the base of the nest [45, 57], however on one occasion, eggs have been also laid directly by the naris of a nestling [45]. A genetic study of P. downsi larvae estimated that 1-6 adult females (average ~3 females) oviposit within a single nest, supporting previous observations of different sized larval groups within nests and suggesting repeated nest infestations throughout the nestling period [7, 65].

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5.3 Effects of host species on Philornis behaviour and microbiome

Philornis downsi is one of the most generalist species within the genus, known to infest 38 host species across avian taxa [5, 6, 76]. However, this high host number may reflect the large number of studies focused on *P. downsi* due to its invasive status on the Galápagos Islands [15, 16].

It is currently unclear how *Philornis* species in general or *P. downsi* in particular find their hosts. Preliminary studies into the role of semiochemicals and volatiles in host nests as an attractant for *P. downsi* have produced inconclusive results [70]. Long-term ornithological field studies have provided some hints that the intensity of host cues may be relevant for P. downsi search behaviour, or alternatively that the density of host nests influences P. downsi oviposition behaviour. Aggregated host nests may attract P. downsi females due to an increase in olfactory or visual cues. These aggregated nests also provide a greater opportunity for *P. downsi* females to infest multiple nests. Indeed, small tree finch nests (Camarhynchus parvulus) Gould (Passeriformes: Thraupidae) with close neighbours contained more P. downsi larvae compared to solitary, more isolated nests [16]. Nests in areas of lower nesting density (i.e., lowlands) have been more likely to contain the offspring of a single P. downsi female than nests in areas of higher nesting density (i.e., highlands) that are more likely to contain the offspring of many P. downsi females [65]. Video recordings of adult P. downsi have been made inside the nests of the small ground finch (Geospiza fuliginosa) Gould (Passeriformes: Thraupidae), medium ground finch (G. fortis) Gould (Passeriformes: Thraupidae), small tree finch (C. parvulus) and Galápagos flycatcher (*Myiarchus magnirostris*) Gould (Passeriformes: Tyrannidae) [45, 64] (Pike et al. in prep). However, despite a combination of video recorders inside or outside the nest across studies, the recordings did not reveal information about P. downsi search behaviour from its flight behaviour.

A metagenomic study into *P. downsi* larval microbiome sampled from different host species found an effect of host diet on the gut bacterial community of *P. downsi* larvae [77]. Larvae retrieved from strictly insectivorous warbler finch (*Certhidea olivacea*) Gould (Passeriformes: Thraupidae) nests have a different microbiome structure compared with larvae parasitising hosts with broader dietary preferences (ground and tree finches, *Geospiza* and *Camarhynchus* sp., respectively) [77]. The gut microbiome also differed between *P. downsi* larvae (blood diet) and adults (plant diet), supporting the hypothesis that *P. downsi* microbiome changes during development and according to diet [77]. Further behavioural, biochemical and genetic studies are needed to understand *P. downsi* oviposition across host species, host locating behaviour and host specificity.

6. Changes in P. downsi behaviour since colonising the Galápagos Islands

6.1 Age of larval cohort in host nests

There is evidence that the oviposition behaviour of female *P. downsi* has changed since its discovery on the Galápagos archipelago. *Philornis downsi* flies are now known to oviposit during any stage of the nesting cycle [45]. In the first decades following initial discovery of *P. downsi* in Darwin's finch nests, changes in the proportions of instar classes among *P. downsi* have been observed, with evidence that oviposition occurred earlier and more synchronously in the nesting phase in the later years of the study [54]. Synchronisation in oviposition date may lead to an increase in larval competition for host resources, and as a consequence result in increased virulence for nestlings that must contend with a greater number of large,

mature larvae at a younger age [16]. The fitness consequences of female oviposition behaviour are further supported by observations in other *Philornis* systems. Host nests that are infested later in the nesting cycle are more likely to have higher fledging success than nests parasitized early in the nesting cycle [50, 78].

6.2 Larval feeding on adult birds

Philornis larvae are generally exclusive parasites of developing nestlings, whether they be subcutaneous or free-living semi-hematophagous species. Infestation of host nests can happen quickly and is often observed within 24 h of the first nestling hatching [41, 43, 50, 79]. Many studies on Philornis species in their native range found no evidence of larvae present during incubation [47, 48, 80, 81]. There have been a few cases of larvae feeding on adults in subcutaneous species [82–84], however these reports are rare, with generally only a few larvae per adult. For this reason, larval feeding on adults is generally regarded as opportunistic [2]. More data are needed to examine the oviposition behaviour of Philornis species to determine whether larvae are present during the incubation phase.

On the Galápagos Islands between 1998 and 2005, there have been no reported cases of P. downsi larvae present in host nests with eggs that would suggest that larvae also feed on incubating females. Two studies during this time period specifically stated that no *P. downsi* larvae have been found during host incubation (**Table 2**) [21, 85]. On Santa Cruz Island during 1998–2010, published studies report findings for 38 nests with eggs that have been inspected for the presence of P. downsi and found no larvae (Table 2) [21, 85]. In 2012, Cimadom and colleagues first observed P. downsi larvae in host nests during incubation where larvae have been found present in 17 of the 26 nests inspected [85]. Since this initial observation, the prevalence of *P. downsi* in host nests with eggs has increased to 80% in some species and years on Santa Cruz Island, with larvae and puparia found in 70 of 177 nests inspected with eggs [86]. Concurrently across this time period, brooding Darwin's finch females have P. downsi antibodies that are associated with decreased P. downsi intensity, but not increased fledging success [87, 88]. This suggests that P. downsi larvae on the Galápagos Islands may have switched to feed on adult finches at some stage [87]. On Floreana Island, inspection of nests that failed during incubation during 2006 and 2016 found P. downsi larvae in 4 of 72 (5.6%) nests with host eggs (Table 2). In 2006, three medium ground finch (G. fortis) nests with eggs in the arid lowlands have P. downsi larvae and puparia, and in 2010 one highland small tree finch (C. parvulus) has P. downsi larvae during the egg stage. During a period of intense drought from 2003 until 2006 with less than 300 mm of rain per year in the lowlands, there were very few active host nests available for oviposition, which may be an explanation for a shift in *P. downsi* female oviposition and larval feeding on incubating females at the end of the drought during 2006. Notably, smaller larvae and eggs are not easily visible in nests and it is possible that P. downsi is present, but not detected during incubation in the early years of study.

In laboratory trials, *P. downsi* hatching success is found to be the same in nests with host eggs and nests with finch hatchlings (*Lonchura striata domestica*) Linnaeus (Passeriformes: Estrildidae) [89]. In these trials, there is even a fitness benefit for *P. downsi* that hatched during incubation and hence earlier during the host cycle, as they survived for longer [89]. Other than *P. downsi*, there is one report of an unidentified *Philornis* species parasitising adults in the pearly-eyed thrasher (*Margarops fuscatus*) Vieillot (Passeriformes: Mimidae) studied in Puerto Rico [49]. About 46% of incubating and brooding females and 13% of attending adult males sustained subcutaneous *Philornis* [49]. It has been suggested that this *Philornis* species may have invaded Puerto Rico, as the patterns of prevalence and host

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Ref#	Year (s) of study	Island	Host species	Total no. of nests examined/no. inspected during egg phase	P. downsi larvae during the egg phase	Comments
[21]	1998, 2000	SC	ST, LT, SG, MG, WF, WP, CF, SBA, YW, VF, DBC, GM	105/17	No	Larvae not found in 17 SG, ST, WF and WP nests that failed during incubation
[85]	1998–2010	SC	ST, WF	na/21	No	Larvae not found in 21 S' and WF nests abandoned during incubation (reported as part of a study during 2012–2015 listed below [86])
[90]	2004	SC, FL, IS	SG	24/na		
[91]	2000, 2004	SC	SG, MG	27/na		Larvae not found in SG and MG nests depredated shortly after host hatch
[44]	2000, 2004, 2005	SC	SG, MF, CF	63/na		
[92]	1998, 2000, 2001, 2002, 2004, 2005	SC	SG, MG, ST, LT, WP, WF	249/na		
[93]	1998, 2000, 2003, 2004, 2005	13 islands incl. SC and FL		515/na		
[87]	2004, 2005, 2006	SC	MG	63/na		
[94]	2000, 2001, 2002, 2004	SC	ST, LT, SG, WF, WP	43/na		
[87]	2008	SC, DMj	MG			Brooding female MG had <i>P. downsi</i> -specific antibodies, suggesting nesting females are
					/ ())	parasitised
[45]	2008	FL	ST, SG, MG	11/5	No	Larvae not found in 4 SG and 1 ST nests abandoned with eggs
[62]	2006, 2008	FL	ST, MT	63/2	No	Larvae not found in 2 MT nests depredated during egg phase
[95]	2004, 2005, 2006	FL	SG	71/na		
Kleindorfer (unpubl. data)	2006	FL	MT, SG, MG	129/27	Yes	Larvae and puparia found in 3 MGF nests abandoned with eggs in the arid lowlands
Kleindorfer (unpubl. data)	2010	FL	ST, MT, SG	153/38	Yes	Larvae found in 1 ST nest depredated with eggs in the highlands

Ref#	Year (s) of study	Island	Host species	Total no. of nests examined/no. inspected during egg phase	P. downsi larvae during the egg phase	Comments
[97]	2009	SC	MG	61/na		
[88]	2010	SC	MG	43/na		Female MG in parasitised nests had more <i>P. downsi</i> antibodies and spent more time standing
						upright when brooding
	57/2					than non-parasitised nests
[98]	2010	SC	MG	30/na		
[63]	2005–2010	FL	ST, MT	43/na		
[54]	2004, 2006, 2008, 2010, 2012, 2013	FL	ST, MT, SG	561/na		Evidence that P. downsi oviposition behaviour occurred more synchronously and earlier in nesting phase in later years of the study
[99]	2013	SC	ST, SG, MG, VGF	26/na		
[46]	2010	FL	SG	14/na		
[57]	2014	SC	GF	1/na		
[100]	2012, 2013	SC	MG, GM	127/na		
[58]	2004, 2005, 2006, 2008, 2010, 2012, 2013, 2014	FL	ST, MT, SG	254/na		
[101]	2013, 2014	SC	VGF	11/na		
[64]	2015	SC	GF	2/na		
[102]	2013	SC	MG, GM	37/na		
[86]	2012, 2014, 2015, 2016, 2017	SC	ST, WF	850/177	Yes	Larvae and puparia found in 18/72 ST nests and 52/105 WF nests that failed during egg phase;
						range in prevalence across species and years was 0-80% of nests
[103]	2012, 2013, 2015, 2016	SC	GM	131/na		
[104]	2010, 2013, 2014	FL	ST, MT	27/na		

The islands are abbreviated as Santa Cruz (SC), Floreana (FL), Isabela (IS), Daphne Major (DMj). The 'total number of nests examined' refers to all active nests monitored over the course of the study and 'number inspected during egg phase' is the sample size for the sub-set of nests examined during host incubation (usually following abandonment or predation) where 'na' denotes that nests have been not sampled during the egg phase. The column 'P. downsi larvae during the egg phase' states 'yes/no' referring only to nest inspections that occurred during the egg phase. Host species are abbreviated as small tree finch (ST), large tree finch (Camarhynchus psittacula) (LT), small ground finch (SG), medium ground finch (MG), woodpecker finch (Cactospiza pallida) (WP), warbler finch (Certhidea olivacea) (WF), cactus finch (Geospiza scandens) (CF), Galápagos mockingbird (GM), smooth billed ani (Crotophaga ani) (SBA), yellow warbler (Dendroica petechia) (YW), dark billed cuckoo (Coccyzus melacoryphus) (DBC), vermillion flycatcher (Pyrocephalus rubinus) (VF), vegetarian finch (Platyspiza crassirostris) (VGF), and Galápagos flycatcher (Myiarchus magnirostris) (GF).

Table 2. Evidence of Philornis downsi larvae present in nests during incubation and before nestling hatching in studies on the Galápagos Islands.

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mortality mirror that of the *P. downsi* invasion in the Galápagos Islands [6, 48, 49]. *Philornis* consumption of attending adult hosts may be an oviposition tactic that is more prevalent under conditions of resource limitation. Resource limitation could be influenced by resource termination such as early host death, resource availability when there is a limited supply of host nests (e.g., during drought), and resource accessibility, for example when competition within and between fly cohorts changes [54].

7. Conclusions

As one of three avian nest parasitic genera in Diptera, the genus Philornis provides a useful system to explore shifts in larval feeding behaviour in native and invasive species. Philornis downsi has been accidentally introduced to the Galápagos Islands and first observed in the nests of Galápagos land birds in 1997. In this chapter, we explored similarities and differences between P. downsi larval development and behaviour with what is known from the other 52 Philornis species. More basal Philornis (aitkeni-group) species have free-living coprophagous larvae and more recently evolved *Philornis* (angustifrons-group) tend to have subcutaneous hematophagous larvae with the exception of *P.* downsi that has free-living semi-hematophagous larvae. Since its introduction to the Galápagos Islands, there have been documented changes in the behaviour of P. downsi. During the early years after initial discovery of P. downsi on the Galápagos Islands, oviposition behaviour was asynchronous across the nesting cycle and larvae appeared to have fed exclusively on developing nestlings until 2005. In later years, P. downsi oviposition behaviour was earlier in the nesting cycle and more synchronous, and since 2006, larvae have also been recorded to feed on incubating females. The first records of P. downsi larvae in host nests with eggs rather than hatchlings occurred at the end of a four-year drought on the Galápagos in 2006. Since 2012, up to 80% of host nests with eggs may contain P. downsi larvae on Santa Cruz Island. Larval feeding by P. downsi on adult birds has been observed in laboratory finches and in one Philornis system (species unknown) in Puerto Rico. In light of changes in P. downsi larval feeding behaviour, we provided a description and photos of the larval instars for use in field identification. We compiled the observations to date of Philornis behaviour and ontogeny within a broad taxonomic framework and summarised patterns of change in the oviposition behaviour of P. downsi in its (presumably) novel habitat on the Galápagos Islands. By examining P. downsi in relation to other Philornis species, we provided a broad phylogenetic context for the potential behavioural repertoire of an invasive species under conditions of intense natural selection in a novel environment.

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References

- [1] Couri MS. Myiasis caused by obligatory parasites. 5a. Philornis Meinert (Muscidae). Myiasis in man and animals in the Neotropical region. In: Editora Pleiade. Brazil: Sao Paulo; 1999. pp. 44-70
- [2] Teixeira DM. Myiasis caused by obligatory parasites. 5b. General observations on the biology of species of the genus Philornis Meinert, 1890 (Diptera, Muscidae). Myiasis in man and animals in the Neotropical Region. In: Editora Pleiade. Brazil: Sao Paulo; 1999. pp. 71-96
- [3] Sabrosky CW, Bennett GF, Whitworth TL. Bird Blow Flies (*Protocalliphora*) in North America (Diptera: Calliphoridae) with Notes on Palearctic Species. Smithsonian Institution Press; 1989
- [4] Pont AC. Revision of the genus *Passeromyia* Rodhain & Villeneuve (Diptera: Muscidae). In: Bulletin of the British Museum (Natural History) Entomology. 1974
- [5] Bulgarella M, Heimpel GE. Host range and community structure of avian nest parasites in the genus *Philornis* (Diptera: Muscidae) on the island of Trinidad. Ecology and Evolution. 2015;5(17):3695-3703. DOI: 10.1002/ece3.1621
- [6] McNew SM, Clayton DH. Alien invasion: Biology of *Philornis* flies highlighting *Philornis downsi*, an introduced parasite of Galápagos birds. Annual Review of Entomology. 2018;63:369-387. DOI: 10.1146/annurev-ento-020117-043103
- [7] Dudaniec RY, Kleindorfer S. Effects of the parasitic flies of the genus *Philornis* (Diptera: Muscidae) on birds. Emu-Austral Ornithology. 2006;**106**(1):13-20. DOI: 10.1071/MU04040

- [8] Bulgarella M, Quiroga MA, Heimpel GE. Additive negative effects of *Philornis* nest parasitism on small and declining Neotropical bird populations. Bird Conservation International. 2018;**29**(3):1-22. DOI: 10.1017/ S0959270918000291
- [9] Skidmore P. The biology of the Muscidae of the world (Series Entomologica). 1st ed. 550 pp. Dordrecht: Dr W; 1985
- [10] Couri MS, Carvalho CD. Systematic relations among *Philornis* Meinert, *Passeromyia* Rodhain & Villeneuve and allied genera (Diptera, Muscidae). Brazilian Journal of Biology. 2003;63(2):223-232. DOI: 10.1590/S1519-69842003000200007
- [11] Kutty SN, Pont AC, Meier R, Pape T. Complete tribal sampling reveals basal split in Muscidae (Diptera), confirms saprophagy as ancestral feeding mode, and reveals an evolutionary correlation between instar numbers and carnivory.

 Molecular Phylogenetics and Evolution. 2014;78:349-364. DOI: 10.1016/j. ympev.2014.05.027
- [12] Haseyama KL, Wiegmann BM, Almeida EA, de Carvalho CJ. Say goodbye to tribes in the new house fly classification: A new molecular phylogenetic analysis and an updated biogeographical narrative for the Muscidae (Diptera). Molecular Phylogenetics and Evolution. 2015;89:1-2. DOI: 10.1016/jympev.2015.04.006
- [13] Edworthy AB, Langmore NE, Heinsohn R. Native fly parasites are the principal cause of nestling mortality in endangered Tasmanian pardalotes. Animal Conservation. 2010;22(1):96-103
- [14] Fessl B, Couri MS, Tebbich S. *Philornis downsi* Dodge & Aitken, new to the

Galápagos Islands (Diptera, Muscidae). Studia Dipterologica. 2001;8(1):317-322

[15] Fessl B, Heimpel GE, Causton CE. Invasion of an avian nest parasite, *Philornis downsi*, to the Galápagos Islands: Colonization history, adaptations to novel ecosystems, and conservation challenges. In: Disease Ecology. Cham: Springer; 2018. pp. 213-266. DOI: 10.1007/978-3-319-65909-1_9

[16] Kleindorfer S, Dudaniec RY. Host-parasite ecology, behavior and genetics: A review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. BMC Zoology. 2016;1(1):1. DOI: 10.1186/s40850-016-0003-9

[17] Lethier H, Bueno P. Report on the Reactive Monitoring Mission to Galápagos Islands World Heritage Site (Ecuador). IUCN. 2018. Available from: https:// whc.unesco.org/en/documents/167914/ [Accessed: 2019-06-29]

[18] Toral-Granda MV, Causton CE, Jäger H, Trueman M, Izurieta JC, Araujo E, et al. Alien species pathways to the Galapagos Islands, Ecuador. PLoS One. 2017;12(9):e0184379. DOI: 10.1371/journal.pone.0184379

[19] Watkins G, Cruz F. Helmsley Charitable Trust's Galapagos Strategic Plan 2012.

[20] Causton CE, Cunninghame F, Tapia W. Management of the avian parasite *Philornis downsi* in the Galápagos Islands: A collaborative and strategic action plan. Galápagos Report. 2012;**2013**:167-173

[21] Fessl B, Tebbich S. *Philornis downsi*—A recently discovered parasite on the Galápagos archipelago—A threat for Darwin's finches? Ibis. 2002;**144**(3):445-451. DOI: 10.1046/j.1474-919X.2002.00076.x

[22] Causton CE, Peck SB, Sinclair BJ, Roque-Albelo L, Hodgson CJ, Landry B.

Alien insects: Threats and implications for conservation of Galápagos Islands. Annals of the Entomological Society of America. 2006;**99**(1):121-143. DOI: 10.1603/0013-8746(2006)099[0121:AIT AIF]2.0.CO;2

[23] Kleindorfer S, Sulloway FJ. Naris deformation in Darwin's finches: Experimental and historical evidence for a post-1960s arrival of the parasite *Philornis downsi*. Global Ecology and Conservation. 2016;7:122-131. DOI: 10.1016/j.gecco.2016.05.006

[24] Macquart J. Notice sur une nouvelle espèce d Aricie, diptère de la tribu des Anthomyzides. Annales de la Société Entomologique de France. 1854;3(1):657-660

[25] Meinert F. Philornis molesta, en paa Fugle snyltend Tachinarie. Videnskabelige Meddelelser fra den Naturhistoriske Forening I Kjøbenhavn. 1890;1(5):304-317

[26] Becker T, Bezzi M, Kertész K, Stein P. Katalog der Paläarktischen Dipteran. Cyclorrapha Aschiza. Acyclorrapha Schizophora: Schizommetopa. Budapest: Hódmezövåsárhely, Wesselényi; 1907;3:1-597. Availabe from: https://archive.org/details/ katalogderpala03beck/page/n5

[27] Malloch JR. Notes on some of van der Wulp's species of north American Anthomyiidae (Diptera). Entomological News. 1921;32:40-45

[28] Aldrich JM. The genus *Philornis*-a bird-infesting group of Anthomyiidae. Annals of the Entomological Society of America. 1923;**16**(4):304-309. DOI: 10.1093/aesa/16.4.304

[29] Dodge HR. A new *Philornis* with coprophagous larva, and some related species (Diptera: Muscidae). Journal of the Kansas Entomological Society. 1963:239-247

- [30] Dodge HR, Aitken TH. *Philornis* flies from Trinidad (Diptera: Muscidae). Journal of the Kansas Entomological Society. 1968;41(1):134-154
- [31] Couri MS. Notes and descriptions of *Philornis* flies (Diptera, Muscidae, Cyrtoneurininae). Revista Brasileira de Entomologia. 1984;**43**(3):297-310
- [32] Savage J, Vockeroth JR. Muscidae (house flies, stable flies). In: Brown BV, Borkent A, Cumming JM, Wood DM, Woodley NE, Zumbado MA, editors. Manual of Central American Diptera. Vol. 2. NRC Research Press; 2010. pp. 1281-1295
- [33] Couri MS, De Carvalho CJB, Löwenberg-Neto P. Phylogeny of *Philornis* Meinert species (Diptera: Muscidae). Zootaxa. 2007;**1530**:19-26
- [34] Couri MS, Antoniazzi LR, Beldomenico P, Quiroga M. Argentine *Philornis* Meinert species (Diptera: Muscidae) with synonymic notes. Zootaxa. 2009;**2261**(5262):77132
- [35] Quiroga MA, Monje LD, Arrabal JP, Beldomenico PM. New molecular data on subcutaneous *Philornis* (Diptera: Muscidae) from southern South America suggests the existence of a species complex. Revista Mexicana de Biodiversidad. 2016;87(4):1383-1386. DOI: 10.1016/j.rmb.2016.10.018
- [36] Teixeira DM, Couri MS, Luigi G. Notes on the biology of *Philornis* rufoscutellaris Couri, 1983 (Diptera, Muscidae) and on its association with nestling birds. Revista Brasileira de Entomologia. 1990;34(2):271-275
- [37] Couri MS, Murphy TG, Hoebeke R. *Philornis fasciventris* (Wulp) (Diptera: Muscidae): Description of the male, larva and puparium, with notes on biology and host association. Neotropical Entomology. 2007;36(6):889-893. DOI: 10.1590/ S1519-566X2007000600009

- [38] Leite GA, Matsui QY, Couri MS, Monteiro AR. New association between *Philornis* Meinert (Diptera: Muscidae) and Falconidae (Aves: Falconiformes). Neotropical Entomology. 2009;38(5):686-687. DOI: 10.1590/ S1519-566X2009000500021
- [39] Bulgarella M, Quiroga MA, Boulton RA, Ramírez IE, Moon RD, Causton CE, et al. Life cycle and host specificity of the parasitoid *Conura annulifera* (hymenoptera: Chalcididae), a potential biological control agent of *Philornis downsi* (Diptera: Muscidae) in the Galápagos Islands. Annals of the Entomological Society of America. 2017;110(3):317-328. DOI: 10.1093/aesa/saw102
- [40] Proudfoot GA, Teel PD, Mohr RM. Ferruginous pygmy-owl (*Glaucidium brasilianum*) and eastern screech-owl (*Megascopes asio*): New hosts for *Philornis mimicola* (Diptera: Muscidae) and *Ornithodoros concanensis* (Acari: Argasidae). Journal of Wildlife Diseases. 2006;**42**(4):873-876. DOI: 10.7589/0090-3558-42.4.873
- [41] Spalding MG, Mertins JW, Walsh PB, Morin KC, Dunmore DE, Forrester DJ. Burrowing fly larvae (*Philornis porteri*) associated with mortality of eastern bluebirds in Florida. Journal of Wildlife Diseases. 2002;38(4):776-783. DOI: 10.7589/0090-3558-38.4.776
- [42] Le Gros A, Stracey CM, Robinson SK. Associations between northern mockingbirds and the parasite *Philornis porteri* in relation to urbanization. The Wilson Journal of Ornithology. 2011;**123**(4):788-796. DOI: 10.1676/10-049.1
- [43] Kinsella JM, Winegarner CE. Notes on the life history of *Neomusca porteri* (Dodge), parasitic on nestlings of the great crested flycatcher in Florida. Journal of Medical Entomology. 1974;11(5):633

- [44] Fessl B, Sinclair BJ, Kleindorfer S. The life-cycle of *Philornis downsi* (Diptera: Muscidae) parasitizing Darwin's finches and its impacts on nestling survival. Parasitology. 2006;**133**(6):739-747. DOI: 10.1017/S0031182006001089
- [45] O'Connor JA, Robertson J, Kleindorfer S. Video analysis of host– parasite interactions in nests of Darwin's finches. Oryx. 2010;44(4):588-594. DOI: 10.1017/S0030605310000086
- [46] O'Connor JA, Robertson J, Kleindorfer S. Darwin's finch begging intensity does not honestly signal need in parasitised nests. Ethology. 2014;**120**(3):228-237. DOI: /10.1111/ eth.12196
- [47] Young BE. Effects of the parasitic botfly *Philornis carinatus* on nestling house wrens, *Troglodytes aedon*, in Costa Rica. Oecologia. 1993;**93**(2):256-262. DOI: 10.1007/BF00317679
- [48] Arendt WJ. *Philornis* ectoparasitism of pearly-eyed thrashers. I. Impact on growth and development of nestlings. The Auk. 1985;**102**(2):270-280. DOI: 10.2307/4086769
- [49] Arendt WJ. *Philornis* ectoparasitism of pearly-eyed thrashers. II. Effects on adults and reproduction. The Auk. 1985;**102**(2):281-292. DOI: 10.2307/4086770
- [50] Rabuffetti FL, Reboreda JC. Early infestation by bot flies (*Philornis seguyi*) decreases chick survival and nesting success in chalk-browed mockingbirds (*Mimus saturninus*). The Auk. 2007;124(3):898-906. DOI: 10.1642/0004-8038(2007)124[898:EIBB FP]2.0.CO;2
- [51] Quiroga MA, Reboreda JC. Lethal and sublethal effects of botfly (*Philornis seguyi*) parasitism on house wren nestlings. The Condor. 2012;**114**(1): 197-202. DOI: /10.1525/cond.2012.110152

- [52] Silvestri L, Antoniazzi LR, Couri MS, Monje LD, Beldomenico PM. First record of the avian ectoparasite *Philornis downsi* Dodge & Aitken, 1968 (Diptera: Muscidae) in Argentina. Systematic Parasitology. 2011;80(2):137. DOI: 10.1007/s11230-011-9314-y
- [53] Cimadom A, Ulloa A, Meidl P, Zöttl M, Zöttl E, Fessl B, et al. Invasive parasites, habitat change and heavy rainfall reduce breeding success in Darwin's finches. PLoS One. 2014;9(9):e107518. DOI: 10.1371/journal.pone.0107518
- [54] Kleindorfer S, Peters KJ, Custance G, Dudaniec RY, O'Connor JA. Changes in *Philornis* infestation behavior threaten Darwin's finch survival. Current Zoology. 2014;**60**(4):542-550. DOI: 10.1093/ czoolo/60.4.542
- [55] Uhazy LS, Arendt WJ. Pathogenesis associated with philornid myiasis (Diptera: Muscidae) on nestling pearly-eyed thrashers (Aves: Mimidae) in the Luquillo Rain Forest, Puerto Rico. Journal of Wildlife Diseases. 1986;22(2):224-237. DOI: 10.7589/0090-3558-22.2.224
- [56] Lincango P, Causton C. Crianza en cautiverio de *Philornis downsi*, en las Islas Galápagos. Charles Darwin Fourndation: Informe interno; 2008
- [57] Lincango P, Causton C, Cedeño D, Castañeda J, Hillstrom A, Freund D. Interactions between the avian parasite, *Philornis downsi* (Diptera: Muscidae) and the Galápagos flycatcher, *Myiarchus magnirostris* Gould (Passeriformes: Tyrannidae). Journal of Wildlife Diseases. 2015;**51**(4):907-910. DOI: 10.7589/2015-01-025
- [58] Kleindorfer S, Peters KJ, Hohl L, Sulloway FJ. Flight behaviour of an introduced parasite affects its Galápagos Island hosts: *Philornis downsi* and Darwin's finches. Chapter 10 In:

- Weis JS, Sol D, editors. Biological Invasions and Animal Behaviour. Cambridge: Cambridge University Press; 2016
- [59] Cha DH, Mieles AE, Lahuatte PF, Cahuana A, Lincango MP, Causton CE, et al. Identification and optimization of microbial attractants for Philornis downsi, an invasive fly parasitic on Galápagos birds. Journal of Chemical Ecology. 2016;42(11):1101-1111. DOI: 10.1007/s10886-016-0780-1
- [60] Bateman AJ. Intra-sexual selection in *Drosophila*. Heredity. 1948;2(3):349-368
- [61] Fowler K, Partridge L. A cost of mating in female fruitflies. Nature. 1989;338(6218):760. DOI: 10.1038/338760a0
- [62] O'Connor JA, Sulloway FJ, Robertson J, Kleindorfer S. *Philornis downsi* parasitism is the primary cause of nestling mortality in the critically endangered Darwin's medium tree finch (*Camarhynchus pauper*). Biodiversity and Conservation. 2010;**19**(3):853-866. DOI: 10.1007/s10531-009-9740-1
- [63] Kleindorfer S, O'Connor JA, Dudaniec RY, Myers SA, Robertson J, Sulloway FJ. Species collapse via hybridization in Darwin's tree finches. The American Naturalist. 2014;183(3):325-341. DOI: 10.1086/674899
- [64] Ramirez I. *Philornis downsi* interactions with its host in the introduced range and its parasitoids in its native range [thesis]. The University of Minnesota; 2018
- [65] Dudaniec RY, Gardner MG, Kleindorfer S. Offspring genetic structure reveals mating and nest infestation behaviour of an invasive parasitic fly (*Philornis downsi*) of Galápagos birds. Biological Invasions. 2010;**12**(3):581-592. DOI: 10.1007/s10530-009-9464-x

- [66] Carlson DA, Mayer MS, Silhacek DL, James JD, Beroza M, Bierl BA. Sex attractant pheromone of the house fly: Isolation, identification and synthesis. Science. 1971;174(4004):76-78. DOI: 10.1126/ science.174.4004.76
- [67] Chapman JW, Knapp JJ, Howse PE, Goulson D. An evaluation of (Z)-9-tricosene and food odours for attracting house flies, *Musca domestica*, to baited targets in deeppit poultry units. Entomologia Experimentalis et Applicata. 1998;89(2):183-192. DOI: 10.1046/j.1570-7458.1998.00398.x
- [68] Jiang Y, Lei CL, Niu CY, Fang YL, Xiao C, Zhang ZN. Semiochemicals from ovaries of gravid females attract ovipositing female houseflies, *Musca domestica*. Journal of Insect Physiology. 2002;48(10):945-950. DOI: 10.1016/S0022-1910(02)00162-2
- [69] Collignon RM. Semiochemicals of *Philornis downsi* (Dipter: Muscidae), a Parasite of Passerine Birds of the Galápagos Islands. State University of New York College of Environmental Science and Forestry; 2011
- [70] Doherty KM. Chemical attractants of *Philornis downsi* (Diptera: Muscidae), an invasive parasite of birds in the Galápagos Islands [honors thesis]. SUNY College of Environmental Science and Forestry; 2012
- [71] Collignon R, Boroczky K, Mieles AE, Causton CE, Lincango MP, Teale SA. Cuticular lipids and mate attraction in the avian parasite *Philornis downsi* (Diptera: Muscidae). In: International Society of Chemical Ecology; Poster; 8-12 July 2014. University of Illinois at Urbana-Champaign
- [72] Lance DR, McInnis DO. Biological basis of the sterile insect technique. In: Sterile Insect Technique. Dordrecht:

- Springer; 2005. pp. 69-94. DOI: 10.1007/1-4020-4051-2_3
- [73] Meier R, Kotrba M, Ferrar P. Ovoviviparity and viviparity in the Diptera. Biological Reviews. 1999;74(3):199-258
- [74] Patitucci LD, Quiroga M, Couri MS, Saravia-Pietropaolo MJ. Oviposition in the bird parasitic fly *Philornis torquans* (Nielsen, 1913) (Diptera: Muscidae) and eggs' adaptations to dry environments. Zoologischer Anzeiger. 2017;267:15-20. DOI: 10.1016/j.jcz.2017.01.004
- [75] Lahuatte PF, Lincango MP, Heimpel GE, Causton CE. Rearing larvae of the avian nest parasite, *Philornis downsi* (Diptera: Muscidae), on chicken blood-based diets. Journal of Insect Science. 2016;**16**(1):84. DOI: 10.1093/jisesa/iew064
- [76] Couri MS, Barbosa L, Marini MÂ, Duca C, Pujol-Luz JR. A new host for *Philornis torquans* (Diptera, Muscidae) from the Brazilian Cerrado. Papéis Avulsos de Zoologia. 2018;58:e20185857. DOI: 10.11606/1807-0205/2018.58.57
- [77] Ben-Yosef M, Zaada DS, Dudaniec RY, Pasternak Z, Jurkevitch E, Smith RJ, et al. Host-specific associations affect the microbiome of *Philornis downsi*, an introduced parasite to the Galápagos Islands. Molecular Ecology. 2017;**26**(18):4644-4656. DOI: 10.1111/ mec.14219
- [78] Segura LN, Reboreda JC. Botfly parasitism effects on nestling growth and mortality of red-crested cardinals. The Wilson Journal of Ornithology. 2011;123(1):107-115. DOI: 10.1676/10-053.1
- [79] Couri MS, Rabuffetti FL, Reboreda JC. New data on *Philornis* seguyi Garcia (1952)(Diptera, Muscidae). Brazilian Journal of Biology. 2005;**65**(4):631-637. DOI: 10.1590/ S1519-69842005000400010

- [80] Nores AI. Botfly ectoparasitism of the Brown Cacholote and the firewood-gatherer. The Wilson Bulletin. 1995;107(4):734-738
- [81] De la Peña MR, Beldoménico PM, Antoniazzi L. Pichones de aves parasitados por larvas de *Philornis* sp. (Diptera: Muscidae) en un sector de la provincia biogeográfica del Espinal de Santa Fe, Argentina. Revista FAVE— Ciencias Veterinarias. 2003;2:141-146
- [82] Oniki Y. Notes on fly (Muscidae) parasitism of nestlings of south American birds. Gerfaut. 1983;73:281-286
- [83] Mendonça ED, Couri MS. New associations between *Philornis* Meinert (Diptera, Muscidae) and Thamnophilidae (Aves, Passeriformes). Revista Brasileira de Zoologia. 1999;**16**(4):1223-1225. DOI: 10.1590/S0101-81751999000400030
- [84] Herrera JM, Bermúdez SE. Miasis ocasionada por *Philornis* spp. (Diptera: Muscidae) in *Dendroica castanea* (Aves: Parulidae) en Panamá. Revista mexicana de biodiversidad. 2012;83(3):854-855. DOI: 10.7550/ rmb.25650
- [85] Cimadom A, Causton C, Cha DH, Damiens D, Fessl B, Hood-Nowotny R, et al. Darwin's finches treat their feathers with a natural repellent. Scientific Reports. 2016;6:34559. DOI: 10.1038/srep34559
- [86] Cimadom A, Jäger H, Schulze CH, Hood-Nowotny R, Wappl C, Tebbich S. Weed management increases the detrimental effect of an invasive parasite on arboreal Darwin's finches. Biological Conservation. 2019;233:93-101. DOI: 10.1016/j. biocon.2019.02.025
- [87] Huber SK, Owen JP, Koop JA, King MO, Grant PR, Grant BR, et al. Ecoimmunity in Darwin's finches: Invasive

parasites trigger acquired immunity in the Medium Ground Finch (*Geospiza fortis*). PLoS One. 2010;5(1):e8605. DOI: 10.1371/journal.pone.0008605

[88] Koop JA, Owen JP, Knutie SA, Aguilar MA, Clayton DH. Experimental demonstration of a parasite-induced immune response in wild birds: Darwin's finches and introduced nest flies. Ecology and Evolution. 2013;3(8):2514-2523. DOI: 10.1002/ece3.651

[89] Sage R, Boulton RA, Lahuatte PF, Causton CE, Cloutier R, Heimpel GE. Environmentally cued hatching in the bird-parasitic nest fly *Philornis downsi*. Entomologia Experimentalis et Applicata. 2018;**166**(9):752-760. DOI: 10.1111/eea.12721

[90] Dudaniec RY, Kleindorfer S, Fessl B. Effects of the introduced ectoparasite *Philornis downsi* on haemoglobin level and nestling survival in Darwin's small ground finch (Geospiza fuliginosa). Austral Ecology. 2006;**31**(1):88-94. DOI: 10.1111/j.1442-9993.2006.01553.x

[91] Fessl B, Kleindorfer S, Tebbich S. An experimental study on the effects of an introduced parasite in Darwin's finches. Biological Conservation. 2006;127(1):55-61. DOI: 10.1016/j. biocon.2005.07.013

[92] Dudaniec RY, Fessl B, Kleindorfer S. Interannual and interspecific variation in intensity of the parasitic fly, *Philornis downsi*, in Darwin's finches. Biological Conservation. 2007;139(3-4):325-332. DOI: 10.1016/j. biocon.2007.07.006

[93] Wiedenfeld DA, Fessl B, Kleindorfer S, Valarezo JC. Distribution of the introduced parasitic fly Philornis downsi (Diptera, Muscidae) in the Galápagos Islands. Pacific Conservation Biology. 2007;13(1):14-19. DOI: 10.1071/ PC070014 [94] Kleindorfer S, Dudaniec RY. Love thy neighbour? Social nesting pattern, host mass and nest size affect ectoparasite intensity in Darwin's tree finches. Behavioral Ecology and Sociobiology. 2009;63(5):731-739. DOI: 10.1007/s00265-008-0706-1

[95] O'Connor JA, Dudaniec RY, Kleindorfer S. Parasite infestation and predation in Darwin's small ground finch: Contrasting two elevational habitats between islands. Journal of Tropical Ecology. 2010;26(3):285-292. DOI: 10.1017/S0266467409990678

[96] Koop JA, Huber SK, Laverty SM, Clayton DH. Experimental demonstration of the fitness consequences of an introduced parasite of Darwin's finches. PLoS One. 2011;6(5):e19706. DOI: 10.1371/journal. pone.0019706

[97] Koop JA, Le Bohec C, Clayton DH. Dry year does not reduce invasive parasitic fly prevalence or abundance in Darwin's finch nests. Reports in Parasitology. 2013;3:11-17. DOI: 10.2147/RIP.S48435

[98] Knutie SA, Koop JA, French SS, Clayton DH. Experimental test of the effect of introduced hematophagous flies on corticosterone levels of breeding Darwin's finches. General and Comparative Endocrinology. 2013;193:68-71. DOI: 10.1016/j. ygcen.2013.07.009

[99] Knutie SA, McNew SM, Bartlow AW, Vargas DA, Clayton DH. Darwin's finches combat introduced nest parasites with fumigated cotton. Current Biology. 2014;24(9):R355-R356. DOI: 10.1016/j.cub.2014.03.058

[100] Knutie SA, Owen JP, McNew SM, Bartlow AW, Arriero E, Herman JM, et al. Galápagos mockingbirds tolerate introduced parasites that affect Darwin's finches. Ecology. 2016;97(4):940-950. DOI: 10.1890/15-0119

[101] Heimpel GE, Hillstrom A, Freund D, Knutie SA, Clayton DH. Invasive parasites and the fate of Darwin's finches in the Galápagos Islands: The case of the vegetarian finch (*Platyspiza crassirostris*). The Wilson Journal of Ornithology. 2017;**129**(2):345-349. DOI: 10.1676/16-050.1

[102] Knutie SA. Relationships among introduced parasites, host defenses, and gut microbiota of Galapagos birds. Ecosphere. 2018;9(5):e02286. DOI: 10.1002/ecs2.2286

[103] McNew SM, Knutie SA, Goodman GB, Theodosopoulos A, Saulsberry A, Yépez RJ, et al. Annual environmental variation influences host tolerance to parasites. Proceedings of the Royal Society B. 2019;**286**(1897):20190049. DOI: 10.1098/rspb.2019.0049

[104] Peters KJ, Evans C, Aguirre JD, Kleindorfer S. Genetic admixture predicts parasite intensity: Evidence for increased hybrid performance in Darwin's tree finches. Royal Society Open Science. 2019;6(4):181616. DOI: 10.1098/rsos.18161

Appendix 2

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RESEARCH PAPER



Evidence for rapid downward fecundity selection in an ectoparasite (Philornis downsi) with earlier host mortality in Darwin's finches

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Abstract

Fecundity selection is a critical component of fitness and a major driver of adaptive evolution. Trade-offs between parasite mortality and host resources are likely to impose a selection pressure on parasite fecundity, but this is little studied in natural systems. The 'fecundity advantage hypothesis' predicts female-biased sexual size dimorphism whereby larger females produce more offspring. Parasitic insects are useful for exploring the interplay between host resource availability and parasite fecundity, because female body size is a reliable proxy for fecundity in insects. Here we explore temporal changes in body size in the myiasis-causing parasite Philornis downsi (Diptera: Muscidae) on the Galápagos Islands under conditions of earlier innest host mortality. We aim to investigate the effects of decreasing host resources on parasite body size and fecundity. Across a 12-year period, we observed a mean of c. 17% P. downsi mortality in host nests with 55 \pm 6.2% host mortality and a trend of $\it c.$ 66% higher host mortality throughout the study period. Using specimens from 116 Darwin's finch nests (Passeriformes: Thraupidae) and 114 traps, we found that over time, P. downsi pupae mass decreased by c. 32%, and male (c. 6%) and female adult size (c. 11%) decreased. Notably, females had c. 26% smaller abdomens in later years, and female abdomen size was correlated with number of eggs. Our findings imply natural selection for faster P. downsi pupation and consequently smaller body size and lower parasite fecundity in this newly evolving host-parasite system.

KEYWORDS

abdomen size, body size, Darwin's finches, Diptera, Galápagos Islands, host-parasite

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1 | INTRODUCTION

Fecundity selection affects fitness by favouring traits associated with increased reproductive output (Roff, 2001). Few studies examine fecundity selection (Pincheira-Donoso & Hunt, 2017) and those that do generally focus on traits that increase fecundity (upward selection) (Orozco & Bell, 1974; Preziosi & Fairbairn, 1996; Saino et al., 2017; Välimäki & Kaitala, 2007). Although traits that increase or decrease fecundity covary, far fewer studies have observed downward selection on traits leading to decreased fecundity (Nunney, 1996: Orozco & Bell, 1974; Quintero-Fong et al., 2018; Reeve & Fairbairn, 1999). To better understand the role of fecundity selection on variation in biological fitness, we need case studies that identify temporal patterns and processes of fecundity change. Host-parasite systems make excellent candidates for such case studies given their tight co-evolutionary interactions that depend on fecundity and survival. Thus, the relationship between parasite virulence and host mortality can be explored to understand the drivers and direction of fecundity selection.

The 'fecundity advantage hypothesis' was originally formulated by Darwin (1871) to explain the common occurrence of large female body size (Cox, Skelly, & John-Alder, 2003; Shine, 1989). Across taxa, female body size is positively associated with fecundity (Pincheira-Donoso & Hunt, 2017), as larger-bodied females can physically accommodate more offspring and can store more energy to invest in reproduction (Calder, 1996). Strong positive fecundity selection can generate directional selection for increased female body size in insects (Andersen, 1994; Hurlbutt, 2008; Sivinski & Dodson, 1992; Teder & Tammaru, 2005) and other taxa (Braña, 1996; Scharf & Meiri, 2013), and can also result in the increased size of particular body regions (i.e. trunk or abdomen) that are functionally linked to fecundity (Olsson, Shine, Wapstra, Ujvari, & Madsen, 2002; Parker et al., 2011: Preziosi, Fairbairn, Roff, & Brennan, 1996: Winkler, Stölting, & Wilson, 2012). Parasitic insects provide useful systems to test ideas about effects of body size on fecundity because parasite diets can be tracked through host availability (Nijhout, 2003; Lahuatte, Lincango, Heimpel, & Causton, 2016). In this way, parasitic insects can provide insights into changing body size and fecundity with altered nutritional conditions.

Parasites must balance virulence and fitness with maximizing host resource use to ensure life cycle completion before host death (Hatcher, Dick, & Dunn, 2012). Increased host exploitation may lead to larger body size and higher fecundity, but could result in early termination of the host and eventually population collapse as host populations are exhausted (Hatcher et al., 2012). Recent host-parasite associations undergoing co-evolutionary interactions are therefore ideal case studies for examining changing fecundity selection under unstable host resource pressures.

Here we focus on natural selection for small body size in the fly, Philornis downsi (Diptera: Muscidae) (Dodge and Aitken), which is an invasive myiasis-causing parasite of Darwin's finches on the Galápagos Islands. Philornis downsi larvae consume the blood and tissue of nestling birds, causing up to 100% in-nest mortality in some of its Darwin's finch hosts (Dudaniec & Kleindorfer, 2006; Fessl, Heimpel, & Causton, 2018; Kleindorfer, Peters, Custance, Dudaniec, & O'Connor, 2014; O'Connor, Sulloway, Robertson, & Kleindorfer, 2010). The adult fly has been present in the Galápagos since at least 1964 (Causton et al., 2006), but its larvae were first reported in Darwin's finch nests on Santa Cruz Island in 1997 (Fessl. Couri, & Tebbich, 2001) despite long-term field study into Darwin's finches on other islands since 1973 (Grant & Grant, 2002). Field research found *P. downsi* requires *c.* 4–7 days to develop through three instar stages and reach pupation (Common, Dudaniec, Colombelli-Négrel, & Kleindorfer, 2019; Kleindorfer, Peters, et al., 2014). In this newly evolving host-parasite system, mortality has been high in both P. downsi and its Darwin's finch hosts. On average, about 17% of P. downsi larvae die in the host nest and about 55 ± 6.2% of Darwin's finch nestlings die in the nest from P. downsi parasitism (Kleindorfer & Dudaniec, 2016). In addition to the high mortality it exerts, P. downsi parasitism has on average been killing nestling hosts at an earlier age of 5.4 ± 0.3 days post-hatch in 2014 compared to 10.6 ± 0.5 days post-hatch in 2004 (Kleindorfer, Peters, et al., 2014; O'Connor, Sulloway, et al., 2010). Questions remain as to how this earlier termination in parasite resources (nestling hosts) affects life cycle completion, body size and fecundity in P. downsi, and in turn, how the evolution of virulence may be affected.

In this study, we use 9 years of field data spanning a 12-year period to examine changes in body size (an indirect measure of fecundity) in the dipteran ectoparasite, P. downsi, in response to the increasingly earlier death of its host. Given that there is a strong correlation between insect body size and fecundity (Armbruster & Hutchinson, 2002; Honěk, 1993; Preziosi et al., 1996; Tammaru, Esperk, & Castellanos, 2002), we analyse body size in adult P. downsi flies and pupae as indicators of P. downsi fecundity across years. If natural selection favours faster pupation and smaller body size as the consequence of earlier host mortality, we predict (a) smaller size in P. downsi pupae and adult flies from 2004 to 2016. If natural selection for smaller body size favours lower fecundity via trade-offs between virulence and host resources, then we predict (b) a larger decrease in female body size relative to male body size in P. downsi adults. Together, this knowledge contributes to our understanding of how shifting host mortality in the natural environment directly selects for parasite body size as the consequence of faster pupation, which may lead to an indirect selection pressure on female fecundity.

2 | MATERIALS AND METHODS

2.1 | Study site and study species

We collected data from long-term field study sites on the islands of Santa Cruz (Cimadom et al., 2014; Kleindorfer, 2007; Kleindorfer, Chapman, Winkler, & Sulloway, 2006) and Floreana (Kleindorfer, Peters, et al., 2014; O'Connor, Sulloway, et al., 2010) in the Galápagos Archipelago. We conducted field work during nine Darwin's finch breeding seasons spanning the months of

February to April over 12 years: 2004, 2005, 2006, 2008, 2010, 2012, 2013, 2014 and 2016. On each island, study sites were located in both the arid lowland zone (El Garrapatero, -0.686479. -90.223775, and El Barranco, -0.739068, -90.301467 on Santa Cruz: habitat surrounding the town of Puerto Velasco Ibarra and La Loberia, -1.279932, -90.485927, on Floreana Island) and in highland Scalesia forest (Los Gemelos, -0.625982, -90.384829, on Santa Cruz; sites along the trail at the base of Cerro Pajas volcano, -1.299974, -90.452710, on Floreana Island). We sampled P. downsi from the following host species: small tree finch (Camarhynchus parvulus), hybrid Camarhynchus tree finch (cross between C. pauper and C. parvulus as well as introgressed individuals) (Kleindorfer, O'Connor, et al., 2014; Peters, Myers, Dudaniec, O'Connor, & Kleindorfer, 2017), medium tree finch (C. pauper), woodpecker finch (C. pallidus), small ground finch (Geospiza fuliginosa) and medium ground finch (G. fortis) (Table S1). For analysis, we tested effects of host species and host genus (Camarhynchus, Geospiza) on P. downsi body size.

Adult P. downsi flies are vegetarian and feed on decaying plant material, so they do not pose a direct threat to Darwin's finches (Couri, 1985; Skidmore, 1985). However, the fly oviposits in active finch nests when the attending female is absent (Lahuatte et al., 2016; O'Connor, Robertson, & Kleindorfer, 2010; O'Connor, Robertson, & Kleindorfer, 2014), and multiple female flies may oviposit in a single nest (Dudaniec, Gardner, & Kleindorfer, 2010). After P. downsi eggs hatch, 1st-instar larvae enter the nares and body cavities of the nestling and reside there to feed on blood and tissue (Fessl, Sinclair, & Kleindorfer, 2006). During the night, 2nd- and 3rd-instar larvae emerge from the nest base to feed internally and externally on the body of nestlings (Fessl et al., 2006; Kleindorfer & Sulloway, 2016; O'Connor et al., 2014). After feeding for c. 4-7 days, 3rd-instar larvae pupate in the nest base, forming a frothy cocoon, and adult flies emerge after 7-14 days (Kleindorfer, Peters, et al., 2014; Lahuatte et al., 2016). Although field research has found that P. downsi requires c. 4-7 days to develop through three instar stages and reach pupation (Kleindorfer, Peters, et al., 2014), laboratory studies have found that pupation occurs at c. 7-10 days (Bulgarella et al., 2017; Lahuatte et al., 2016). Philornis downsi parasitism causes higher than average nestling mortality in 10 out of 17 Darwin's finch species in which the interaction has been studied (Fessl et al., 2018; Kleindorfer & Dudaniec, 2016), with surviving nestlings commonly showing physical deformation of the paris into adulthood (Galligan & Kleindorfer, 2009; Heimpel, Hillstrom, Freund, Knutie, & Clayton, 2017: Kleindorfer, Custance, Peters Katharina, & Sulloway Frank, 2019: Kleindorfer & Dudaniec, 2016).

2.2 | Philornis downsi collection from Darwin's finch nests

We monitored 116 Darwin's finch nests for nesting outcome using our well-established field protocols (Kleindorfer, Peters, et al., 2014)

in all sampling years except 2005. Upon nesting termination (fledging or death of the last nestling), each nest was collected in a sealed plastic bag, and all *P. downsi* larvae, pupae, empty puparia and adult flies were counted within 1–24 hr of collection. All *P. downsi* samples were stored in 90% ethanol immediately after counting. *Philornis downsi* intensity in the nest was measured as the total number of larvae, pupae, puparia and adult flies present upon collection of the nest. The sample size per year and host genus (*Camarhynchus*, *Geospiza*) is provided in Table S1.

2.3 | Philornis downsi collection from McPhail traps

We placed a total of 114 McPhail Traps in the lowlands and highlands of Santa Cruz and Floreana Island to sample adult $P.\ downsi$ flies in the years 2004, 2005, 2012, 2013 and 2014 (for details see Table S1). The McPhail traps were baited with a liquid lure of blended papaya, water and white sugar (following trapping protocol developed by P. Lincango and C. Causton) that was replaced every 7 days. Traps were hung in trees along 4×90 m transects, and flies were collected twice per week and stored in ethanol. In 2014 on Floreana Island, we placed 28 McPhail traps along four transects, seven traps per transect, at heights of 2–7 m. In other years and locations, traps were placed ad hoc every 50 m within $100 \ \text{m} \times 200 \ \text{m}$ plots spanning a 2 km transect within study sites. We analysed data from 46 lowland traps and 68 highland traps (Table S1).

2.4 | Pupa mass and size

Mass (g), length and width (mm) were measured for each pupa, as these measurements are known to be highly correlated with adult fly size (Gauld & Fitton, 1987; Quiroga & Reboreda, 2013; Shingleton, Mirth. & Bates, 2008; Stillwell, Dworkin, Shingleton, & Frankino, 2011), and can therefore be an indirect indicator of an individuals' fecundity upon maturity (Orozco & Bell, 1974; Preziosi & Fairbairn, 1996; Saino et al., 2017; Välimäki & Kaitala, 2007). Pupae cannot be sexed: therefore, these data could not be used for sexual dimorphism analysis but are useful when looking at general temporal shifts in body size in the P. downsi population. All pupae were removed from ethanol and placed on filter paper to dry for 30 s before taking measurements (Armbruster & Hutchinson, 2002). We measured the total mass of all intact pupae per nest and divided this by the number of pupae to calculate average pupa mass (Thomas, Fadul, Keller, & Chaudhury, 2018). The pupae were weighed to the nearest 0.001 g using an A&D HR-200 Digital Analytical Balance. The length (mm) and width (mm) of the largest pupa per nest was measured using digital callipers. For analysis, we used the average mass per nest. Pupa mass was measured from the nests of 19 C. parvulus (268 pupae), 10 hybrid Camarhynchus tree finch (55 pupae), 25 C. pauper (332 pupae), 57 G. fuliginosa (816 pupae) and 5 G. fortis (52 pupae) (Table S1).

2.5 | Adult P. downsi size

We measured body size for 38 male and 38 female adult P. downsi from nests, and 34 male and 85 female adult P. downsi from McPhail traps. From the 43 nests and 114 McPhail traps sampled. we measured one male and one female adult fly unless there was only one sex present, in which case we used one sample per nest or trap. We visually sorted all fly specimens per sex for each nest or trap from smallest to largest and selected the median-sized fly as the specimen for analysis. This approach was used because we measured the average pupa mass per nest and also to avoid any possible pseudoreplication due to genetic relatedness among the fly specimens. For each specimen, we used callipers with 0.1 mm accuracy to measure head length (mm), thorax length (mm) and abdomen length (mm), all measured with the specimen ventral side up; wing length (mm), measured from the base of the basicosta to the tip of the wing; and body length (mm), which was calculated from the values of head, thorax and abdomen length combined. For seven specimens, the head was missing due to previous DNA extractions; for 23 specimens, we only have data on body length as the specimens were destroyed for a separate study (Dudaniec et al., 2010). Therefore, sample size for head length (N = 188) and body length (N = 211) versus thorax, abdomen and wing length (N = 195) differ.

2.6 | Philornis downsi body size and fecundity

To assess if the overall pattern of association between abdomen size/body size and number of eggs in P. downsi is comparable with the pattern reported in other Diptera studies, we collated published r and r^2 values across 17 studies (Table S2). Collated values were used to calculate average r^2 and 95% CI, and compared to the pattern found in P. downsi. We randomly sampled and dissected 10 female P. downsi specimens collected from McPhail traps at 4 m in the study area on Floreana Island in 2014 (Kleindorfer, Peters, Hohl, & Sulloway, 2016). One specimen was collected from a different trap and/or different collection week to ensure independence of data. Specimens were stored in 70% ethanol at room temperature for at least 24 hr before dissection and were dissected under a stereomicroscope at 16× magnification to count the total number of eggs present in ovaries (Malmqvist, Adler, & Strasevicius, 2004). We limit the sample size as the specimens are valuable intact for our long-term study, and our aim is to test for an already established pattern of association in Diptera.

2.7 | Statistical analysis

Data were analysed with SPSS version 25.0. The summary data are presented as mean ± standard error, unless otherwise stated. Data were checked for normality to satisfy requirements of parametric tests. We tested the association between abdomen size/body

size and the number of eggs present in ovaries using linear regression analysis. We completed principal component analysis (PCA) on mean pupae mass, length and width to assess overall changes in pupae size. One principal component was retained, pupae size, which explained 85.96% of the total variation within these variables (Eigenvalue = 2.579) (Table S4). We used a generalized linear mixed model (GLMM) to test for an effect of year on pupae size with PC pupae size as the dependent variable, year, island and habitat as fixed factors, and species as a random factor. We then used linear regression to test for changes in pupae mass, length and width separately to investigate whether each variable displays a different pattern of change across time.

We explored adult fly size across years and in relation to sex (male, female). To assess overall changes in adult body size, we completed a PCA on abdomen length and body length. One principal component, fly size, was extracted which explained 91.4% of the variation within these three variables (Eigenvalue = 1.828) (Table S5). We used a GLMM to test for an effect of year on adult fly size using PC fly size as the dependent variable, and year, sex, year \times sex as fixed factors and island as a random factor. There was no difference in body size of adult flies collected from Darwin's finch nests or McPhail traps (independent t test: head length: t = -0.003, p = .998; thorax length: t = -0.188, p = .851; abdomen length: t = 1.804, p = .074; wing length: t = 0.629, p = .530). Therefore, nest and trap data were pooled to test for the effect of year on P. downsi head, thorax, abdomen, wing and body length separately using linear regression analysis. We conducted linear regression analyses separated by sex to examine for sex differences. We derive all statistical conclusions from the GLMM analyses, but present individual regression analyses for comparative purposes.

3 | RESULTS

3.1 | Pupae size and mass

Only the fixed factor year had a significant effect on pupae size ($F_{1,112}$ = 30.814, p < .001); no other covariate or interaction term was related to P. downsi size (Table 1). There was no effect of species on pupae size (Wald Z = 0.540; p = .589). Using regression analysis, P. downsi pupae mass was negatively correlated with year $(F_{1,115} = 30.709, r = -.461, p < .001, N = 115)$ (Figure 1), as was length ($F_{1.115}$ = 12.086, r = -.310, p = .001, N = 115) and width $(F_{1.115} = 33.450, r = -.476, p < .001, N = 115)$. Pupae mass decreased up to 32.9% (0.073 \pm 0.003 g to 0.049 \pm 0.006 g), pupae length by 5.8% (10.09 ± 0.12 to 9.50 ± 0.39 mm) and pupae width by 10.6% $(4.17 \pm 0.06 \text{ to } 3.73 \pm 0.15 \text{ mm})$. Since 2004, P. downsi pupae have become significantly lighter, shorter and narrower (Table S3). We found the same pattern when analysing the data separately for pupae collected from the nests of Camarhynchus finches (N = 53; mass: $F_{1.53}$ = 17.476, r = -.502, p < .001; length: $F_{1.53}$ = 10.476, r = -.409, p = .002; width: $F_{1.53} = 28.045$, r = -.592, p < .001) and Geospiza

TABLE 1 Coefficients of the generalized linear mixed model of pupae size and adult fly size. The test statistic was t for fixed factors and Z for random factors

Response variable	Final model	Coefficients	Estimate	SE	Test statistic	p-value
Pupae size						
PC pupae size	Year Island Habitat	Intercept	286.370	51.596	5.550	<.001
		Year	-0.143	0.026	-5.551	<.001
		Island	0.501	0.348	1.438	.153
		Habitat	0.103	0.227	0.453	.651
		Species	0.021	0.038	0.540	.589
Adult fly size						
PC adult size	Year	Intercept	325.560	65.523	4.969	<.001
	Year × Sex	Year	-0.162	0.033	-4.972	<.001
	Sex Island	Sex (female) ^a	-211.557	101.934	-2.075	.039
		Year × Sex	0.105	0.051	2.082	.039
		Island	0.090	0.142	-0.629	.530

^aFor Sex, male was set to zero.

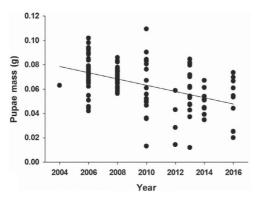


FIGURE 1 Mass (g) of *Philornis downsi* pupae collected from the nests of Darwin's finches between 2004 and 2016

finches (N = 61; mass: $F_{1,61}$ = 15.286, r = -.451, p < .001; length: $F_{1,61}$ = 4.197, r = -.256, p = .045; width: $F_{1,61}$ = 13.219, r = -.425, p = .001).

3.2 | Adult P. downsi size

We found a correlation between number of eggs and female body length ($F_{1,9}=7.085$, $r^2=.47$, p=.029, N=10) (Figure S1) and abdomen length ($F_{1,9}=5.917$, $r^2=.43$, p=.041, N=10) (Figure S2). We calculated the coefficient of determination (r^2) from 17 studies on Diptera (S2) that published the association between abdomen size and body size and the number of eggs. The overall r^2 in Diptera was .39 (95% CI 0.28–0.50), and hence, our values are in line with previous studies.

We found an effect of year and sex on *P. downsi* adult size (Year: $F_{1.184}$ = 19.435, t = -4.972, p < .001; Sex: t = -2.075,

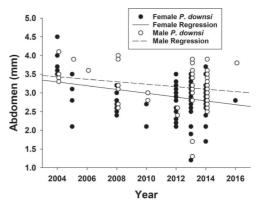


FIGURE 2 Abdomen length (mm) of male and female *Philornis downsi* adult flies collected from the nests of Darwin's finches and McPhail Traps between 2004 and 2016

p=.039) (Table 1). Next, we explored each $P.\ downsi$ body size variable. Combining adult males and females, there was a significant negative correlation between year and $P.\ downsi$ head length ($F_{1.187}=8.394, r=-.208, p=.004, N=188)$, thorax length ($F_{1.194}=12.438, r=-.246, p=.001, N=195)$, abdomen length ($F_{1.194}=13.321, r=-.254, p<.001, N=195)$ (Figure 2), wing length ($F_{1.194}=33.335, r=-.384, p<.001, N=195)$ and total body length ($F_{1.187}=18.459, r=-.650, p<.001, N=211)$. Adult flies were 7.6% smaller across the study period (8.44 \pm 0.05 to 7.80 \pm 0.17 mm), with abdomen length decreasing 7.2% (3.74 \pm 0.11 to 3.47 \pm 0.33 mm). We found similar patterns when analysing the data separately for $P.\ downsi$ adults collected from McPhail traps (sexes pooled; N=119; head: $F_{1.114}=4.356, r=-.193, p=.039$; thorax: $F_{1.118}=3.423, r=-.169, p=.067$; abdomen: $F_{1.118}=21.433, r=-.393, p<.001$; wing: $F_{1.118}=6.236, r=-.225, p=.014$; total

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body length: $F_{1.75}$ = 23.140, r = -.412, p < .001) and P. downsi adults reared from pupae collected from Darwin's finch nests (sexes pooled; N = 72; head: $F_{1.72} = 5.263$, r = -.263. p = .025; thorax: $F_{1.75}$ = 14.598, r = -.406, p < .001; abdomen: $F_{1.75}$ = 3.397, r = -.210, p = .069; wing: $F_{1.75} = 24.595$, r = -.499, p < .001; total body length: $F_{1,72}$ = 8.503, r = -.327, p = .005).

3.3 | Male versus female adult fly size across years

Due to the significant interaction of year \times sex on fly size $(F_{1.184} = 4.336, t = 2.082, p = .039)$, we explored the differences in body size between the sexes in more detail. From 2004 to 2016, adult male P. downsi wing length and head length became smaller (wing: $F_{1,72}$ = 19.147, r = -.463, p < .001, N = 72; head: $F_{1.69}$ = 4.989, r = -.261, p = .029, N = 70) but there was no effect of year on male thorax length ($F_{1,69}$ = 2.393, r = -.182, p = .126, N = 72) or abdomen length ($F_{1,71} = 3.223, r = -.210, p = .077,$ N = 72) (Figure 2). Male head length decreased 15.0% across the study period (1.53 \pm 0.10 to 1.30 \pm 0.10 mm), and wing length decreased 7.0% (8.70 \pm 0.25 to 8.09 \pm 0.07 mm). Although not significant, there was a trend for smaller body length in adult males ($F_{1.69}$ = 3.866, r = -.232, p = .53, N = 82). In adult female P. downsi, there was a negative correlation between year and wing length ($F_{1,121}$ = 20.045, r = -.378, p < .001, N = 122), thorax length $(F_{1.121} = 12.776, r = -.310, p = .001, N = 122)$, abdomen length $(F_{1,121} = 12.591, r = -.308, p = .001, N = 122)$ (Figure 2) and body length ($F_{1,117}$ = 20.058, r = -.384, p < .001, N = 129), but no effect of year on female head length ($F_{1,117}$ = 3.533, r = .172, p = .063, N = 118). Across the study period, female thorax length decreased 18.0% (3.21 ± 0.18 to 2.63 ± 0.06 mm), abdomen length decreased by 25.6% (3.83 \pm 0.14 to 2.85 \pm 0.06 mm), and wing length decreased by 12.9% (8.59 \pm 0.17 to 7.48 \pm 0.09 mm).

4 | DISCUSSION

Our findings show a change in P. downsi pupae and adult body size between 2004 and 2016 that is coincident with increasing in-nest mortality in both parasite and host (Kleindorfer & Dudaniec, 2016) in a newly evolving host-parasite system. Across the time period sampled, we found up to a 25% reduction in P. downsi pupae and adult size but a greater size reduction in females than in males. Therefore, these results support evidence that natural selection favours faster pupation and smaller body size as a consequence of earlier host mortality in both sexes, and also that natural selection for smaller body size may favour lower fecundity because only abdomen size was smaller in females. Abdomen length in female insects is a trait functionally linked with fecundity. Female abdomen length decreased across years, whereas male abdomen length did not, which underscores fecundity changes in this system. Under conditions of early host death and high risk of in-nest P. downsi mortality, natural selection favours larvae that pupate earlier and at a smaller body

size. This smaller size at pupation results in adult flies with lower fecundity, supported by a correlation between female body size and the number of eggs. We do not know whether environmental plasticity or genetic changes explain variation in pupa and adult size, but both processes can be shaped by natural or sexual selection (Blanckenhorn, 2000: Perry, Schield, & Castoe, 2018), Smaller P. downsi body size and lower fecundity may have implications for parasite competition within host nests and the evolution of host virulence in Darwin's finches.

The impact of P. downsi on native and endemic Galápagos bird species cannot be overstated: nestlings are being heavily parasitized, nestling hosts experience intense competition within nests to avoid being parasitized, and most die in the nest (O'Connor, Robertson, et al., 2010; O'Connor et al., 2014). An average of 45% of parasitized birds fledge (Kleindorfer & Dudaniec, 2016) but those that survive often have bill abnormalities due to early instar larval feeding, which has implications for song characteristics and mate choice (Kleindorfer et al., 2019; Kleindorfer & Dudaniec, 2016; Kleindorfer & Sulloway, 2016). With the prediction that lower parasite fecundity should covary with lower virulence, Kleindorfer and Dudaniec (2016) found that the number of P. downsi in finch nests increased by 46% across the decade but that patterns of host mortality on both Floreana and Santa Cruz Island remained stable at a high c. 55% per year (Kleindorfer & Dudaniec, 2016; Kleindorfer, O'Connor, et al., 2014; Kleindorfer, Peters, et al., 2014). This suggests that forms of parasite resistance could be evolving in the host, or P. downsi is evolving to be less virulent-perhaps with the benefit of securing host resources for longer. Our data support the latter suggestion, with evidence for smaller P. downsi and lower P. downsi fecundity corresponding with earlier pupa-

Given that P. downsi requires between 4 and 7 days to pupate in the field, the early death of host nestlings at c. 5 days post-hatch is likely to exert strong selection pressures on larval development (Kleindorfer, Peters, et al., 2014; Lahuatte et al., 2016). Insect larvae are generally required to reach a critical mass in order to pupate, after which they can pupate immediately or continue to grow (Nijhout & Callier, 2015). Larvae can pupate faster and at a smaller size when starved after reaching that critical mass (Nijhout & Callier, 2015). Shorter development times have been linked with decreased body sizes in Dipterans (Butlin & Day, 1984; Lehmann et al., 2006), and larvae with resource termination or fewer resources during development were smaller as adults (Singh & Bala, 2009; Williams & Richardson, 1983). In P. downsi, earlier termination of host resources has likely led to shorter developmental periods, resulting in the smaller pupa size we observed. Understanding P. downsi developmental biology is critical for developing control strategies, with recent research gaining new insights into conditions that stimulate egg hatching in the field (Sage et al., 2018) and the effect of larval diet on pupal mass and developmental duration in a laboratory setting (Lahuatte et al., 2016). In the absence of a host, first-instar P. downsi survived for up to 5 days, suggesting that larvae have the capacity to exploit and survive under conditions of unpredictable resources (Sage et al., 2018); however, body size and condition after starvation are not yet known.

Although decreasing P. downsi body size is coincident with early host termination, there may be other factors driving body size in this system. Density-dependent parasite competition for limited resources may also affect developmental rate, body size and hence fecundity, a process that is well documented in Dipteran flies (Lieske & Zwick, 2008; Peckarsky & Cowan, 1991; Shiao & Yeh, 2008), In nests of Darwin's finches, P. downsi intensity varies considerably (Kleindorfer & Dudaniec, 2016), and the genetic relatedness of larvae indicates that multiple adult female flies oviposit eggs in a single nest (mean = 3.04 ± 0.21), and multiple males (mean = 1.97 ± 0.08) sire the offspring of each female, with an average of five offspring per female (range 1-24 offspring per female) (Dudaniec et al., 2010). Relatedness among larvae in finch nests is therefore very low, whereas studies have found that decreased genetic relatedness can increase competitive interactions within species, which in turn may compromise fitness (Frank, 1994). However, such interactions and any concurrent shifts in the genetic relatedness of P. downsi are yet to be examined.

Host switching by parasitizing more than one host life stage may increase development time due to suboptimal resources. Previously, P. downsi larvae were only present in Darwin's finch nests once the host nestlings had hatched (Fessl & Tebbich, 2002; O'Connor et al., 2014). However, in recent years, there have been a growing number of observations of P. downsi larvae in nests during the incubation phase suggesting that larvae are feeding on incubating females (Cimadom et al., 2016: Common et al., 2019). Incubating female finches have been found to express P. downsi-specific antibodies (Huber et al., 2010), and females with higher antibody levels were found to have fewer parasites in their nest (Knutie et al., 2016; Koop, Owen, Knutie, Aguilar, & Clayton, 2013). Parasitizing incubating female finches may provide compromised nutrition for larvae due to the presence of P. downsi-specific antibodies, protective feathers and behavioural adaptations such as the consumption and removal of larvae from nests (O'Connor, Robertson, et al., 2010). Despite these potential costs, earlier host infestation during female incubation may be an attempt to prolong larval developmental period due to the narrowing window of nestling resources imposed by earlier host mortality.

Male and female P. downsi showed different size trajectories as adults, with evidence for strong downward selection on abdomen size in females, but not in males. Findings from multiple studies have reported substantial benefits of being a larger-bodied female (Blanckenhorn, 2000; Esperk, 2006), such as the associated increase in fecundity (Calder, 1996; Head, 1995; Honěk, 1993; Tammaru et al., 2002). Notably, the significant decrease in female abdomen length we observed suggests an impact of earlier pupation on fly fecundity. Natural selection for faster pupation can have fecundity impacts when body size and specific body regions linked with reproductive output are affected by development time (Olsson et al., 2002; Pincheira-Donoso & Hunt, 2017; Wickman & Karlsson, 1989; Winkler et al., 2012). Downward fecundity selection has been documented far less frequently than upward fecundity selection (Nunney, 1996; Preziosi & Fairbairn, 1996; Quintero-Fong et al., 2018; Reeve & Fairbairn, 1999). and most evidence for downward selection comes from laboratory studies rather than natural systems (Preziosi & Fairbairn, 1996). It is

important to note limitations with using the magnitude of female-biased sexual size dimorphism to determine the strength of fecundity selection as discussed by Pincheira-Donoso and Hunt (2017). Due to the effects of sexual selection on sexual size dimorphism (Cox & Calsbeek, 2009), research into the strength of sexual selection in *P. downsi* populations should be conducted to determine the driving factors for changing male and female body size.

Host-parasite co-evolution is rarely observed in natural systems, and biological invasions by parasites offer an opportunity to explore co-evolutionary processes (Feis, Goedknegt, Thieltges, Buschbaum, & Wegner, 2016). Understanding the effects of downward fecundity selection on female oviposition behaviour, larval competition within nests and virulence patterns in Darwin's finches will further unravel the host-parasite co-evolutionary dynamics occurring in this system. This study provides further understanding of host-parasite co-evolution during invasion and parasite trade-offs of fecundity and nutrition under strong natural selection.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Andersen, N. M. (1994). The evolution of sexual size dimorphism and mating systems in water striders (Hemiptera: Gerridae): A phylogenetic approach. Écoscience, 1, 208–214. https://doi.org/10.1080/11956 860.1994.11682244

Armbruster, P., & Hutchinson, R. A. (2002). Pupal mass and wing length as indicators of fecundity in Aedes albopictus and Aedes geniculatus

- (Diptera: Culicidae), Journal of Medical Entomology, 39, 699-704 https://doi.org/10.1603/0022-2585-39.4.699
- Blanckenhorn, W. U. (2000). The evolution of body size: What keeps organisms small? The Quarterly Review of Biology, 75, 385-407. https:// doi.org/10.1086/393620
- Braña, F. (1996). Sexual dimorphism in lacertid lizards: Male head increase vs female abdomen increase? Oikos, 75, 511-523, https://doi. org/10.2307/3545893
- Bulgarella, M., Quiroga, M. A., Boulton, R. A., Ramírez, I. E., Moon, R. D., Causton, C. E., & Heimpel, G. E. (2017). Life Cycle and Host Specificity of the Parasitoid Conura annulifera (Hymenoptera: Chalcididae), a Potential Biological Control Agent of Philornis downsi (Diptera: Muscidae) in the Galápagos Islands. Annals of the Entomological Society of America, 110, 317-328. https://doi.org/10.1093/aesa/
- Butlin, R. K., & Day, T. H. (1984). The effect of larval competition on development time and adult size in the seaweed fly, Coelopa frigida. Oecologia, 63, 122-127. https://doi.org/10.1007/BF00379793
- Calder, W. A. (1996). Size, function, and life history. Mineola, NY: Courier Corporation.
- Causton, C. E., Peck, S. B., Sinclair, B. J., Roque-Albelo, L., Hodgson, C. J., & Landry, B. (2006). Alien Insects: Threats and Implications for Conservation of Galápagos Islands. Annals of the Entomological Society of America, 99, 121–143. https://doi.org/10.1603/0013-8746 (2006)099[0121:AITAIF]2.0.CO;2
- Cimadom, A., Causton, C., Cha, D. H., Damiens, D., Fessl, B., Hood-Nowotny, R., ... Tebbich, S. (2016). Darwin's finches treat their feathers with a natural repellent. Scientific Reports, 6, 34559. https://doi. org/10.1038/srep34559
- Cimadom, A., Ulloa, A., Meidl, P., Zöttl, M., Zöttl, E., Fessl, B., ... Tebbich, S. (2014). Invasive parasites, habitat change and heavy rainfall reduce breeding success in Darwin's finches. PLoS ONE, 9, e107518. https:// doi.org/10.1371/journal.pone.0107518
- Common, L. K., Dudaniec, R. Y., Colombelli-Négrel, D., & Kleindorfer, S. (2019). Taxonomic shifts in Philornis larval behaviour and rapid changes in *Philornis downsi* Dodge & Aitken (Diptera: Muscidae): An invasive avian parasite on the Galápagos Islands. In M. Sarwar (Ed.), Life cycle and development of Diptera, London, UK: IntechOpen, https:// ://doi.org/10.5772/intechopen.88854
- Couri, M. (1985). Considerações sobre as relações ecológicas das larvas de Philornis Meinert, 1890 (Diptera, Muscidae) com aves. Revista Brasileira De Entomologia, 29, 17-20.
- Cox, R. M., & Calsbeek, R. (2009). Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. The American Naturalist, 173, 176-187. https://doi. org/10.1086/595841
- Cox, R. M., Skelly, S. L., & John-Alder, H. B. (2003). A comparative test of adaptive hypothesis for sexual size dimorphism in lizards. Evolution 57, 1653-1669. https://doi.org/10.1111/j.0014-3820.2003.tb003 71.x
- Darwin, C. (1871). The descent of man and selection in relation to sex London, UK: John Murray.
- Dudaniec, R. Y., Gardner, M. G., & Kleindorfer, S. (2010). Offspring ge netic structure reveals mating and nest infestation behaviour of an invasive parasitic fly (Philornis downsi) of Galápagos birds. Biological Invasions, 12, 581-592. https://doi.org/10.1007/s10530-009-9464-x
- Dudaniec, R. Y., & Kleindorfer, S. (2006). Effects of the parasitic flies of the genus Philornis (Diptera: Muscidae) on birds. Emu - Austral Ornithology, 106, 13-20. https://doi.org/10.1071/MU04040
- Esperk, T. (2006). Larval instar as a key element of insect growth schedules. Doctor of Philosophy (pp. 180). University of Tartu, Tartu University Press.
- Feis, M. E., Goedknegt, M. A., Thieltges, D. W., Buschbaum, C., & Wegner, K. M. (2016). Biological invasions and host-parasite coevolution: Different coevolutionary trajectories along separate parasite

- invasion fronts. Zoology, 119, 366-374. https://doi.org/10.1016/j. zool.2016.05.012
- Fessl, B., Couri, M. S., & Tebbich, S. (2001). Philornis downsi Dodge & Aitken, new to the Galapagos Islands (Diptera, Muscidae). Studia Dipterologica, 8, 317-322.
- Fessl, B., Heimpel, G. E., & Causton, C. E. (2018). Invasion of an avian nest parasite, Philornis downsi, to the Galapagos Islands: Colonization history, adaptations to novel ecosystems, and conservation challenges. In P. G. Parker (Ed.), Disease ecology: Galapagos birds and their parasites (pp. 213-266). Cham, Switzerland: Springer International Publishing.
- Fessl, B., Sinclair, B. J., & Kleindorfer, S. (2006). The life-cycle of Philornis downsi (Diptera: Muscidae) parasitizing Darwin's finches and its impacts on nestling survival. Parasitology, 133, 739-747. https://doi. org/10.1017/S0031182006001089
- Fessl, B., & Tebbich, S. (2002). Philornis downsi- a recently discovered parasite on the Galápagos archipelago - a threat for Darwin's finches? Ibis, 144, 445-451. https://doi.org/10.1046/j.1474-919X.2002.00076.x
- Frank, S. A. (1994). Kin selection and virulence in the evolution of protocells and parasites. Proceedings of the Royal Society of London. Series B: Biological Sciences, 258, 153-161, https://doi.org/10.1098/ rspb.1994.0156
- Galligan, T. H., & Kleindorfer, S. (2009). Naris and beak malformation caused by the parasitic fly, Philornis downsi (Diptera: Muscidae), in Darwin's small ground finch, Geospiza fuliginosa (Passeriformes Emberizidae). Biological Journal of the Linnean Society, 98, 577-585. https://doi.org/10.1111/i.1095-8312.2009.01309.x
- Gauld, I. D., & Fitton, M. G. (1987). Sexual dimorphism in Ichneumonidae: A response to Hurlbutt. Biological Journal of the Linnean Society, 31, 291-300. https://doi.org/10.1111/j.1095-8312.1987.tb01994.x
- Grant, P. R., & Grant, B. R. (2002). Unpredictable evolution in a 30-year study of Darwin's finches. Science, 296, 707. https://doi.org/10.1126/ science.1070315
- Hatcher, M. J., Dick, J. T. A., & Dunn, A. M. (2012). Disease emergence and invasions. Functional Ecology, 26, 1275-1287. https://doi. org/10.1111/j.1365-2435.2012.02031.x
- Head, G. (1995). Selection on the fecundity and variation in the degree of sexual size dimorphism among spider species (Class Araneae). Evolution, 49, 776-781. https://doi.org/10.1111/j.1558-5646.1995. tb02313.x
- Heimpel, G. E., Hillstrom, A., Freund, D., Knutie, S. A., & Clayton, D. H. (2017). Invasive parasites and the fate of Darwin's finches in the Galapagos Islands: The case of the Vegetarian Finch (Platyspiza crassirostris). The Wilson Journal of Ornithology, 129, 345-349. https://doi. org/10.1676/16-050.1
- Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: A general relationship. Oikos, 66, 483-492. https://doi. org/10.2307/3544943
- Huber, S. K., Owen, J. P., Koop, J. A. H., King, M. O., Grant, P. R., Grant, B. R., & Clayton, D. H. (2010). Ecoimmunity in Darwin's finches: Invasive parasites trigger acquired immunity in the medium ground finch (Geospiza fortis). PLoS ONE, 5, e8605. https://doi.org/10.1371/ journal.pone.0008605
- Hurlbutt, B. (2008). Sexual size dimorphism in parasitoid wasps Biological Journal of the Linnean Society, 30, 63-89. https://doi. org/10.1111/j.1095-8312.1987.tb00290.x
- Kleindorfer, S. (2007). The ecology of clutch size variation in Darwin's Small Ground Finch Geospiza fuliginosa: Comparison between lowland and highland habitats. Ibis, 149, 730-741. https://doi. org/10.1111/j.1474-919X.2007.00694.x
- Kleindorfer, S. M., Chapman, T. W., Winkler, H., & Sulloway, F. (2006). Adaptive divergence in contiguous populations of Darwin's Small Ground Finch (Geospiza fuliginosa). Evolutionary Ecology Research, 8, 357-372.
- Kleindorfer, S., Custance, G., Peters Katharina, J., & Sulloway Frank, J. (2019). Introduced parasite changes host phenotype, mating signal

- and hybridization risk: Philornis downsi effects on Darwin's finch song. Proceedings of the Royal Society B: Biological Sciences, 286, 20190461. https://doi.org/10.1098/rspb.2019.0461
- Kleindorfer, S., & Dudaniec, R. Y. (2016). Host-parasite ecology, behavior and genetics: A review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. *BMC Zoology*, 1, 1. https://doi.org/10.1186/s40850-016-0003-9
- Kleindorfer, S., O'Connor, J. A., Dudaniec, R. Y., Myers, S. A., Robertson, J., & Sulloway, F. J. (2014). Species collapse via hybridization in Darwin's tree finches. The American Naturalist, 183, 325–341. https://doi.org/10.1086/674899
- Kleindorfer, S., Peters, K. J., Custance, G., Dudaniec, R. Y., & O'Connor, J. A. (2014). Changes in *Philornis* infestation behavior threaten Darwin's finch survival. *Current Zoology*, 60, 542–550. https://doi. org/10.1093/czoolo/60.4.542
- Kleindorfer, S., Peters, K. J., Hohl, L., & Sulloway, F. J. (2016). Flight behaviour of an introduced parasite affects its Galapagos Island hosts: Philornis downsi and Darwin's finches. In S. Judith, & D. S. Weis (Eds.), Biological invasions and animal behaviour (pp. 158–179). Cambridge, UK: Cambridge University Press.
- Kleindorfer, S., & Sulloway, F. J. (2016). Naris deformation in Darwin's finches: Experimental and historical evidence for a post-1960s arrival of the parasite *Philornis downsi*. Global Ecology and Conservation, 7, 122–131. https://doi.org/10.1016/j.gecco.2016.05.006
- Knutie, S. A., Owen, J. P., McNew, S. M., Bartlow, A. W., Arriero, E., Herman, J. M., ... Clayton, D. H. (2016). Galápagos mockingbirds tolerate introduced parasites that affect Darwin's finches. *Ecology*, 97(4), 940–950. https://doi.org/10.1890/15-0119
- Koop, J. A. H., Owen, J. P., Knutie, S. A., Aguilar, M. A., & Clayton, D. H. (2013). Experimental demonstration of a parasite-induced immune response in wild birds: Darwin's finches and introduced nest files. *Ecology and Evolution*, 3, 2514–2523. https://doi.org/10.1002/ece3.651
- Lahuatte, P. F., Lincango, M. P., Heimpel, G. E., & Causton, C. E. (2016). Rearing larvae of the avian nest parasite, *Philornis downsi* (Diptera: Muscidae), on chicken blood-based diets. *Journal of Insect Science*, 16(1), 84. https://doi.org/10.1093/jisesa/iew064
- Lehmann, T., Dalton, R., Kim, E. H., Dahl, E., Diabate, A., Dabire, R., & Dujardin, J. P. (2006). Genetic contribution to variation in larval development time, adult size, and longevity of starved adults of Anopheles gambiae. Infection, Genetics and Evolution, 6, 410–416. https://doi.org/10.1016/j.meegid.2006.01.007
- Lieske, R., & Zwick, P. (2008). Effects of intraspecific competition on the life cycle of the stonefly, Nemurella pictetii (Plecoptera: Nemouridae). BMC Ecology, 8, 5. https://doi.org/10.1186/1472-6785-8-5
- Malmqvist, B., Adler, P. H., & Strasevicius, D. (2004). Testing hypotheses on egg number and size in black flies (Diptera: Simuliidae). *Journal of Vector Ecology*, 29, 248–256.
- Nijhout, H. F. (2003). The control of body size in insects. *Developmental Biology*, 261(1), 1–9.
- Nijhout, H. F., & Callier, V. (2015). Developmental mechanisms of body size and wing-body scaling in insects. Annual Review of Entomology, 60, 141-156. https://doi.org/10.1146/annur ev-ento-010814-020841
- Nunney, L. (1996). The response to selection for fast larval development in *Drosophila melanogaster* and its effect on adult weight: An example of a fitness trade-off. *Evolution*, 50, 1193–1204. https://doi.org/10.1111/j.1558-5646.1996.tb02360.x
- O'Connor, J. A., Robertson, J., & Kleindorfer, S. (2010). Video analysis of host-parasite interactions in nests of Darwin's finches. *Oryx*, 44, 588–594. https://doi.org/10.1017/S0030605310000086
- O'Connor, J. A., Sulloway, F. J., Robertson, J., & Kleindorfer, S. (2010).

 **Philornis downsi parasitism is the primary cause of nestling mortality in the critically endangered Darwin's medium tree finch

- (Camarhynchus pauper). Biodiversity and Conservation, 19, 853-866. https://doi.org/10.1007/s10531-009-9740-1
- O'Connor, J. A., Robertson, J., & Kleindorfer, S. (2014). Darwin's finch begging intensity does not honestly signal need in parasitised nests. *Ethology*, 120, 228–237. https://doi.org/10.1111/eth.12196
- Olsson, M., Shine, R., Wapstra, E., Ujvari, B., & Madsen, T. (2002). Sexual dimorphism in lizard body shape: The roles of sexual selection and fecundity selection. Evolution, 56, 1538–1542. https://doi.org/10.1111/j.0014-3820.2002.tb01464.x
- Orozco, F., & Bell, A. E. (1974). Reciprocal recurrent selection compared to within-strain selection for increasing rate of egg lay of *Tribolium* under optimal and stress conditions. *Genetics*, 77, 143.
- Parker, T. H., Wilkin, T. A., Barr, I. R., Sheldon, B. C., Rowe, L., & Griffith, S. C. (2011). Fecundity selection on ornamental plumage colour differs between ages and sexes and varies over small spatial scales. *Journal of Evolutionary Biology*, 24, 1584–1597. https://doi.org/10.1111/j.1420-9101.2011.02289.x
- Peckarsky, B. L., & Cowan, C. A. (1991). Consequences of larval intraspecific competition to stonefly growth and fecundity. *Oecologia*, 88, 277–288. https://doi.org/10.1007/BF00320823
- Perry, B. W., Schield, D. R., & Castoe, T. A. (2018). Evolution: Plasticity versus selection, or plasticity and selection? *Current Biology*, 28, R1104–R1106. https://doi.org/10.1016/j.cub.2018.07.050
- Peters, K. J., Myers, S. A., Dudaniec, R. Y., O'Connor, J. A., & Kleindorfer, S. (2017). Females drive asymmetrical introgression from rare to common species in Darwin's tree finches. *Journal of Evolutionary Biology*, 30, 1940–1952. https://doi.org/10.1111/jeb.13167
- Pincheira-Donoso, D., & Hunt, J. (2017). Fecundity selection theory: Concepts and evidence. Biological Reviews, 92, 341–356. https://doi. org/10.1111/brv.12232
- Preziosi, R. F., & Fairbairn, D. J. (1996). Sexual size dimorphism and selection in the wild in the waterstrider Aquarius remigis: Body size, components of body size and male mating success. Journal of Evolutionary Biology, 9, 317–336. https://doi.org/10.104 6/j.1420-9101.1996.9030317.x
- Preziosi, R. F., Fairbairn, D. J., Roff, D. A., & Brennan, J. M. (1996). Body size and fecundity in the waterstrider Aquarius remigis: A test of Darwin's fecundity advantage hypothesis. Oecologia, 108, 424–431. https://doi.org/10.1007/BF00333717
- Quintero-Fong, L., Toledo, J., Ruiz-Montoya, L., Rendón, P., Orozco-Dávila, D., Valle-Mora, J., & Liedo, P. (2018). Demography of a genetic sexing strain of Anastrepha ludens (Diptera: Tephritidae): Effects of selection based on mating performance. Agricultural and Forest Entomology, 20, 1–8. https://doi.org/10.1111/afe.12223
- Quiroga, M. A., & Reboreda, J. C. (2013). Sexual differences in life history traits of Philornis seguyi (Diptera: Muscidae) parasitizing house wrens (Troglodytes aedon). Annals of the Entomological Society of America, 106, 222–227. https://doi.org/10.1603/AN12084
- Reeve, J. P., & Fairbairn, D. J. (1999). Change in sexual size dimorphism as a correlated response to selection on fecundity. *Heredity*, 83, 697– 706. https://doi.org/10.1046/j.1365-2540.1999.00616.x
- Roff, D. A. (2001). Life history evolution. Oxford, UK: Oxford University Press.
- Sage, R., Boulton, R. A., Lahuatte, P. F., Causton, C. E., Cloutier, R., & Heimpel, G. E. (2018). Environmentally cued hatching in the bird-parasitic nest fly Philornis downsi. Entomologia Experimentalis Et Applicata, 166, 752-760. https://doi.org/10.1111/eea.12721
- Saino, N., Ambrosini, R., Caprioli, M., Liechti, F., Romano, A., Rubolini, D., & Scandolara, C. (2017). Wing morphology, winter ecology, and fecundity selection: Evidence for sex-dependence in barn swallows (Hirundo rustica). Oecologia, 184, 799–812. https://doi.org/10.1007/s00442-017-3918-0
- Scharf, I., & Meiri, S. (2013). Sexual dimorphism of heads and abdomens: Different approaches to 'being large' in female and male lizards.

- Biological Journal of the Linnean Society, 110, 665-673. https://doi. org/10.1111/bii.12147
- Shiao, S.-F., & Yeh, T.-C. (2008). Larval competition of Chrysomya megacephala and Chrysomya rufifacies (Diptera: Calliphoridae): Behavior and ecological studies of two blow fly species of forensic significance. Journal of Medical Entomology, 45, 785-799. https://doi. org/10.1093/jmedent/45.4.785
- Shine, R. (1989). Ecological causes for the evolution of sexual dimorphism: A review of the evidence. The Quarterly Review of Biology, 64, 419-461. https://doi.org/10.1086/416458
- Shingleton, A. W., Mirth, C. K., & Bates, P. W. (2008). Developmental model of static allometry in holometabolous insects. Proceedings of the Royal Society B: Biological Sciences, 275, 1875-1885, https://doi. org/10.1098/rspb.2008.0227
- Singh, D., & Bala, M. (2009). The effect of starvation on the larval behavior of two forensically important species of blow flies (Diptera: Calliphoridae). Forensic Science International, 193, 118-121. https:// doi.org/10.1016/j.forsciint.2009.09.022
- Sivinski, J. M., & Dodson, G. (1992). Sexual dimorphism in Anastrepha suspensa (Loew) and other tephritid fruit flies (Diptera: Tephritidae): Possible roles of developmental rate, fecundity, and dispersal. Journal of Insect Behavior, 5, 491–506. https://doi.org/10.1007/BF01058194
- Skidmore, P. (1985). The biology of the Muscidae of the world. Dordrecht, The Netherlands: Springer Science & Business Media.
- Stillwell, R. C., Dworkin, I., Shingleton, A. W., & Frankino, W. A. (2011). Experimental manipulation of body size to estimate morphological scaling relationships in Drosophila. JoVE, 56, e3162. https://doi. org/10.3791/3162
- Tammaru, T., Esperk, T., & Castellanos, I. (2002). No evidence for costs of being large in females of Orgyia spp. (Lepidoptera, Lymantriidae): Larger is always better. Oecologia, 133, 430-438. https://doi. org/10.1007/s00442-002-1057-7
- Teder, T., & Tammaru, T. (2005). Sexual size dimorphism within species increases with body size in insects. Oikos, 108, 321-334. https://doi. org/10.1111/j.0030-1299.2005.13609.x

- Thomas, J. K., Fadul, G. J., Keller, G. P., & Chaudhury, M. F. (2018). The use of dried bovine hemoglobin and plasma for mass rearing new world screwworm. Journal of Insect Science, 18(3), 12. https://doi. org/10.1093/jisesa/iey052
- Välimäki, P., & Kaitala, A. (2007). Life history tradeoffs in relation to the degree of polyandry and developmental pathway in Pieris napi (Lepidoptera, Pieridae). Oikos, 116, 1569-1580. https://doi. org/10.1111/j.0030-1299.2007.15733.x
- Wickman, P.-O., & Karlsson, B. (1989). Abdomen size, body size and the reproductive effort of insects. Oikos, 56, 209-214.
- Williams, H., & Richardson, A. M. M. (1983). Life history responses to larval food shortages in four species of necrophagous flies (Diptera: Calliphoridae). Australian Journal of Ecology, 8, 257-263. https://doi. org/10.1111/j.1442-9993.1983.tb01323.x
- Winkler, J. D., Stölting, K. N., & Wilson, A. B. (2012). Sex-specific responses to fecundity selection in the broad-nosed pipefish. Evolutionary Ecology, 26, 701-714. https://doi.org/10.1007/s10682-011-9516-4

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Avian vampire fly (*Philornis downsi*) mortality differs across Darwin's finch host species

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In invasive parasites, generalism is considered advantageous during the initial phase of introduction. Thereafter, fitness costs to parasites, such as host-specific mortality, can drive parasites towards specialism to avoid costly hosts. It is important to determine changes in host specificity of invasive populations to understand host-parasite dynamics and their effects on vulnerable host populations. We examined changes in mortality in the introduced avian vampire fly (*Philornis downsi*) (Diptera: Muscidae), a generalist myasis-causing ectoparasite, between 2004 and 2020 on Floreana Island (Galápagos). Mortality was measured as the proportion of immature larvae found upon host nest termination. Over the time period, the avian vampire fly was most abundant and had low mortality in nests of the critically endangered medium tree finch (*Camarhynchus pauper*) and had the highest mortality in nests of hybrid tree finches (*Camarhynchus* spp.). Low larval mortality was also found in small tree (*Camarhynchus parvulus*) and small ground finch (*Geospiza fuliginosa*) nests. Selection could favour avian vampire flies that select medium tree finch nests and/or avoid hybrid nests. Overall, the finding of differences in avian vampire fly survival across host species is parsimonious with the idea that the introduced fly may be evolving towards host specialisation.

Niche breadth is a fundamental concept that underpins key hypotheses in species ecology¹⁻³. The breadth of a niche is the set of conditions in which a species can persist, and can include dimensions such as habitat diversity and climatic variation⁴. In parasitic organisms, niche breadth is often synonymous with host specificity—i.e., the number of host species a parasite can infect, and parasites range from highly host specific to generalist⁵⁻⁷. Host specificity is mediated by host-parasite co-evolutionary processes⁸. Hosts and parasites enter an arms race in which they adapt and counter-adapt reciprocally at the expense of the other⁹. Hosts are selected to evade or resist the parasite, whereas parasites evolve to more efficiently exploit their host⁶. Higher virulence (damage to the host) and host specificity can lead to increased exploitation of the host by the parasite¹⁰. Selection for greater host exploitation may break down when parasite fitness is reduced, whereby high exploitation of hosts leads to premature host mortality, leading to decreased parasite growth and focundity or increased parasite mortality.

host exploitation may break down when parasite fitness is reduced, whereby high exploitation of hosts leads to premature host mortality, leading to decreased parasite growth and fecundity, or increased parasite mortality. Host specificity presents trade-offs for the parasite. Generalist parasites tend to occur on host species that are phylogenetically closely related. Nonetheless, they incur the cost of maintaining variation in life history, genetic and behavioural traits that enable exploitation of different host species. This relationship can be further complicated in host hybrid zones, where hybrids can be more or less resilient to parasite populations. Despite high host encounter rates due to wide host ranges, generalist parasite populations exhibit slower geographic expansion rates compared to specialist populations. The occurrence of parasite generalism or specialism is influenced by the costs and benefits inherent to occupying different host ranges, including mortality rates in parasite populations associated with particular host species. For blood feeding parasites, for example, the costs of generalist feeding can push species towards specialisation because of the variation in host blood properties and nutritional value for the parasite! High mortality risk or a lack of nutritional value in specific hosts can drive parasites to specialise on hosts that optimise their fitness? Generalist parasites may have a selective advantage when colonising novel environments given their capacity to switch hosts if a primary host population declines, which can increase their chance of persistence despite a range of establishment challenges.

which can increase their chance of persistence despite a range of establishment challenges^{21,22}.

When a generalist parasite colonises a novel environment and suite of potential host species, the differences in fitness due to altered selection creates a window of opportunity to study niche and host specialisation shifts

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under changing evolutionary pressures. While selection may initially favour a generalist strategy to maximise initial spread upon colonisation, specialisation is favoured in parasites that are capable of host choice^{23,24}. In fact, generalism is rare compared to specialism, with most species parasitising only one or a few host species^{20,25,26}. Compared with generalists, specialist parasites have been shown to evolve more quickly in response to evolving host defences and have fewer deleterious alleles present in their gene pool^{9,27}. Fitness differences and resulting mortality of parasites in certain host species can drive parasites to specialise on ideal hosts that minimise energetic and fitness costs. When a given optimal host species is abundant, predictable²⁶, accessible²⁶, and energetically efficient for the parasite²⁴, host specialisation is expected to evolve rapidly²³. Host specialisation can be favoured when fitness trade-offs are present and in the presence of multiple viable hosts that vary in fitness costs for the parasite^{23,29}.

Here, we consider the case of the avian vampire fly (*Philornis downsi*) (Diptera: Muscidae) (Dodge and Aitken 1968), a generalist myiasis-causing invasive parasitic fly of the Galápagos Islands. First observed in a Darwin's finch nest in 1997 on Santa Cruz Island, the avian vampire fly is currently known to parasitise nestlings in all 18 studied bird species on the Galápagos Islands^{30,31}. Low host defences due to immunological naivety to this type of parasite^{32,35} and close taxonomic clustering of Darwin's finches⁷ may have facilitated its rapid spread across hosts and the archipelago^{31,34}. Avian vampire fly larvae consume the blood and tissue of developing birds in the nest³⁵, causing high in-nest mortality in their hosts^{31,36}. Nestlings that survive the parasitism often have permanently deformed nares, affecting their song and foraging strategy^{37,38} (Kleindorfer et al. unpublished). Given the apparent ubiquity of the avian vampire fly across Galápagos passerine species and the increasing number of avian vampire fly larvae and pupae per host nest in studies carried out between 2000 to 2013^{36,39}, theory predicts that parasite generalism should prevail if there are negligible resource differences (i.e. nutritional value or fitness costs to parasites) between host species. However, host specialisation or host preference should occur if there are differences in fitness costs for the avian vampire fly between host species.

Nestling mortality in Darwin's finches caused by blood-sucking avian vampire fly larvae can be high (55% on average), with hosts dying younger in recent years 36,39. Yet, some Darwin's finch species appear better able than others to tolerate the impacts of avian vampire fly parasitism 36,40-42, perhaps because of differences in brood size, such as between ground (*Geospiza*) and tree (*Camarhynchus*) finches 43. Smaller broods have higher parasite loads per nestling and hence suffer higher nestling mortality 44,45. On Santa Cruz Island, nestling mortality caused by avian vampire fly larvae has shifted across the past decade in warbler finch (*Certhidea olivaceae*) and small tree finch (*Camarhynchus parvulus*) 46,47. Initially, during 2000–2005, warbler finches had on average more larvae per nest than small tree finches (41 ± 6 compared to 23 ± 3) 45,48, with the pattern reversing during 2010–2014 46. During 2012–2014, 56% of small tree finch nestlings died due to vampire fly parasitism, 71% of nests lost the whole brood before nestlings reached 7 days old, compared to 37% mortality in warbler finch nestlings 46. The differences in host mortality and intensity (total number of parasites present in the nest) between warbler finches compared with small tree finches on Santa Cruz Island might be due to changes in the oviposition behaviour by the vampire fly or the behaviour of the host, which remains to be further explored (but see 49).

Host tolerance of parasitism is also dependent on environmental conditions. For example, droughts and heavy rainfall may exacerbate the negative impact of avian vampire fly parasitism when hosts are unable to compensate with increased nestling feeding rates or experience elevated numbers of parasites in nests ^{16,50}. Periods of high rainfall, such as El Niño years, have been associated with increased numbers of avian vampire fly larvae in nests across host species ^{36,45,51}. High rainfall years have also been associated with increased hybrid recruitment in Camarhynchus tree finches on Floreana Island ⁵². Current hybridisation patterns occur as female medium tree finches (*C. pauper*) pair with male small tree finches (*C. parvulus*), resulting in sex-specific gene flow and the existence of a hybrid swarm ⁵³. Hybrid nests have significantly fewer avian vampire fly larvae with up to 60% fewer parasites per nest than their parental species ⁴². Parents of nests that had the greatest genetic admixture (therefore, greater hybrid assignment probability) had the fewest avian vampire fly larvae. However, the mechanisms driving these intensity differences are not yet known ⁴².

Here, we explore changes in avian vampire fly larval mortality across time and host species. We suggest that if there are changes in avian vampire fly larval mortality in different host species, this could indicate selection on the avian vampire fly to diverge and specialise, or to avoid particular hosts. Our long-term data offer a unique opportunity to describe early co-evolutionary processes in a generalist parasite across a suite of hosts in its invasive range, which has implications for the evolution of host specificity. We analyse data spanning non-consecutive 17-years of avian vampire fly specimens collected from Floreana Island, Galápagos, to examine changes in in-nest mortality and survival when parasitising three host species and a hybrid cluster: small ground finch (Geospiza fuliginosa), small tree finch (C. parvulus), medium tree finch (C. pauper), and the hybrid tree finch (C. pauper × C. parvulus, including hybrids that have backcrossed to one of the parent species⁵³). In a comparison of samples collected from host nests between 2004 and 2020, we predict that (1) avian vampire fly intensity (i.e. total number per nest) has increased over time regardless of the host species based on previous research⁵⁹; (2) avian vampire fly larval mortality and nestling age at death (the age at which the last nestling dies), as early host death (i.e. early termination of resources)⁵⁹ results in younger parasites upon nest termination; (3) avian vampire fly larval mortality will increase with increasing annual rainfall, as heavy rainfall decreases host survival^{46,50} and, (4) avian vampire fly larval mortality differs between host species due to (a) differences in brood size between small ground finch and the tree finch species⁴⁵ and (b) differences in parasite-induced nestling mortality between these host species⁴².

Methods

Study system. This study was conducted on Floreana Island, Galápagos Archipelago, and followed long-term field protocols as described below³⁹. The field work was conducted during the Darwin's finch breeding season in the highlands (01° 17′ S, 090° 27′ W) between the months of January and April in ten non-consecutive seasons spanning 17 years: 2004, 2005, 2006, 2008, 2010, 2012, 2013, 2014, 2016 and 2020. We collected avian vampire fly specimens from the nests of the small ground finch, small tree finch, medium tree finch, and the hybrid *Camarhynchus* tree finch^{53–55}. Host species were first determined morphologically, and hybrid tree finches were retrospectively confirmed via genetic analyses^{53,54}. Due to this approach, data for hybrid *Camarhynchus* finches are only available for the years 2006, 2010, 2012, 2013 and 2014. Floreana rainfall data (sum of annual rainfall; mm) were collected via satellite sourced from CPC Global Unified Precipitation Data provided by NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, downloaded from the Galápagos Vital Signs website by the Galápagos Conservancy⁵⁶.

Study species. The avian vampire fly is an obligate myiasis-causing parasite of birds that feeds on the blood and tissue of developing nestlings⁴⁴. Non-parasitic adult flies feed upon decaying vegetable matter, ovipositing their eggs in active bird nests^{57–59}. Upon hatching, first and early second instar larvae move to the naris and ear canals of nestlings to feed on blood and keratin³⁵. Late second and third instar larvae feed externally on nestlings at night, residing in the base of the nest during the day^{35,60,61}. Reports of development times of larval instars vary between field and lab reared specimens, with pupation occurring after 4–10 days of feeding^{39,62}. Upon host fledging or death, third instar larvae pupate in the base of the nest, forming frothy cocoons and emerging as adults within 7–18 daya^{39,62}.

Nest monitoring and vampire fly collection. We analysed data from 280 Darwin's finch nests on Floreana Island with all avian vampire fly specimens per nest collected and stored in ethanol following well-established field protocols³⁹. Small tree finch (n = 64), hybrid tree finch (n = 34), medium tree finch (n = 55) and small ground finch (n = 127) nests were monitored for activity and brood size every 3 days during incubation and every 2 days during the nestling phase. Males of these host species build new display nests at which they sing for each new nesting event⁶³. The female either selects a display nest or selects a male and they build a new display nest together⁶³. Incubation lasts ~14 days and, if successful, nestlings fledge the nest approximately 12–14 days after hatching. Brood size was determined using a borescope to view inside the nest once nestlings had hatched. After nesting activity had finished (i.e., nest termination, either through death of the nestling or fledging), the nest was collected and dismantled within 24 h to count the number of avian vampire fly offspring within the nest. Nestling age at death was known for a subset of nests (n = 105) from hatching date or visual aging of the nestlings via borescope. In all sampling years (except for four nests in 2004 and 2005), nestlings found dead in the nest were soaked in 70% ethanol for 24 h to allow first instar larvae within the nestling nares or ear canals to float and be collected. We generally only collect ~ 8 1st and 2nd instar using this method from ~ 8% of nestlings soaked. The 1st instar larvae reside inside the nares for the first two days post-hatch and nestlings tended to survive until d7 post-hatch during 2004 and 2005³⁹. All avian vampire fly larvae, pupae, puparia and adult flies were stored in 70% ethanol within 24 h of collection.

Larval specimens of 241 nests were assigned an age class via observation using a dissecting microscope, following instar identification protocols^{35,49}. Parasite intensity was calculated as the total number of larvae, pupae, puparia and adult flies within a nest. Mortality in the avian vampire fly larvae was measured as the proportion of immature (first and second instar) larvae in the nest at the time of host resource termination⁶⁴. This measure accounts for the possibility of third instars and pupae fully developing into adult flies following host termination⁶⁵. This measure also provides an estimate of parasite mortality per host nest, given that first and second larval instars are unable to continue development in the absence of nutrition^{65,66}.

Statistical analysis. All models were fitted using R version $4.0.3^{67}$ with the packages lme 4^{68} , MASS⁶⁹, and car⁷⁰, and were visualised with lattice⁷¹, ggplot2⁷² and effects⁷³. Total number of avian vampire fly offspring per nest (log transformed to fulfil the assumption of normality) was analysed in relation to study year, annual rainfall and the Darwin's finch species and the interaction between year and rainfall as fixed effects with a linear regression model on the full data set (n = 280).

For the corresponding analyses considering different age classes of the parasite, we had a smaller dataset of n=241. We repeated the analysis of total intensity in relation to year and rainfall on this subset of data to confirm the same pattern across both data sets. We analysed the total number of first and second instar larvae, third instar larvae, total larvae, pupae and puparia in relation to year and annual rainfall similar to the total number of vampire flies, but as count data with Generalized Linear Models (GLMs) with negative binomial distribution and log link function to correct for overdispersion. Throughout we tested for linear and quadratic relationships of year and rainfall and their additive and interactive effects on the total avian vampire fly intensity, first and second instar larvae, third instar larvae, total larvae, pupae and puparia. Based on the principles of parsimony (the largest amount of variance explained with the minimum number of predictors²⁴), we then selected the model structure that best described our measures of parasite loads at different developmental stages.

Avian vampire fly larval mortality was modelled using the column bind ('cbind') function specifically designed to fit proportion data in logistic regression models with the number of larvae in the first and second larval instar as binomial denominator, and a quasibinomial distribution and a logit link function to correct for overdispersion. We fitted the key response variable avian vampire fly larval mortality to two different data sets: (1) considering all Darwin's finch nests on Floreana Island for which we identified larvae to age class (n = 241); and (2) considering nests where nestling age at death was known (n = 106), with nestling age at death as an additional co-variate.

Philornis downsi intensity (n = 280)	Estimate	SE	t value	Sum Sq	df	P-value	Sign
Intercept	1.401	0.045	30.857			< 0.001	***
Year	0.012	0.022	0.549	0.040	1	0.583	
Hybrid ^a	- 0.086	0.078	- 1.115				
Medium tree finch	0.254	0.067	3.796	3.038	3	< 0.001	***
Small ground finch	0.041	0.056	0.728				

Table 1. Linear model for avian vampire fly (*Philornis downsi*) intensity in relation to year and host species collected between 2004 and 2020 from Darwin's finch nests on Floreana. 'Rain' did not feature into the most parsimonious model. Avian vampire flies collected from Darwin's finch nests over 10 years across a 17-year period on Floreana Island. "Species 'small tree finch' was used as a reference category. Note the response variable *Philornis downsi* intensity was log transformed to achieve normality and all quantitative input variables were scaled and centred. Intercept presented in italics. Sign = significance levels: '*** < 0.001.

This second analysis accounts for changes in parasite intensities within nests according to nestling age at death 44 ; and highlights interspecific host differences in vampire fly larval mortality even when accounting for host age at death 39 . We fitted the study year, annual rainfall and the Darwin's finch species and the interactions (year × rainfall) as fixed effects. Initially, we also controlled for brood size, but this additional predictor did not reveal any significant result and was dropped from the final model to improve sample size (from n = 191 to n = 241 in the data set without brood size). We removed non-significant interaction terms from the models to simplify the statistical approach and interpretation of the results and to ensure a valid interpretation of the remaining additive effects. The effect of host genus (*Geospiza* and *Camarhynchus*), excluding hybrid tree finches to remove the effect of hybridisation (n = 213), was analysed using the full model of mortality in relation to year and annual rainfall and their interaction as fixed effects.

All quantitative variables were scaled (standardized to mean = 0 and standard deviation = 1) to bring the variables to comparable dimensions and to facilitate the correct interpretation of effect sizes for interaction terms⁷⁵. Residual distributions of the models were inspected visually to assess model fit (diagnostic plots produced by the 'plot' function in the 'base' package: residuals versus fitted values and normal Q-Q plot displaying the theoretical quantiles versus standard Pearson residuals). Throughout, we report model effect sizes (estimates \pm SE, derived from the summary function); presented $\chi 2$ and p-values are based on an ANOVA Table of Deviance using Type III Wald $\chi 2$ tests (ANOVA function in 'car' package). No random factors were considered, as there were no repeated measurements in the data. We tested for correlations of fixed effects beforehand but did not find any indication for co-linearity in our data.

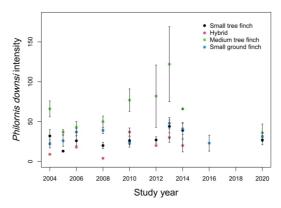
Permits. Permission to conduct this study was given by the Galápagos National Park and Charles Darwin Research Center, permit no. MAE-DNB-CM-2016-0043, and Flinders University, permit no. E480/19.

Results

Avian vampire fly intensity. We found no effect of year on avian vampire fly intensity (i.e., total number of parasites per nest) across the entire study period (LM, $F_{1,275} = 0.040$, estimate 0.012 ± 0.02 , p = 0.583, Table 1, Fig. 1). There was an effect of species: medium tree finches had the highest intensity of avian vampire flies per nest (57.1 \pm 5.4 vampire flies per nest compared to *C. parvulus*: 29.9 ± 2.3 ; *Camarhynchus* hybrids: 26.6 ± 3.3 ; *G. fuliginosa*: 36.2 ± 2.0) (LM, $F_{3,275} = 3.038$, estimate 0.254 ± 0.07 , p < 0.001, Table 1; raw data in supplementary Table (2)

Age class distribution and abundance. Analysis of avian vampire fly age classes revealed a significant quadratic relationship with year and the number of first and second instar larvae (GLM, 'year' term estimate: -0.51 ± 0.14 , p=0.002, Table 2b, Figure S1), with numbers of first and second instar larvae peaking in approximately 2013. The number of first and second instar larvae was consistently higher in hybrid tree finches (Table S1). Furthermore, across all species, third instar larvae and the total number of larvae per nest increased until 2013 and decreased thereafter (GLM, third instar: 'year' term estimate: -0.32 ± 0.10 , p=0.010; total larvae: 'year' term estimate: -0.38 ± 0.10 , p=0.01, Table 2c,d, Figure S1). Conversely, the number of pupae and puparia per nest showed the opposite pattern, decreasing until 2013 and 2014 onwards (GLM, 'year' term estimate: 0.46 ± 0.10 , p<0.001; 'year' term estimate: 0.78 ± 0.24 , p=0.002, respectively, Table 2e,f, Figure S1). There was no significant effect of rainfall on the total intensity in these nests (LM, 'rainfall' term estimate: -0.04 ± 0.03 , p=0.110, Table 2a, Figure S1) or on any other age class considered (i.e., rainfall did not feature in any other parsimonious model, neither in the linear nor quadratic relationship).

Larval mortality. For all samples combined, there was an increase in the proportion of avian vampire fly larval mortality over time ('year' term estimate: 0.48 ± 0.19 , p=0.012, Table 3a) and during years with higher annual rainfall ('rainfall' term estimate: 0.40 ± 0.13 , p=0.002, Table 3a). However, these additive effects should be interpreted with caution due to their involvement in a significant interaction term (estimate 0.58 ± 0.18 , p=0.002; Fig. 2a). Earlier in the study period (2004–2008), when the years were drier (e.g., ~360 mm), vampire fly mortality was lower; while later in the study period (2010–2020), when the years were wetter (e.g., 400–650 mm), avian



 $\textbf{Figure 1.} \ \ \text{Number (mean \pm SE) of a vian vampire flies (\textit{Philornis downsi}) per nest of Darwin's finch species per year on Floreana Island. Each Darwin's finch species is denoted by a different colour.$

	Estimate	SE	t-value	Sum Sq	Df	P-value	Sign
(a) Philornis down	si intensity	(n = 241	; log trans	formed)			
(Intercept)	1.42	0.03	54.73			< 0.001	***
Rain	- 0.04	0.03	- 1.60	0.42	1	0.110	
	Estimate	SE	z-value	LR χ ²	Df	P-value	
(b) Philornis down	si first and	second	larval insta	ar (n=241)			
(Intercept)	1.72	0.14	12.43				
Year (linear)	- 0.06	0.14	- 0.45	12.69	2	0.002	**
Year (quadratic)	- 0.51	0.14	- 3.60				
(c) Philornis down	si third larv	al insta	r (n=241)				
(Intercept)	2.47	0.10	23.76				
Year (linear)	0.01	0.10	0.12	9.13	2	0.010	*
Year (quadratic)	- 0.32	0.10	- 3.10				
(d) Philornis down	ısi total larv	ae (n=2	241)				
(Intercept)	2.86	0.10	28.49				
Year (linear)	0.00	0.10	- 0.03	13.90	2	0.001	***
Year (quadratic)	- 0.38	0.10	- 3.79				
(e) Philornis down	si pupae (n	= 241)					
(Intercept)	2.45	0.10	24.98				
Year (linear)	0.01	0.10	0.07	20.22	2	< 0.001	***
Year (quadratic)	0.46	0.10	4.71				
(f) Philornis down	si puparia (n = 241)				,	
(Intercept)	0.80	0.24	3.39				
Year (linear)	- 0.52	0.24	- 2.22	12.40	2	0.002	**
Year (quadratic)	0.78	0.24	3.31	-			

Table 2. Linear Models exploring the effects of rain and year on avian vampire fly (*Philornis downsi*) intensity and age class. (a) response variable P.downsi infection intensity, log transformed to achieve normality; and Generalized Linear Models (negative binomial distribution) of (b) first and second larval instar; (c) third larval instar; (d) total number of larvae; (e) total number of pupae; and (f) total number of puparia for the different life stages of P.downsi in relation to year and rainfall (fitted in a linear or quadratic relationship). We show the most parsimonious model after considering linear and quadratic relationships of year and rainfall and their interaction. Analysis of Deviance Table (Type III tests). Avian vampire flies collected from Darwin's finch nests over 10 years across a 17-year period (2004–2020) on Floreana Island. Note all quantitative input variables were scaled and centred. A full intercept is only displayed for Linear Models and cannot be derived with the ANOVA function for Generalized linear models. Intercept presented in italics. Sign = significance levels: "*** < 0.001; "** < 0.05; " < 0.1.

(a) Philornis downsi in-nest mortality (n = 241)	Estimate	SE	t-value	$LR \chi^2$	df	P-value	Sign
Intercept	- 1.659	0.245	- 6.758			< 0.001	***
Year	0.477	0.188	2.533	6.660	1	0.010	*
Rain	0.402	0.128	3.128	9.997	1	0.002	**
Hybrida	1.436	0.366	3.924				
Medium tree finch	0.084	0.316	0.267	23.483	3	< 0.001	***
Small ground finch	- 0.093	0.304	- 0.305				
Year × Rainfall	0.577	0.182	3.170	10.496	1	0.001	**
(b) Ismeans between species	Probability	SE	LCL	UCL			
Small tree finch	0.16	0.03	0.11	0.24			
Hybrid	0.45	0.07	0.32	0.58			
Medium tree finch	0.17	0.03	0.12	0.23			
Small ground finch	0.15	0.02	0.11	0.20			
(c) Post-hoc contrast	Odds ratio	SE	z-ratio	P-value	Sign		
Small tree finch/Hybrid	0.24	0.09	- 3.92	0.001	**		
Small tree finch/Medium tree finch	0.92	0.29	- 0.27	0.993			
Small tree finch/Small ground finch	1.10	0.33	0.31	0.990			
Hybrid/Medium tree finch	3.87	1.27	4.12	0.000	***		
Hybrid/Small ground finch	4.62	1.54	4.59	<.0001	***		
Medium tree finch/Small ground finch	1.19	0.33	0.65	0.915			

Table 3. Generalized linear model for (a) avian vampire fly (*Philornis downsi*) in-nest mortality in relation to year and rainfall (interaction term) and species; (b) Ismeans (least squares means; extracted with the 'emmeans' package) and (c) post-hoc contrasts for vampire fly in-nest mortality between Darwin's finch species. Avian vampire flies collected from Darwin's finch nests in 10 sampling years across a 17-year period (2004–2020) on Floreana Island. "Species 'small tree finch' was used as a reference category. × indicates an interaction term Dispersion Parameter for quasibinomial family taken to be 12.381. Intercept presented in italics. Sign = significance levels: '***' < 0.001; '**' < 0.01; '**' < 0.05; Ismeans intervals are back-transformed from the logit scale and post-hoc contracts were performed on the log-odds ratio scale following the tukey method.

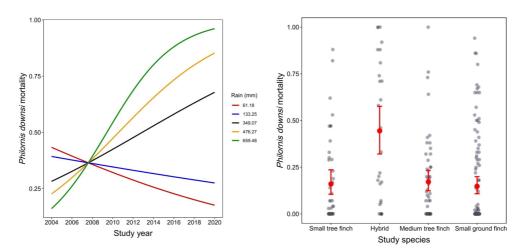


Figure 2. The relationship between avian vampire fly (*Philornis downsi*) in-nest larval mortality and (a) the interaction between study year and annual rainfall (sum in mm); and (b) the different Darwin's finch species. Note the interaction is plotted for min (rainfall=61.18 mm, red line), 1st quantile (rainfall=132.25 mm), median (rainfall=349.07 mm), 3rd quantile (rainfall=476.27 mm) and max (rainfall=659.48 mm, black dashed line) values; while the additive effect is plotted as effect sizes plus 95% CIs. Model details provided in Table 3.

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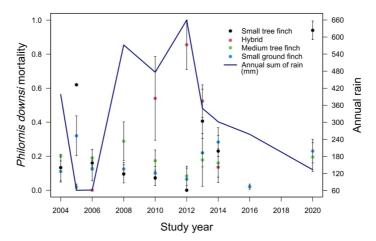


Figure 3. The relationship between avian vampire fly (*Philornis downsi*) in-nest mortality and year across the Darwin's finch host species, with cumulative annual rainfall on Floreana Island. Dots represent the proportion of vampire fly larvae that died upon termination of the host and are labelled according to the four different Darwin's finch species.

Philornis downsi in-nest mortality (n = 213)	Estimate	SE	t-value	$LR \chi^2$	df	P-value	Sign
Intercept	- 1.605	0.151	- 10.614			< 0.001	***
Year	0.462	0.192	2.405	6.007	1	0.014	*
Rain	0.377	0.130	2.894	8.467	1	0.004	**
Ground finch (Geospiza fuliginosa)	- 0.150	0.240	- 0.625	0.392	1	0.531	
Year × Rain	0.562	0.184	3.059	9.763	1	0.002	**

Table 4. Generalized linear model for avian vampire fly (*Philornis downsi*) in-nest mortality in relation to year and rainfall (interaction term); and genus (*Camarhynchus* sp. n = 108; *Geospiza* sp. n = 115), excluding *Camarhynchus* hybrids. Avian vampire flies collected from Darwin's finch nests in 10 sampling years over a 17-year period (2004–2020) on Floreana Island. ^aGenus *Camarhynchus* sp. 'tree finch' was used as a reference category. × indicates an interaction term. Note all quantitative input variables were scaled and centred. Dispersion Parameter for quasibinomial family taken to be 12.421. Intercept presented in italics. Sign = significance levels: '***' < 0.001; '**' < 0.01; '**' < 0.05.

vampire fly mortality was higher (Fig. 1a, Fig. 3). Larval mortality did not differ between the small tree finch, medium tree finch or small ground finch, but was significantly higher in hybrid nests (least square means and post-hoc contrasts Table 3b,c, Fig. 2b). Larval mortality did not differ between *Camarhynchus* and *Geospiza* host nests when excluding hybrid tree finches (estimate 'genus' term – 0.15 ± 0.24, p. = 0.532; Table 4).

when excluding hybrid tree finches (estimate 'genus' term -0.15 ± 0.24 , p=0.532; Table 4). When analysing nests where the nestling age at death was known, we found a strong effect of nestling age at death on larval mortality. Larval mortality increased as nestling age at death decreased (estimate -0.34 ± 0.15 , p=0.025, Table 5, Figure S2c). The interaction effect of year and rainfall on mortality was marginally non-significant (estimate 0.38 ± 0.20 , p=0.056, Table 5, Figure S2a). Avian vampire fly mortality was significantly higher in hybrid hosts (estimate 1.36 ± 0.41 , t=3.30, p<0.001, Table 5, Figure S2b). Larval mortality did not differ between the small ground, small tree, or medium tree finch (Figure S2b).

Discussion

In this study, we tested patterns of larval mortality in avian vampire fly, a generalist myiasis-causing parasite of Darwin's finches, across time and host species. We did not find a significant increase in parasite mortality across time, but there were clear differences in parasite mortality across host species. Parasite mortality was lowest in nests of the medium tree finch, and highest in hybrid finch nests, even when accounting for chick age at death³⁹. If host-specific selection pressures on larval mortality continue or increase, the avian vampire fly may be selected to ovinosit in optimal host nests, which may result in host specialisation.

to oviposit in optimal host nests, which may result in host specialisation.

Our results provide some support for the idea that *Camarhynchus* hybridisation may be an adaptive host response to thwart a novel parasite, in line with previous findings⁴². The Red Queen hypothesis is a powerful

Philornis downsi in-nest mortality	Estimate	SE	t-value	LR χ²	df	P-value	Sign
Intercept	- 1.128	0.283	- 3.985			< 0.001	***
Year	0.116	0.228	0.508	0.390	1	0.609	
Rain	0.194	0.168	1.152	0.915	1	0.248	
Hybrid ^a	1.361	0.413	3.296				
Medium tree finch	- 0.189	0.383	- 0.494	21.040	3	< 0.001	***
Small ground finch	- 0.063	0.369	- 0.170				
Nestling age at death	- 0.344	0.152	- 2.270	5.500	1	0.018	*
Year × Rainfall	0.378	0.201	1.877	4.112	1	0.056	

Table 5. Generalized linear model for avian vampire fly (Philornis downsi) in-nest mortality in relation to year and rainfall (interaction term) and species, including the co-variate 'nestling age at death, ranging from 1 to 14 days). Including this co-variate reduces our data set to n = 106. Avian vampire flies collected from Darwin's 14 days). Including this co-variate reduces our data set to n = 100. Avian vampure has concern from Dalling finch nests in 10 sampling years over a 17-year period (2000–2020) on Floreana Island. Significant estimates indicated in bold. "Species 'small tree finch' was used as a reference category.x indicates an interaction term. Note all quantitative input variables were scaled and centred. Dispersion Parameter for quasibinomial family taken to be 12.273. Intercept presented in italics. Sign = significance levels: "*** < 0.001; "** < 0.05. taken to be 12.273. Intercept presented in italics. Sign = significance levels: '*** < 0.001;

theoretical framework to predict host-parasite coevolutionary dynamics, and one expects that host-impacting change caused by the parasite is countered by the host, and vice versa9. The newly evolving Darwin's finch and avian vampire fly system is consistent with the idea of oscillating evolutionary dynamics in the wild but requires additional research into genetic and behavioural mechanisms to more fully understand these patterns. Previous research has shown that: (1) during the first part of the decade from 2004 to 201339, the average number of avian vampire flies per host nest increased and then stabilised; (2) one host species, the medium tree finch, consistently has the most avian vampire flies in the nest compared with other host species⁴⁰; (3) the proportion of hybrid birds increased from 12% in 1998 to between 27 and 55% in later years, and hybrid hosts have the fewest avian vampire flies compared with other host species⁵²; and here we show that (4) avian vampire fly mortality was highest in hybrid finch nests and lowest in the nests of the other host species (small ground, small tree and medium tree finches), even when accounting for nestling age at death. From the perspective of the parasite, it should avoid hybrid finch nests. The mechanisms that may drive host-seeking versus host-avoidance behaviours by the parasite are unknown. However, this study uncovers two concurrent scenarios whereby both parasite intensity and parasite mortality across hosts differed, especially between medium tree finch and hybrid tree finch nests during the early co-evolutionary stages of a host-parasite interaction.

In parasites that use multiple host species for different life stages, host generalism is the optimal strategy⁷⁶. The avian vampire fly lives its parasitic life stages in a single host environment, and in this case, specialist offspring are predicted to be optimal to maximise arithmetic mean fitness76. The observation that different Darwin's finch host species have different average numbers of avian vampire flies per nest, even immediately after host hatching, is in line with the idea of differentiated oviposition in certain hosts \$^{42,46}\$. Despite specialisation, some specialist lineages hedge their bets by ovipositing in suboptimal hosts 76,77 . In the case of the avian vampire fly, genetic evidence has shown oviposition by multiple females in one host nest; also, females frequently lay fewer eggs than they are able to oviposit at a time, which the supports the idea of bet hedging by ovipositing in multiple nests⁷⁸. However, it is currently unknown if females oviposit preferentially in specific host nests or whether there could be host-specific lineages of avian vampire fly.

Given that high rainfall is associated with more avian vampire flies in host nests (0.45.51), we would expect to see an increase in competition between larvae during high rainfall years as more parasites compete for the same amount of resources^{79,80}. Increased competition may lead to increased parasite mortality. However, in this study, we found lowest parasite mortality in nests of the host species with the most parasites, the critically endangered medium tree finch. The extreme fluctuations in rainfall within parasite lifetimes and across generations on the Galápagos Islands may favour environmental generalists that maintain optimal fitness levels with rainfall fluctuation. Selection pressures from introduced pathogens can lead to swamping of local environmental adaptation in favour of immune response loci. Therefore, there may be a trade-off between achieving optimal parasite fitness across multiple host species and the parasite's capacity to tolerate environmental variation. In the avian vampire fly, such relationships are yet to be explored.

Host specialisation may ease the burden of parasitism in some host species yet may heighten the threat for other neighbouring species, particularly in host-limited, geographically restricted habitats, as occurring on Floreana Island⁸². Smaller, endangered populations, such as the medium tree finch, are more likely to have low genetic diversity with a reduced capacity to evolve in response to parasites⁸³. The threat posed by the parasite is further exacerbated by the high intensity of avian vampire fly larvae found in medium tree finch nests. In comparison, the hybrid tree finch that is the result of recombination between the small and the medium tree finch may have increased genetic variation, which may offer novel genes on which selection can act to evolve resistance to parasitism^{84,85}. Given the observation that female medium tree finch frequently pair with male small or hybrid tree finches rather than medium tree finch, and the potential for increased hybrid resilience^{42,53}, the medium tree finch population may continue to decline, eventually resulting in only a hybrid swarm¹⁷. Hybrid recruitment, as measured by the proportion of yearling birds in the population, has remained stable across years since 2005,

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whereas medium tree finch recruitment rates declined across the same period, suggesting hybrid nestlings and/ or fledglings may have a selective advantage over medium tree finch offspring⁵². Understanding host-specific parasite fitness in this system highlights the need for directed conservation efforts to more exploited hosts or those less likely to evolve parasite resistance mechanisms. Our results suggest that such mechanisms may be evolving in the *Camarhynchus* hybrid group, but at a cost to the medium tree finch population.

evolving in the *Camarhynchus* hybrid group, but at a cost to the medium tree finch population.

The effects of host hybridisation on both host and parasite fitness have mainly been documented in plant-parasite systems, as hybridisation is common in plant species⁸⁶. These effects vary between systems. Host hybridisation can, for example, increase susceptibility to parasites, resulting in increased numbers of parasites and decreased hybrid fitness^{17,867,88}. In other cases, host hybridisation increases host resistance and tolerance, decreasing parasite loads and increasing host fitness^{17,869}. We see this latter pattern in the Darwin's finch system, where hybrid tree finches tend to have fewer parasites per nest than their parent species¹². In this study, using a sub-sample of nests for which we have accurate data on parasite age class, we also found a pattern of fewer parasites per nest in hybrid cast, though the different linear than the parasite age class, we also found a pattern of fewer parasites per nest in hybrid nests, though the difference in number of parasites across host species was not statistically significant. There is not much available data on parasite fitness in hybrid versus non-hybrid hosts, which is a research gap that requires attention. In a study on fungal pathogens infecting hybrid plant hosts, pathogens had a fitness advantage in hybrid hosts that was contingent on pathogen hybridisation. In addition, the role of host hybrid fitness is expected to affect parasite fitness. If host resistance and tolerance to parasites increases as the consequence of hybridisation, then parasite fitness could be higher in hybrid hosts able to sustain the parasite, or conversely, parasite fitness could be lower in hybrid hosts that deter parasites from ovipositing. More research is needed to explore different host-parasite evolutionary pathways under conditions of genetic introgression in host and/or parasite.

We don't know why avian vampire fly larval mortality differed across hosts species in this study, but it is known that blood properties of host species can vary in nutritional gain for the parasite^{19,28}. Mortality in second and third instar avian vampire fly larvae reared on chicken blood did not differ between formulated diets of varying nutrition, however development time to pupation was fastest on the diet with the highest nutritional value⁶². Decreased developmental time is advantageous when resources can be terminated quickly, such as when Darwin's finch nestlings die young³⁹, allowing more larvae to reach pupation faster and hence survive to adulthood in nutritionally optimal hosts. High mortality was found in first instar larvae reared on artificial diets and the possible contamination of the blood with pathogenic bacteria such as Serratia may be driving this high mortality⁶². Serratia, a genus with pathogenic species that affects myiasis-causing and muscid flies, was found to be uniquely associated with avian vampire flies parasitising warbler finches in a microbiome analysis of the fly⁹². Warbler finches in recent years had fewer avian vampire flies and lower host mortality compared to tree finches ⁴⁶. Research has further shown that the avian vampire fly microbiome differs significantly across Darwin's finch host species, which is suspected to be associated with differences in finch diets within and across habitats^{55,92-95}. Overall, the findings of this and previous research suggest that larval mortality may be driven by multiple factors, including host nutritional quality, habitat, and microbiome.

We found high parasite mortality in hybrid avian hosts, which we document in a generalist and recently introduced parasite to the Galapagos archipelago. The parasite did best in nests of the Floreana Island endemić, the medium tree finch. Theory predicts that the vampire fly should be selected to oviposit preferentially in medium tree finch nests, given that it has the highest pupation success in medium tree finch nests, and avoid hybrid finch nests where most of its offspring fail to pupate. Understanding the mechanisms by which the avian vampire fly avoids or selects host nests, invests in generalist or specialist offspring, or alters its strategy to survive in prevailing environmental conditions are at the forefront of research into this rapidly evolving host-parasite interaction system on the Galápagos Islands. Our study provides evidence for differential fitness of an invasive parasite in nests of different host species.

Data availability

All data analysed within this paper are available through the Dryad Digital Repository: https://doi.org/10.5061/dryad.9ghx3ffhw.

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References

- Hutchinson, G. E. Cold Spring Harbor Symposia on Quantitative Biology. Concluding Remarks 22 415–427 (1957).
 Smith, E. P. Niche breadth, resource availability, and inference. Ecology 63, 1675–1681. https://doi.org/10.2307/1940109 (1982).
- Leibold, M. A. The niche concept revisited: Mechanistic models and community context. *Ecology* 76, 1371–1382. https://doi.org/10.2307/1938141 (1995).
 Sexton, J. P., Montiel, J., Shay, J. E., Stephens, M. R. & Slatyer, R. A. Evolution of ecological niche breadth. *Annu. Rev. Ecol. Evol.*
- Syst. 48, 183-206, https://doi.org/10.1146/annurev-ecolsys-110316-023003 (2017)
- 3931-30, 163-200. https://doi.org/10.1140/annurev-ecoisys-110310-023003 (2017).
 Jaenike, J. Hots specialization in phytophagous insects. *Annu. Rev. Ecol. Syst.* 21, 243-273. https://doi.org/10.1146/annurev.es.21. 110190.001331 (1990).
- 6. Thompson, J. N. *The Coevolutionary Process* (University of Chicago Press, 1994).
- Krasnov, B. R., Mouillot, D., Shenbrot, G. I., Khokhlova, I. S. & Poulin, R. Geographical variation in host specificity of fleas (Siphonaptera) parasitic on small mammals: The influence of phylogeny and local environmental conditions. *Ecography* 27, 787–797. https://doi.org/10.1111/j.0906-7590.2004.04015.x (2004).
- Poullain, V., Gandon, S., Brockhurst, M. A., Buckling, A. & Hochberg, M. E. The evolution of specificity in evolving and coevolving antagonistic interactions between bacteria and its phage. Evolution 62, 1–11. https://doi.org/10.1111/j.1558-5646.2007.00260.x
- Whitlock, M. C. The Red Queen beats the Jack-Of-All-Trades: The limitations on the evolution of phenotypic plasticity and niche breadth. Am. Nat. 148, S65–S77. https://doi.org/10.1086/285902 (1996).

- 10. Gandon, S. Local adaptation and the geometry of host-parasite coevolution. Ecol. Lett. 5, 246-256. https://doi.org/10.1046/j. 1461-0248,2002,00305,x (2002),
- Alizon, S. & Michalakis, Y. Adaptive virulence evolution: The good old fitness-based approach. *Trends Ecol. Evol.* 30, 248–254. https://doi.org/10.1016/j.tree.2015.02.009 (2015).
 Frank, S. A. & Schmid-Hempel, P. Mechanisms of pathogenesis and the evolution of parasite virulence. *J. Evol. Biol.* 21, 396–404.
- https://doi.org/10.1111/j.1420-9101.2007.01480.x (2008).
 Beadell, J. S. et al. Global phylogeographic limits of Hawaii's avian malaria. Proc. R. Soc. B: Biol. Sci. 273, 2935–2944. https://doi.org/10.1098/rspb.2006.3671 (2006).
- 14. Krasnov, B. R. Functional and Evolutionary Ecology of Fleas: A Model for Ecological Parasitology (Cambridge University Press, 2008)
- Poulin, R. Evolutionary Ecology of Parasites (Princeton University Press, 2011).
- 16. Välimäki, P. et al. Geographical variation in host use of a blood-feeding ectoparasitic fly: Implications for population invasiveness. Oecologia 166, 985–995. https://doi.org/10.1007/s00442-011-1951-y (2011).

 7. Theodosopoulos, A. N., Hund, A. K. & Taylor, S. A. Parasites and host species barriers in animal hybrid zones. Trends Ecol. Evol. 34, 19–30. https://doi.org/10.1016/j.tree.2018.09.011 (2019).
- Mackenzie, A. A trade-off for host plant utilization in the black bean aphid, Aphis fabae. Evolution 50, 155–162. https://doi.org/10.1111/j.1558-5646.1996.lb04482.x (1996).
 Harrington, L. C., Edman, J. D. & Scott, T. W. Why do female Aedes aegypti (Diptera: Culicidae) feed preferentially and frequently on human blood?: J. Med. Entomol. 38, 411–422. https://doi.org/10.1603/0022-2585-38.3.411 (2001).

- on numan blood: J. Nea. Entomol. 38, 411–422. https://doi.org/10.1003/0022-2585-38.3.411 (2001).

 20. Dick, C. W. & Patterson, B. D. Against all odds: Explaining high host specificity in dispersal-prone parasites. Int. J. Parasitol. 37, 871–876. https://doi.org/10.1016/j.ijpara.2007.02.004 (2007).

 21. Torchin, M. E. & Mitchell, C. E. Parasites, pathogens, and invasions by plants and animals. Front. Ecol. Environ. 2, 183–190. https://
- doi.org/10.1890/1540-9295(2004)002[0183:PPAIBP]2.0.CO;2 (2004).

 22. Clark, N. J. & Clegg, S. M. The influence of vagrant hosts and weather patterns on the colonization and persistence of blood parasites in an island bird. J. Biogeogr. 42, 641-651. https://doi.org/10.1111/jbi.12454 (2015).
- Kawecki, T. J. Red Queen meets Santa Rosalia: Arms races and the evolution of host specialization in organisms with parasitic lifestyles. Am. Nat. 152, 635–651. https://doi.org/10.1086/286195 (1998).
 Egas, M., Dieckmann, U. & Sabelis, M. W. Evolution restricts the coexistence of specialists and generalists: The role of trade-off
- structure. Am. Nat. 163, 518–531. https://doi.org/10.1086/382599 (2004).

 25. Poulin, R. & Keeney, D. B. Host specificity under molecular and experimental scrutiny. Trends Parasitol. 24, 24–28. https://doi.org/10.1016/j.pt.2007.10.002 (2008).
- 26. Lyimo, I. N. & Ferguson, H. M. Ecological and evolutionary determinants of host species choice in mosquito vectors. *Trends*
- Parasitol. 25, 189–196. https://doi.org/10.1016/j.pt.2009.01.005 (2009).

 7. Visher, E. & Boots, M. The problem of mediocre generalists: Population genetics and eco-evolutionary perspectives on host breadth evolution in pathogens. Proc. R. Soc. B: Biol. Sci. 287, 20201230. https://doi.org/10.1098/rspb.2020.1230 (2020).
- Sarfati, M. et al. Energy costs of blood digestion in a host-specific haematophagous parasite. J. Exp. Biol. 208, 2489. https://doi.org/10.1242/jeb.01676 (2005).
 Fry, J. D. The evolution of host specialization: Are trade-offs overrated?. Am. Nat. 148, S84–S107. https://doi.org/10.1086/285904
- 30. Fessl, B. et al. Galápagos landbirds (passerines, cuckoos, and doves): Status, threats, and knowledge gaps. Galápagos Rep. 2016.
- 31. Fessl, B., Heimpel, G. E. & Causton, C. E. Invasion of an avian nest parasite, Philornis downsi, to the Galapagos Islands: colonization
- history, adaptations to novel ecosystems, and conservation challenges. In *Disease Ecology: Galapagos Birds and their Parasites* (ed Patricia G. Parker) 213–266 (Springer International Publishing, 2018).

 32. Frankham, R. Do island populations have less genetic variation than mainland populations?. *Heredity* 78, 311–327. https://doi.
- org/10.1038/hdv.1997.46 (1997).
- Reichard, M. et al. The bitterling-mussel coevolutionary relationship in areas of recent and ancient sympatry. Evolution 64, 3047–3056. https://doi.org/10.1111/j.1558-5646.2010.01032.x (2010).
 Wiedenfeld, D. A., Jiménez, G. U., Fessl, B., Kleindorfer, S. & Carlos Valarezo, J. Distribution of the introduced parasitic fly Philornis
- Wiedenierd, D. A., Jimenez, C. C., Pessi, B., Kleindorfer, S. & Carros Vautare20, J. Distribution of the introduced parasitic in *Jerinormis downsi* (Diptera, Muscidae) in the Galápagos Islands. *Pacific Conserv Biol.* 13, 14–19. https://doi.org/10.1017/PG070014 (2007).
 Fessl, B., Sinclair, B. J. & Kleindorfer, S. The life-cycle of *Philornis downsi* (Diptera: Muscidae) parasitizing Darwin's finches and its impacts on nestling survival. *Parasitology* 133, 739–747. https://doi.org/10.1017/S003118200600189 (2006).
 Kleindorfer, S. & Dudaniec, R. Y. Host- parasite ecology, behavior and genetics: A review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. *BMC Zool.* 1, 1. https://doi.org/10.1186/s40850-016-0003-9 (2016).
 Galligan, T. H. & Kleindorfer, S. Naris and beak malformation caused by the parasitic fly, *Philornis downsi* (Diptera: Muscidae), in Darwide and Constant of the Con
- in Darwin's small ground finch, Geospiza fuliginosa (Passeriformes: Emberizidae). Biol. J. Lin. Soc. 98, 577-585. https://doi.org/
- In Dat with Stillan government, a coopera jungment (assertions is an activated by the coopera jungment (assertions is activated by the cooperation) and the cooperation is activated by the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation) and the cooperation (assertions is activated by the cooperation) and the cooper
- O'Connor, J. A., Sulloway, F. J., Robertson, J. & Kleindorfer, S. Philos. Sci. 269, 20190-61. https://doi.org/10.1098/rspb.2019.0461 (2019).
 Kleindorfer, S., Peters, K. J., Custance, G., Dudaniec, R. Y. & O'Connor, J. A. Changes in Philornis infestation behavior threaten Darwin's finch survival. Curr. Zool. 60, 542–550. https://doi.org/10.1093/czoolo/60.4.542 (2014).
 O'Connor, J. A., Sulloway, F. J., Robertson, J. & Kleindorfer, S. Philornis downsi parasitism is the primary cause of nestling mortality in the critically endangered Darwin's medium tree finch (Camarhynchus pauper). Biodivers. Conserv. 19, 853–866. https://doi.org/10.1007/s10531-009-9740-1 (2010).
 Kayati, S. A. et al. (Giorges medicinghird tolgeste introduced parasites that offset Darwin's faches. Federal https://doi.org/10.1007/s10531-009-9740-1 (2010).
- Knutie, S. A. et al. Galápagos mockingbirds tolerate introduced parasites that affect Darwin's finches. Ecology https://doi.org/10. 1890/15-0119 (2016).
- 1890/15-0119 (2016).
 24. Peters, K. J. Evans, C., Aguirre, J. D. & Kleindorfer, S. Genetic admixture predicts parasite intensity: Evidence for increased hybrid performance in Darwin's tree finches. R. Soc. Open Sci. 6, 181616. https://doi.org/10.1098/rsos.181616 (2019).
 35. Kleindorfer, S. The ecology of clutch size variation in Darwin's Small Ground Finch Geospiza fuliginosa: Comparison between lowland and highland habitats. Ibi: 149, 730-741. https://doi.org/10.1111/j.1474-1918.200694x. (2007).
 44. Fessl, B. & Tebbich, S. Philornis downsi- a recently discovered parasite on the Galápagos archipelago: A threat for Darwin's finches?

- Fessl, B. & Tebbich, S. *Philornis downsi-* a recently discovered parasite on the Galápagos archipelago: A threat for Darwin's finches?. *Ibis* 144, 445-451. https://doi.org/10.1046/j.1474-919X.2002.00076.x (2002).
 Dudaniec, R. Y., Fessl, B. & Kleindorfer, S. Interannual and interspecific variation in intensity of the parasitic fly, *Philornis downsi*, Darwin's finches. *Biol. Cons.* 139, 325-332. https://doi.org/10.1016/j.biocon.2007.07.006 (2007).
 Cimadom, A. *et al.* Invasive parasites, habitat change and heavy rainfall reduce breeding success in Darwin's Finches. *PLoS ONE* 9, e107518. https://doi.org/10.1371/journal.pone.0107518 (2014).
 Cimadom, A. *et al.* Weed management increases the detrimental effect of an invasive parasite on arboreal Darwin's finches. *Biol. Cons.* 233, 39-3101. https://doi.org/10.1016/j.biocon.2019.02.025 (2019).
 Kleindorfer, S. & Dudaniec, R. Y. Love thy neighbour? Social nesting pattern, host mass and nest size affect ectoparasite intensity in Darwin's tree finches. *Behav. Ecol. Sociobiol.* 63, 731-739. https://doi.org/10.1007/s00265-008-0706-1 (2009).

- 49. Common, L. K., Dudaniec, R. Y., Colombelli-Négrel, D. & Kleindorfer, S. Taxonomic shifts in Philornis larval behaviour and rapid changes in *Philornis downsi* Dodge & Aitken (Diptera: Muscidae): An invasive avian parasite on the Galápagos Islands. in *Life Cycle and Development of Diptera* (ed Muhammad Sarwar) (IntechOpen, 2019). McNew, S. M. et al. Annual environmental variation influences host tolerance to parasites. *Proc. R. Soc. B: Biol. Sci.* 286, 20190049. https://doi.org/10.1098/rspb.2019.0049 (2019).
- McNew, S. M. & Clayton, D. H. Alien invasion: Biology of *Philornis* flies highlighting *Philornis downsi*, an introduced parasite of Galápagos birds. *Annu. Rev. Entomol.* 63, 369–387. https://doi.org/10.1146/annurev-ento-020117-043103 (2018).
 Kleindorfer, S. & Dudaniec, R. Y. Hybridization fluctuates with rainfall in Darwin's tree finches. *Biol. J. Lin. Soc.* 130, 79–88. https://
- doi.org/10.1093/biolinnean/blaa029 (2020).
- Peters, K. J., Myers, S. A., Dudaniec, R. Y., O'Connor, J. A. & Kleindorfer, S. Females drive asymmetrical introgression from rare to common species in Darwin's tree finches. *J. Evol. Biol.* 30, 1940–1952. https://doi.org/10.1111/jeb.13167 (2017).
 Kleindorfer, S. et al. Species collapse via hybridization in Darwin's Tree Finches. *Am. Nat.* 183, 325–341. https://doi.org/10.1086/
- 674899 (2014)
- 55. Loo, W. T., Dudaniec, R. Y., Kleindorfer, S. & Cavanaugh, C. M. An inter-island comparison of Darwin's finches reveals the impact of habitat, host phylogeny, and island on the gut microbiome. *PLoS ONE* 14, e0226432. https://doi.org/10.1371/journal.pone.02264 32 (2019)
- 32 (2019).
 Galapagos Conservancy. Galapagos Vital Signs: A satellite-based environmental monitoring system for the Galapagos Archipelago, https://galapagosvitalsigns.org (2021).
 Couri, M. Considerações sobre as relações ecológicas das larvas de Philornis Meinert, 1890 (Diptera, Muscidae) com aves. Revista Brasileira de Entomologia 29, 17–20. https://doi.org/10.1017/S00311182006001089 (1985).
 Skidmore, P. The Biology of the Muscidae of the World Vol. 29 (Springer, 1985).
 O'Connor, J. A., Robertson, J. & Kleindorfer, S. Video analysis of host-parasite interactions in nests of Darwin's finches. Oryx 44,

- 588-594. https://doi.org/10.1017/S0030605310000086 (2010).
 O'Connor, J. A., Robertson, J. & Kleindorfer, S. Darwin's finch begging intensity does not honestly signal need in parasitised nests. Ethology 120, 228-237. https://doi.org/10.1111/eth.12196 (2014).

- Ethology 120, 228–237. https://doi.org/10.1111/eth.12196 (2014).

 61. Kleindorfer, S. & Sulloway, F. J. Naris deformation in Darwin's finches: Experimental and historical evidence for a post-1960s arrival of the parasite Philornis downsi. Glob. Ecol. Conserv. 7, 122–131. https://doi.org/10.1016/j.gecco.2016.05.006 (2016).

 62. Lahuatte, P. F., Lincango, M. P., Heimpel, G. E. & Causton, C. E. Rearing larvae of the avian nest parasite, Philornis downsi (Diptera: Muscidae), on chicken blood-based diets. J. Insect Sci. https://doi.org/10.1093/j.isseas/iew064 (2016).

 63. Kleindorfer, S. Nesting success in Darwin's small tree finch, Camarhynchus parvulus: Evidence of female preference for older males and more concealed nests. Anim. Behav. 74, 795–804. https://doi.org/10.1016/j.anbehav.2007.01.020 (2007).

 64. Nijhout, H. F. & Callier, V. Developmental mechanisms of body size and wing-body scaling in insects. Annu. Rev. Entomol. 60, 141–156. https://doi.butsc/
- Nijnout, H. F. & Cainer, V. Developmental mechanisms of body size and wing-body scaining in insects. Annu. Rev. Entomol. 60, 141–156. https://doi.org/10.1146/annurev-ento-101814-020841 (2015).
 Singh, D. & Bala, M. The effect of starvation on the larval behavior of two forensically important species of blow flies (Diptera: Calliphoridae). For. Sci. Int. 193, 118–121. https://doi.org/10.1016/j.forsciint.2009.09.022 (2009).
 Coulson, S. J. & Bale, J. S. Characterisation and limitations of the rapid cold-hardening response in the housefly Musca domestica (Diptera: Muscidae). I. Insect Physiol. 36, 207–211. https://doi.org/10.1016/0022-1910/90)90124-X (1990).
 R Core Team. R: A language and environment for statistical computing. R version 4.0.3 (R Foundation for Statistical Computing, Vianne Austria, 2020).

- Vienna, Austria, 2020)
- Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* https://doi.org/10.18637/jss.v067.i01 (2015).
 Venables, B. & Ripley, B. Modern Applied Statistics with S-PLUS (Springer Science & Business Media, 2002).

- Kenables, B. & Ripley, B. Modern Applied Statistics with S-PLUS (Springer Science & Business Media, 2002).
 Fox, J. & Weisberg, S. An R Companion to Applied Regression (Sage publications, 2011).
 Sarkar, D. Lattice: Multivariate Data Visualization with R (Springer, 2008).
 Wickham, H. ggplot2: Elegant Graphics for Data Analysis (Springer, 2009).
 Fox, J. Effect displays in R for generalised linear models. J. Stat. Softw. https://doi.org/10.18637/jss.v008.i15 (2003).
 Burnham, K. P. & Anderson, D. R. Model Selection and Multimodal Inference: A Practical Information-Theoretic Approach (eds Kenneth P. Burnham & David R. Anderson) 75–117 (Springer New York, 1998).
 Grueber, C. E., Nakagawa, S., Laws, R. J. & Jamieson, I. G. Multimodel inference in ecology and evolution: Challenges and solutions. Level Riol 24, 460–711 https://doi.org/10.1111/j.1429-1011.2010.2019.x. (2011).

- Gruebert, C. E., Nakagawa, S., Laws, R. J. & Jamieson, I. G. Multimoder interface in ecology and evolution: Challenges and solutions. J. Evol. Biol. 24, 699–711. https://doi.org/10.1111/j.it.29-1012.2010.22210.x (2011).
 Haaland, T. R., Wright, J. & Ratikainen, I. I. Generalists versus specialists in fluctuating environments: A bet-hedging perspective. Oikos 129, 879–890. https://doi.org/10.1111/oik.07109 (2020).
 Duvies, N. Cuckoos, Cowbirds and Other Cheats (Bloomsbury Publishing, 2010).
 Dudaniec, R. Y., Gardner, M. G. & Kleindorfer, S. Offspring genetic structure reveals mating and nest infestation behaviour of an invasive parasitic fly (Philornis downsi) of Galápagos birds. Biol. Invas. 12, 581–592. https://doi.org/10.1007/s10530-009-9464-x (2010)
- Fredensborg, B. L. & Poulin, R. Larval helminths in intermediate hosts: Does competition early in life determine the fitness of adult parasites?. Int. J. Parasitol. 35, 1061–1070. https://doi.org/10.1016/j.ijpara.2005.05.005 (2005).
 Begon, M., Harper, J. L. & Townsend, C. R. Ecology: Individuals, Populations and Communities (Blackwell Scientific Publications, 2006).
- Fraik, A. K. et al. Disease swamps molecular signatures of genetic-environmental associations to abiotic factors in Tasmanian devil (Sarcophilus harrisii) populations. Evolution 74, 1392–1408. https://doi.org/10.1111/evo.14023 (2020).
 Dyorak, M. et al. Conservation status of landbirds on Floreana: The smallest inhabited Galápagos Island. J. Field Ornithol. 88, 1202 (2012).

- Dyorak, M. *et al.* Conservation status of anaboritos on Fioreana: the smallest inhabited Galapagos Island. *J. Field Ormithol.* 88, 132–145. https://doi.org/10.1111/jofo.12197 (2017).
 Hedrick, P. W., Kim, T. J. & Parker, K. M. Parasite resistance and genetic variation in the endangered Gila topminnow. *Anim. Conserv.* 4, 103–109. https://doi.org/10.1017/81367943001001135 (2001).
 Lewontin, R. C. & Birch, L. C. Hybridization as a source of variation for adaptation to new environments. *Evolution* 20, 315–336. https://doi.org/10.2307/2406633 (1966).
 Wolinska, J., Lively, C. M. & Spaak, P. Parasites in hybridizing communities: The Red Queen again?. *Trends Parasitol.* 24, 121–126. https://doi.org/10.1016/j.13020.1101.07.0209.
- https://doi.org/10.1016/j.pt.2007.11.010 (2008).

 86. Floate, K. D. & Whitham, T. G. The, "Hybrid Bridge" Hypothesis: Host shifting via plant hybrid swarms. *Am. Nat.* 141, 651–662. https://doi.org/10.1086/285497 (1993).
- 87. Le Brun, N., Renaud, F., Berrebi, P. & Lambert, A. Hybrid zones and host-parasite relationships: Effect on the evolution of parasitic
- specificity. Evolution 46, 56–61. https://doi.org/10.1111/j.1558-5646.1992.tb01984.x (1992).

 88. Fritz, R. S., Moulia, C. & Newcombe, G. Resistance of hybrid plants and animals to herbivores, pathogens, and parasites. Annu. Rev. Ecol. Syst. 30, 565–591. https://doi.org/10.1146/annurev.ecolsys.30.1.565 (1999).
- Moulia, C., Brun, N. L., Loubes, C., Marin, R. & Renaud, F. Hybrid vigour against parasites in interspecific crosses between two mice species. Heredity 74, 48–52. https://doi.org/10.1038/hdy.1995.6 (1995).
 Gibson, A. K., Refrégier, G., Hood, M. E. & Giraud, T. Performance of a hybrid fungal pathogen on pure-species and hybrid host
- plants. Int. J. Plant Sci. 175, 724-730. https://doi.org/10.1086/676621 (2014).

- 91. Arnold, M. L. & Martin, N. H. Hybrid fitness across time and habitats. Trends Ecol. Evol. 25, 530-536. https://doi.org/10.1016/j. tree.2010.06.005 (2010).

- tree.2010.06.005 (2010).

 92. Ben-Yosef, M. et al. Host-specific associations affect the microbiome of Philornis downsi, an introduced parasite to the Galápagos Islands. Mol. Ecol. 26, 4644–4656. https://doi.org/10.1111/mec.14219 (2017).

 93. Loo, W. T., García-Loor, J., Dudaniec, R. Y., Kleindorfer, S. & Cavanaugh, C. M. Host phylogeny, diet, and habitat differentiate the gut microbiomes of Darwin's finches on Santa Cruz Island. Sci. Rep. 9, 18781. https://doi.org/10.1038/s41598-019-54869-6 (2019).

 94. Knutie, S. A. Relationships among introduced parasites, host defenses, and gut microbiota of Galapagos birds. Ecosphere 9, e02286. https://doi.org/10.1002/ecs2.2286 (2018).

 95. Knutie, S. A., Chaves, J. A. & Gotanda, K. M. Human activity can influence the gut microbiota of Darwin's finches in the Galapagos Islands. Mol. Ecol. 28, 2441–2450. https://doi.org/10.1111/mec.15088 (2019).

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Author contributions

S.K. and L.K.C. designed the research; L.K.C., S.K., R.Y.D. and D.C.-N. collected the data and sorted the specimens; L.K.C. and P.S. conducted statistical analysis; L.K.C. wrote the manuscript and all authors discussed the results, provided critical analysis, feedback, and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Appendix 4

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ARTHROPODS AND MEDICAL ENTOMOLOGY - ORIGINAL PAPER



Temporal and spatial variation in sex-specific abundance of the avian vampire fly (*Philornis downsi*)

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Abstract

Understanding the range and behaviour of an invasive species is critical to identify key habitat areas to focus control efforts. Patterns of range use in parasites can differ temporally, across life stages and between sexes. The invasive avian vampire fly, Philornis downsi, spends the larval stage of its life within bird nests, feeding on developing nestlings and causing high levels of mortality and deformation. However, little is known of the ecology and behaviour of the non-parasitic adult fly life stage. Here, we document sex-specific temporal and spatial patterns of abundance of adult avian vampire flies during a single Darwin's finch breeding season. We analyse fly trapping data collected across 7 weeks in the highlands (N=405 flies) and lowlands (N=12 flies) of Floreana Island (Galápagos). Lowland catches occurred later in the season, which supports the hypothesis that flies may migrate from the food-rich highlands to the food-poor lowlands once host breeding has commenced. Fly abundance was not correlated with host nesting density (oviposition site) but was correlated with distance to the agricultural zone (feeding site). We consistently caught more males closer to the agricultural zone and more females further away from the agricultural zone. These sex differences suggest that males may be defending or lekking at feeding sites in the agricultural zone for mating. This temporal and sex-specific habitat use of the avian vampire fly is relevant for developing targeted control methods and provides insight into the behavioural ecology of this introduced parasite on the Galápagos Archipelago.

Keywords Range use · Ectoparasite · Invasive species · Galápagos Islands · Philornis

Introduction

In an era of increasing human and animal global mobility, the proportion of invasive species is rapidly increasing, exacerbated by the effects of climate change, over-exploitation, pollution, and habitat fragmentation (Pelletier and

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Coltman 2018). Invasive parasites that pose risks to public health (González et al. 2017; Ruberanziza et al. 2019) or that negatively impact host species of conservation concern (Olson et al. 2013) warrant monitoring for informing control strategies. A single species may occupy and utilise different areas within its range across seasons, life stages, and between sexes (Bierzychudek and Eckhart 1988; Maxwell et al. 2019; Ruckstuhl and Neuhaus 2006). Understanding the distribution and behaviour of an introduced species is useful to identify seasonally or geospatially restricted habitat areas to focus control and management efforts (Escobar et al. 2019; Mathieu-Bégné et al. 2020; Raghavan et al. 2019; Woodworth et al. 2005).

In parasitic arthropods, studies have found selective spatial and temporal habitat use between the sexes (Papadopoulos et al. 2003; Sciarretta et al. 2018; Warburg and Yuval 1997; Wong and Jim 2018). In general, sexual conflict and sexual dimorphism have been shown to drive sex-specific distributions in arthropods (Foster and Soluk 2006; Romey and Wallace 2007; Stanley et al. 2018). Patterns of male and

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female abundance can differ due to sex-biased dispersal, with extremes where one sex disperses while the other is sedentary, which is especially relevant during range expansions or invasions (Beirinckx et al. 2006; Dudaniec et al. 2021; Miller and Inouye 2013). In other cases, females may aggregate together, away from areas of high male density, to avoid harassment from males, which is seen in systems with high costs to females from multiple mating (Roswell et al. 2019; Stanley et al. 2018; Stone 1995; Warburg and Yuval 1997). Understanding patterns of sex-specific distribution, location of oviposition, and feeding sites in invasive parasite populations is useful to control mating behaviours and frequencies, and to maximise the impact of targeted control interventions (Dunn and Hatcher 2015).

In resource-based mating, which is common in many insects, including parasitic insects (Dodson 1997; Preston-Mafham 2001; Warburg and Yuval 1997; Wilkinson and Johns 2005), males compete to guard a resource, such as a food or oviposition site, and mate with females that are attracted to the resource (Choe and Crespi 1997; Parker 1978). Resources must be predictable and defendable but uncommon enough to attract females. When resources are scattered or ubiquitous, swarm-based mating or mate searching systems, where males actively search for receptive mates, tend to prevail (Emlen and Oring 1977; Wilkinson and Johns 2005). Defended resources differ across species and are commonly food or oviposition sites (Preston-Mafham 2001; Wilkinson and Johns 2005). Males may lek near resources (Hendrichs et al. 1991; Warburg and Yuval 1997: Yuval 2005) or defend non-resource-based territories (Yeates and Dodson 1990) to attract and mate with females. Integrating information on the spatial and temporal distribution of invasive species, their mating systems, and monitoring of female population densities is therefore critical for the success of large-scale eradication programs (Enkerlin et al. 2017; Yamagishi et al. 1993).

The avian vampire fly (Philornis downsi, Dodge and Aitken, 1968) (Diptera: Muscidae) is a generalist invasive ectoparasite whose larvae consume the blood and tissue of developing birds across a range of host species (Dudaniec and Kleindorfer 2006; McNew and Clayton 2018). Introduced to the Galápagos Islands during the 1960s, avian vampire fly larvae were first discovered in Darwin's finch nests in 1997 (Causton et al. 2006; Fessl et al. 2001). Since then, the fly has been detected on 14 islands across the archipelago (Fessl et al. 2018; Wiedenfeld et al. 2007). Adult avian vampire flies are non-parasitic and feed on fruit, nectar, and decaying vegetable matter (Fessl et al. 2018). However, their larvae are obligate parasites of nestlings, feeding both internally and externally on the host (Fessl et al. 2006; O'Connor et al. 2010a). In its invasive range, the avian vampire fly is highly virulent, causing severe in-nest mortality or alternatively, naris deformation in nestlings that persist into adulthood and thus affect song and foraging technique (Kleindorfer et al. 2019; Kleindorfer and Dudaniec 2016; Kleindorfer et al. in review). The effects of avian vampire fly parasitism, such as lowered body condition, naris deformation, and mortality, are of particular concern for declining populations of critically endangered Darwin's finch species (Fessl et al. 2010; Lawson et al. 2017; O'Connor et al. 2010b).

Our understanding of adult avian vampire fly behaviour comes from genetic sources where multiple mating behaviour was established via larval sib-ship reconstructions (Dudaniec et al. 2010), or from video recordings at host nests that showed adult flies entering and leaving nests (Lincango et al. 2015; O'Connor et al. 2010a). Male and female avian vampire flies differ in their minimum longevity determined under laboratory rearing conditions (males ~ 188 days; females ~ 265 days) (Causton et al. 2019). The height at which adult flies were caught differed between the sexes on Floreana Island—females were more commonly caught at lowest and highest heights (2 m and 7 m) where there were fewer males (Kleindorfer et al. 2016). Wild avian vampire fly adults collected from Santa Cruz Island also have sex-specific microbiomes, which suggests the sexes may differ in diet and therefore foraging behaviour, perhaps due to different nutritional needs between sexes (Jose et al. 2021). Despite increasing knowledge on the avian vampire fly and its effects on hosts, we know little about where adult flies feed or mate. Mating behaviour has only been studied in a laboratory setting and has yet to be fully understood in the wild (Causton and Lahuatte pers, observation). In the laboratory, flies mate after being fed an enriched papaya diet and as well as a range of native and introduced plant species that have been offered to them, including the invasive blackberry (Causton and Lahuatte, pers. observation). As such, the agricultural zones of the inhabited islands may be an important area for avian vampire fly feeding because of the abundance of fruiting trees.

As the avian vampire fly is the greatest threat to the survival of all Galápagos land birds, it has been targeted for management and control (Causton et al. 2013). Therefore, it is critical to understand the various drivers of adult abundance and distribution. Host species nesting abundance may drive local avian vampire fly abundances, as adults may be attracted to nests for oviposition, and emerge from nests following development and pupation (Fessl et al. 2018). Although both larval and adult avian vampire fly populations occur ubiquitously across habitats (Causton et al. 2019; Dudaniec et al. 2007; Kleindorfer and Dudaniec 2016), intensity does differ across years and when accounting for host species (Kleindorfer and Dudaniec 2016).

It has been suggested that adult avian vampire fly populations may persist in highland refugia outside the host breeding season, where they can access agricultural crops

and experience higher rainfall, and then disperse to lower, drier elevations once the host breeding season commences (Causton et al. 2013; Kleindorfer and Dudaniec 2016; Wiedenfeld et al. 2007). On Santa Cruz Island, catch rates of males decreased significantly across the non-breeding season in line with a shorter lifespan, while catch rates in females remained comparatively stable across the year (Causton et al. 2019). High rainfall was also shown to suppress daily catch rates of both male and female adult flies, likely due to decreased flight activity (Causton et al. 2019). Seasonal movements, key habitats, catch rates, and sex differences in habitat use in male and female avian vampire flies across islands are currently poorly understood. Due to the fly's geographically widespread occurrence, identifying key sites and times of peak reproductive and dispersal activity within a Darwin's finch breeding season is of special interest for the development of targeted control techniques, such as for mass release of biological control agents or ster-

In this study, we are interested in whether male and female avian vampire flies have different temporal or spatial distribution patterns that could be associated with different resource types (i.e. food resource = fruit from agricultural zone vs. reproductive resource = host nests) across two habitat types: humid, dense highlands, and dry lowlands. We quantify the number of male and female avian vampire flies captured in traps on Floreana Island, Galápagos, during the Darwin's finch breeding season in 2020 and examine patterns of avian vampire fly abundance in relation to (a) date of trapping, (b) distance to the agricultural zone, and (c) the nesting density of Darwin's finches within the highland and lowland study areas. We predict (a) female-biased sex ratio at the onset of breeding due to sex differences in minimum longevity and catch rates documented on Santa Cruz (Causton et al. 2019), (b) increased avian vampire fly abundance closer to the agricultural zone at the start of the breeding season, (c) increased avian vampire fly abundance, particularly female abundance as host nesting density increases, and (d) increased male abundance closer to the agricultural zone near higher densities of fruiting trees.

Materials and methods

Study site and species

We collected adult avian vampire flies from traps on Floreana Island, Galápagos Archipelago, between January 19th and March 6th, 2020, during the Darwin's finch breeding season. Trapping occurred in both the highlands and lowlands (Fig. 1). The highland site receives between 600 and 2300 mm of rain per year (Ben-Yosef et al. 2017; Charles Darwin Researcher Solanda Rea at Bella Vista; Galapagos Conservancy 2021). The highland site is a humid Scalesia forest at an elevation of 300-400 m asl located at the base of Cerro Pajas volcano (01°17'S, 090°27'W), and is adjacent to the agricultural zone (01°18'S, 090°26'W). The lowland site (01°16'S, 90°29'W) receives between 100 and 700 mm of rain per year (Charles Darwin Foundation Researcher Heinke Jäger at Puerto Avora). The lowland is dominated by Palo Santo (Bursera graveolens) and Acacia (Parkinsonia aculeata and Scutia spicata) (Dvorak et al. 2017) with elevation of 0-150 m asl and is adjacent to the town of Puerto Velasco Ibarra (Fig. 1). On Floreana Island, human food production for the population (~110 people) occurs in the highland agricultural zone with only scattered fruiting trees near homes of individual families in the lowlands. Daily highland rainfall data were collected via satellite from CPC Global Unified Precipitation Data provided by NOAA/OAR/ ESRL PSD, Boulder, CO, USA, downloaded from the Galápagos Vital Signs website (Galápagos Conservancy 2021).

The avian vampire fly is a myiasis-causing parasite whose free-living semi-hematophagous larvae feed on the developing nestlings of altricial birds (Dudaniec and Kleindorfer 2006; Fessl and Tebbich 2002). Avian vampire fly eggs are laid inside the host nests (O'Connor et al. 2010a) and once hatched, 1st instar larvae move to the nares and ear canals of newly hatched nestlings to feed on blood and tissue (Fessl et al. 2006). Second and third instar larvae generally reside in the base of the nest during the day, feeding internally (in nares) and externally on the nestlings at night (Fessl et al. 2006; O'Connor et al. 2010a). After 4-10 days of feeding, larvae pupate in a frothy cocoon in the base of the nest and emerge as adults after 7-18 days (Kleindorfer et al. 2014; Lahuatte et al. 2016). Adult flies feed on decaying vegetable matter including fruits and flowers (Fessl et al. 2018; Skidmore 1985), and can be attracted to baited traps using fruit juice lures (Lincango and Causton 2009).

Avian vampire fly trapping

Adult vampire flies were collected using baited McPhail traps hung in trees (Causton et al. 2019; Lincango and Causton 2009). Traps were baited with 150 mL of liquid lure composed of 600-g ripe Hawaiian papaya, 75-g sugar, and 4 L of water, blended and fermented in the sun 3 days prior to use. Trapping occurred in the highland and lowland site. At each site, traps were placed within four study plots, each containing 12 traps separated by 50 m in a three by four trap lattice (Fig. 1). In addition, four traps were placed in two more study plots along a single transect each separated by 50 m (N=32 traps per site, total N=62 traps; Fig. 1). Traps were placed alternatively at 4 and 7 m high to capture potential sex ratio differences of flight height found previously by Kleindorfer et al. (2016). Bait lure was replaced and all specimens collected every



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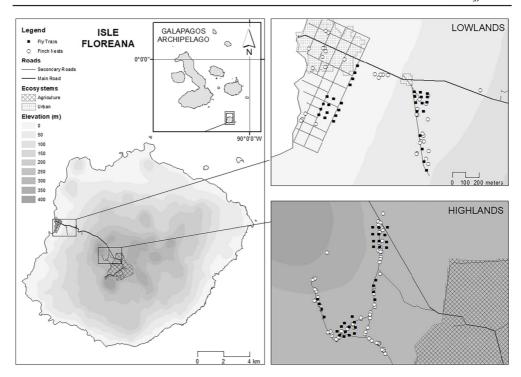


Fig. 1 Map of Floreana Island, Galápagos Archipelago. McPhail trap locations are marked with black squares, location of Darwin's finch nests monitored across the 2020 breeding season (small tree finch, medium tree finch, small ground finch and cactus finch) are marked with white dots

5 days. This was repeated nine times from January 19th to March 5th for a total of 563 trapping events. Collected flies were stored in 70% ethanol, identified, and sexed under a stereomicroscope following morphology described in Kleindorfer et al. (2016).

GPS coordinates were collected for each trap as they were deployed. Distance of each trap to the agricultural zone boundary was calculated using coordinate data. The host nesting density, i.e. the number of active Darwin's finch nests per 200 m \times 100 m study plot, was collected from our long-term nest monitoring protocol (see Kleindorfer et al. 2014), which occurred concurrently with trapping. Search effort for active nests within study plots was equal across highland and lowland sites. The host species monitored were the small ground finch (Geospiza fuliginosa), cactus finch (Geospiza scandens), small tree finch (Camarhynchus parvulus), medium tree finch (C. pauper), and the hybrid tree finch ($C. parvulus \times C. pauper$ as well as introgressed individuals). Each monitored host nest that was with eggs (incubation phase) or nestlings (feeding phase) within each study plot (100 m×200 m) during each trapping period (5 days)

was counted as an active nest, giving a nesting density of Darwin's finch nests per plot for each trapping event.

Mapping

Map figures were prepared using ArcMap 10.8.1 (ESRI 2011), with UTM 15S projection. Primary data obtained via ESRI Web Map included Ecosistemas Galápagos 2016, and Vías (roads) layers (ESRI 2017). The shoreline was obtained from NOAA Shoreline World Vector Shoreline (NOAA 2016; Wessel and Smith 1996) and the Digital Elevation Model (Souris 2018) was used to create elevation vectors at 50-m intervals for display purposes.

Statistical analysis

All models were fitted in R version 4.0.0 (R Core Development Team 2020) using the packages 'lme4' (Bates et al. 2015), 'car' (Fox and Weisberg 2011), and 'effects' (Fox 2003). Results are presented as estimate ± standard error, unless otherwise stated. Total number of avian vampire

flies (N=417) across habitats (lowlands: N=12; highlands: N=405) was analysed using a generalised linear mixed model (GLMM) with negative binomial distribution to account for the non-normal distribution and over-dispersion of the count data. To incorporate the dependency among observations of the same trap, we used 'trap ID' as random intercept. To test for the effects of rainfall in highland avian vampire fly abundance, average daily rainfall was calculated per trapping event (5 days) for both sites. However, due to the strong correlation between average daily rainfall and Julian date (Pearson's correlation test: rho = -0.71), rainfall was excluded from further analysis; patterns of rainfall for highlands are instead described in the results. The number of highland avian vampire flies caught in traps was analysed using negative binomial GLMM in relation to Julian date. distance to agricultural zone and host nest, the interaction term Julian date x distance to agricultural zone, with trap ID as a random intercept (N=417) and a log link function. Corresponding analysis for male (N=199) and female (N=205)highland abundance in relation to Julian date, distance to agricultural zone, host nesting density, and number of the opposite sex caught in the same trap was analysed using GLMMs with negative binomial distribution, log link function, and trap ID as a random intercept. The proportion of male avian vampire flies, representing the sex ratio, was analysed separately for the highlands in relation to Julian date, distance to agricultural zone, and host nesting density using a GLM with the command bind ('cbind') function specifically designed to fit ratio data within the binomial family. The number of male avian vampire flies was the binomial denominator, quasibinomial (correct for overdispersion) distribution, and a logit link function. All quantitative variables were scaled (mean = 0 and standard deviation = 1) to bring variables into comparable scales and allow interpretation

Table 1 (a) Highland and lowland avian vampire flies (*Philornis downsi*) from McPhail traps collected during the Darwin's finch breeding season. Generalized linear mixed model with negative binomial distribution for total number of adult avian vampire flies caught in relation to habitat, Julian date, and host nesting density. Trap ID was used as a random factor (variance=0.145±0.38). (b) General-

of the magnitude of all main effects (Grueber et al. 2011). Here, we report model effect sizes as estimate \pm SE (summary function in 'lme4'; Bates et al. 2015); χ^2 and p-values from the ANOVA Table of Deviance using Type III χ^2 tests (ANOVA function in the package 'car'; Fox and Weisberg 2011).

Results

The number of avian vampire flies was significantly higher in the highlands compared to the lowlands (GLMM, 3.49 ± 0.33 , p < 0.001, Table 1; Fig. 2; Supplementary Fig. 1). Catch rates increased across the breeding season $(0.57 \pm 0.07, p < 0.001, Table 1)$. There was no effect of host nesting density on the number of flies caught $(0.03 \pm 0.07,$ p = 0.715, Table 1) and distance to agricultural zone was not included in the most parsimonious model. Trap ID accounted for 0.145 + 0.38 of the variance in fly abundance. We caught a total of 12 avian vampire flies in the lowlands (0.003 males and 0.005 females per trap per day) and 405 in the highlands $\left(0.135 \text{ males} \text{ and } 0.140 \text{ females per trap per day}\right).$ At the onset of trapping (January 19th), which occurred before the onset of Darwin's finch egg laying and nesting (approximately January 25th in highlands, January 31st in lowlands), we caught 13 males and 15 females from 59 traps in January in the highlands and no males or females from 62 traps in the lowlands. Average daily rainfall decreased across the study period (t = -17.037, df = 282, rho = -0.71, p < 0.001). During the first trapping period in the highlands (January 20th-25th), there was 31.9 mm of rain per day. At the end of the study period (February 29th-March 5th), rainfall was 2.0 mm per day.

ized linear model with quasibinomial distribution for avian vampire fly sex ratio in the highlands in relation to Julian date, distance to agricultural zone, and host nesting density. Sex ratio calculated as the proportion of male flies in relation to female flies. Avian vampire flies collected from McPhail traps on Floreana Island during the 2020 Darwin's finch breeding season

(a) Total number of <i>Philornis downsi</i> (N=417)	Estimate	SE	z-value	LR χ^2	df	P-value
Intercept	-3.384	0.31	- 10.938			< 0.001
Habitat	3.492	0.33	10.582	111.99	1	< 0.001
Julian date	0.586	0.07	8.122	65.96	1	< 0.001
Host nesting density	0.027	0.07	0.365	0.13	1	0.089
(b) Highland <i>P. downsi</i> sex ratio $(N=405)$	Estimate	SE	t-value	LR χ^2	df	P-value
Intercept	-0.130	0.12	-1.077			0.283
Julian date	0.207	0.11	1.810	3.318	1	0.072
Distance to agricultural zone	-0.516	0.11	-4.731	23.686	1	< 0.001
Host nesting density	-0.051	0.12	-0.415	0.173	1	0.678

Dispersion parameter for negative binomial model (a) taken to be 1.981 for quasibinomial model (b) taken to be 0.938



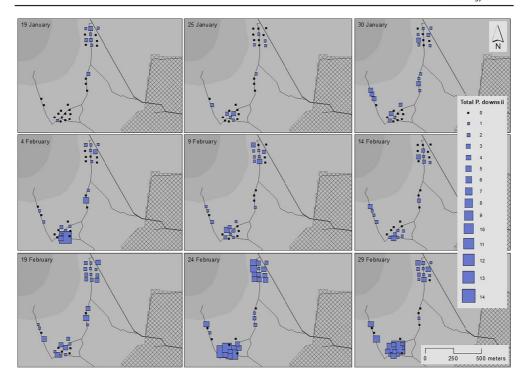


Fig. 2 Avian vampire fly abundance per trapping event (top left of each frame indicates date of trap deployment, trapping events last 5 days) in the highlands of Floreana Island during the 2020 Darwin's Finch breeding season (January 19th to March 5th)

Lowlands

Flies were not collected in the lowlands until the fourth replicate of trapping (February 5th–February 10th; Supplementary Fig. 1), despite equal trapping effort across the season and across habitats. On the contrary, in the highlands, flies were collected in the first replicate of trapping (January 20th–January 25th; Fig. 2). Due to the small sample size of flies collected in the lowlands (N=12) and low statistical power, we are unable to analyse the effects of host nesting density on adult avian vampire fly abundance or change in sex ratio across the season in the lowlands.

Highlands

Highland avian vampire fly abundance (N=405) increased across the breeding season (GLMM, 0.57 ± 0.07 , p<0.001, Table 2, Fig. 2). There was no effect of distance to the agricultural zone $(0.09\pm0.01, p=0.370, \text{Table 2})$ or host nesting density $(0.04\pm0.08, p=0.607, \text{Table 2})$ on the overall abundance in the highlands. There was also no interaction

effect between trapping date and distance to the agricultural zone (-0.08 ± 0.07 , p=0.273). Trap ID accounted for 0.16 ± 0.40 of the variance in highland abundance. The sex ratio did not change significantly across the breeding season (GLM, 0.21 ± 0.12 , p=0.072, Table 1). There was no effect of host nesting density (N=16 total active nests in highland study plots across breeding season, Fig. 3) on sex ratio when accounting for date of capture (-0.05 ± 0.12 , p=0.679, Table 1; Fig. 3). The sex ratio was highly skewed towards males closer to the agricultural zone (-0.51 ± 0.11 , p<0.001, Table 1) and skewed towards females further from the agricultural zone.

Examining abundance patterns in each sex in the highlands separately, male avian vampire fly abundance (N=199) increased across the breeding season (GLMM, 0.54 ± 0.10 , p<0.001, Table 2), and increased closer to the agricultural zone $(-0.27\pm0.10$, p=0.008, 2), with no effect of host nesting density $(0.04\pm0.10$, p=0.653, Table 2). There was a positive association between the number of male flies and female flies collected in the same trap $(0.37\pm0.07, p<0.001$, Table 2), as the number of



Table 2 Highland avian vampire flies (*Philornis downsi*) from McPhail traps collected in 2020 during the Darwin's finch breeding season from Jan to Mar. Generalised linear mixed model for (a) total number of adult avian vampire flies in relation to Julian date, distance to agricultural zone, elevation, and host nesting density (random factor trap ID variance = 0.16 ± 0.40); (b) number of male avian vampire

flies in relation to Julian date, distance to agricultural zone, host nesting density, and number of females (trap ID variance=0.06±0.25); (c) number of female avian vampire flies in relation to Julian date, distance to agricultural zone, host nesting density, and number of males (trap ID variance=3.4×10⁻⁹±5.8×10⁻⁵). N is the raw number of flies caught

a) Total number of <i>Philornis downsi</i> (N=405)	Estimate	SE	z-value	$LR \chi^2$	df	P-value
Intercept	0.106	0.11	1.005	~		0.315
Julian date	0.562	0.07	7.627	58.167	1	< 0.001
Distance to agricultural zone	0.065	0.10	0.648	0.420	1	0.517
Host nesting density	0.042	0.08	0.076	0.300	1	0.584
Julian date × Distance to agricultural zone	-0.081	0.07	-1.096	1.201	1	0.273
b) Male <i>Philornis downsi</i> (N=199)	Estimate	SE	z-value	LR χ^2	df	P-value
Intercept	-0.735	0.12	-6.260			< 0.001
Julian date	0.544	0.10	5.420	29.373	1	< 0.001
Distance to agricultural zone	-0.267	0.10	-2.645	6.994	1	0.008
Host nesting density	0.045	0.10	0.449	0.202	1	0.653
Number of females	0.373	0.07	5.238	27.440	1	< 0.001
c) Female <i>Philornis downsi</i> (N=205)	Estimate	SE	z-value	LR χ^2	df	P-value
Intercept	-0.548	0.09	-6.244			< 0.001
Julian date	0.283	0.09	3.269	10.687	1	< 0.001
Distance to agricultural zone	0.380	0.08	4.676	21.862	1	< 0.001
Host nesting density	0.039	0.08	0.464	0.215	1	0.643
Number of males	0.372	0.06	6.582	43.329	1	< 0.001

Dispersion parameter for negative binomial model (a) taken to be 1.925; (b) taken to be 2.455; (c) taken to be 5.966

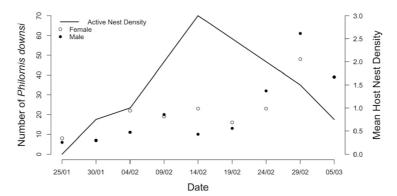


Fig. 3 Highland number of male and female avian vampire flies (*Philomnis downsi*) across date of collection (trapping replicate duration 5 days) with mean host nesting density per study plot ($200~\text{m}\times100~\text{m}$). Open circles represent the number of female avian vampire flies caught in the highlands during a trapping event, closed

circles represent the number of male avian vampire flies caught across the same time period. Avian vampire flies collected from McPhail traps in the highlands of Floreana Island in 2020 during the Darwin's finch breeding season (January 19th to March 5th)

female flies increased, so did the number of male flies. Trap ID accounted for 0.06 ± 0.25 of the variance in male fly abundance. Female avian vampire fly abundance (N=205) increased across the breeding season (GLMM, 0.28 ± 0.09 , p<0.001, Table 2), decreased closer to

the agricultural zone $(0.38\pm0.08,\,p<0.001,\,\text{Table 2})$, and did not increase in relation to host nesting density $(0.04\pm0.08,\,p=0.643,\,\text{Table 2};\,\text{Fig. 3})$. Trap ID only accounted for $3.4\times10^{-9}\pm5.8\times10^{-5}$ of the variance in female abundance.



Discussion

Understanding sex-specific range use, key resources, and habitat use by populations is critical for developing effective control techniques for invasive species. We found significant differences in temporal patterns of abundance of the invasive avian vampire fly across the Darwin's finch breeding season and two habitat types on Floreana Island. The number of flies caught in traps increased significantly across the breeding season (January-March), which might be due to increasing numbers of adult flies emerging from host nests towards the end of the host breeding season. The prevalence of P. downsi in monitored nests with nestlings was 100%; however, if the nests failed during incubation, no avian vampire fly larvae were found. Contrary to our prediction, there was no effect of host nesting density on overall abundance or sex-specific abundance. Our data suggest that avian vampire flies may use the highland Scalesia and nearby agricultural zone as a refugium during the non-breeding season. Adult flies were collected in the highlands during the first trapping event but were not collected in the lowlands until 20 days into trapping. Avian vampire flies may have dispersed from the humid highlands to the arid lowlands as host breeding increased. as there is evidence that adult flies are capable of dispersing large distances (Fessl et al. 2018). We did not find support for our predictions of a female-biased sex-ratio or increased abundance near the agricultural zone at the onset of the breeding season. This contrasts with previous research that found males have a shorter lifespan and are unlikely to survive to the next year's breeding season, resulting in a female-biased sex ratio (Causton et al. 2019). Although the sex ratio remained stable across habitats (highlands vs lowlands) and time, we caught more male avian vampire flies closer to the agricultural zone, with females showing the opposite pattern. Our results highlight the importance of the highland habitat, on Floreana Island, as a key location to plan control measures for the avian vampire fly.

The sex differences in catch numbers close to the agricultural zone raise questions about the mating behaviour of the avian vampire fly. It is possible, though untested, that males near the agricultural zone may be guarding food resources or lekking near fruits to attract females. In many species that display resource-based mating, males defend oviposition sites, with both mating and oviposition occurring at the guarded site (Preston-Mafham 2001; Warburg and Yuval 1997; Wilkinson and Johns 2005). This may not be a preferred option for male avian vampire flies, due to the presence of the incubating or brooding female at the nest (Kleindorfer et al. in review). There is video evidence that avian vampire flies wait outside the host nest,

only entering once the nest is unattended, and leaving once the female returns (Lincango et al. 2015; O'Connor et al. 2010a). There may be a risk of predation by the insectivorous bird if a fly enters or mates near an occupied nest, making the host nest a less attractive 'mate attraction' resource, especially when considering that the length of mating reported under laboratory conditions is often between 5 and 7 min (Causton and Lahuatte et al., pers. comms.). Sex differences in dispersal or climatic tolerance (Enriquez and Colinet 2017; Lyons et al. 2014; Miller and Inouye 2013) are other explanations for the possible differences in spatial patterns we observed. The proximity to the agricultural zone stands out as a key element that warrants further study and points to possible differences in nutritional needs between the sexes or mating behaviour. A key factor known to drive sex-specific ranges is male harassment, which may operate in conjunction with or independently of the mating system (Stanley et al. 2018; Stone 1995). Male harassment, and its associated costs to females, has previously been suggested as one possible explanation for sex-specific micro-habitat use in the avian vampire fly (Kleindorfer et al. 2016), though it is still untested. Due to the limitations of this study, we are unable to determine the cause of the sex-specific distribution in avian vampire fly on Floreana Island during 2020. Future research could test ideas to disentangle the potential role of mating system and foraging ecology in avian vampire flies in relation to proximity to fruiting trees in the agricultural zone.

Although we did not find a correlation between host nesting density and number of avian vampire flies, there may be a time lag between nest termination and fly emergence, and therefore an increase in fly abundance. Due to the restricted sampling period of this study, we do not know how the abundance of the avian vampire fly changes after host breeding has finished on Floreana. On Santa Cruz, female catch rates remained approximately stable across the non-breeding season, with male catch rates decreasing between breeding seasons (Causton et al. 2019). It is possible that the fly population decreases slowly after the breeding season as adult flies die off. This is in contrast to the rapid drop-off in nesting density seen in this study, which may be why we did not find a relationship between fly abundance and host density; however, this remains to be tested. Other host-parasite systems display a parallel pattern of density in host and parasite populations (Byers et al. 2008; Oorebeek and Kleindorfer 2008; Young et al. 2015); however, this pattern is not universal (Cardon et al. 2011). Extending the trapping duration outside of the host breeding season can further explore the sex-specific population dynamics on Floreana Island in relation to host nesting density.

The marked habitat differences in number of flies caught between highlands (N=405) and lowlands (N=12) could



be explained by rainfall, host density, or number of fruiting trees, whereby our patterns stand in marked contrast to those found on Santa Cruz Island (Causton et al. 2019). On Santa Cruz Island, catch rates of avian vampire fly adults, in particular female flies, was higher in lowland sites compared to highland sites (Causton et al. 2019). Differences in catch rates between the sexes in these two studies—0.08 females vs 0.08 males per trap per day in this study compared to 0.25 females vs 0.08 males per trap per day in Causton et al. (2019)—are likely due to the differences in sampling duration (6 weeks versus 1 year). It is also notable that the Santa Cruz lowland sites were sampled near a dense urban area with higher human population and potential access to fruiting trees, fresh water, and human food (INEC 2015). Causton et al. (2019) trapped adults across the entire year and found lower catch rates for male avian vampire flies during the host non-breeding season. In contrast to Causton et al. (2019), we did not catch fewer males at the start of the host breeding season. The number of birds is generally lower in the lowlands than in the highlands on both Santa Cruz and Floreana Islands (Dvorak et al. 2012; Dvorak et al. 2017). In terms of nesting, across the same time period, monitoring effort, and area, we encountered five lowland nests with eggs and 16 highland nests with eggs (Kleindorfer et al. unpublished). While the summary data suggest that host nesting density could explain the different trapping success in lowlands versus highlands, the host nesting density did not predict the number of adult flies caught in highland traps, and so we reject this explanation. We suggest that rainfall, human population, and fruiting trees may be more important factors for avian vampire fly abundance.

Although our results are based on one season of data, the clear patterns combined with conservation urgency call for future research and targeted intervention. For example, the Sterile Insect Technique (Hendrichs et al. 2002), whereby large numbers of sterile insects that produce no offspring are released, could be targeted to high male density areas. Utilising mating or attractant pheromones to disrupt mating (Carde and Minks 1995) can be deployed where mating most commonly occurs. Future research should extend trapping into the non-breeding season and within the agricultural zone at particular fruiting trees to determine patterns of migration across habitat types (Midgarden et al. 2014). Sex-specific population control could be effective in managing and eradicating invasive populations, especially in populations that display sex segregation (Papathanos et al. 2014). If the avian vampire fly is restricted to the highlands and agricultural zone during the non-breeding season, with males primarily distributed in the agricultural zone, this could be a critical area for male population suppression (Hendrichs et al. 2005). Male annihilation techniques, such as those used to decrease fruit fly populations, could be used during the

non-breeding season to decrease male density, which can be used alone or in conjunction with sterile insect release (Vargas et al. 2014). With promising developments on survival rates for reared avian vampire fly larvae in a laboratory setting (Lahuatte et al. 2016), the potential for sterile insect breeding and release is increasing, although more research is needed.

Invasive parasites often pose challenges to biodiversity, but they also represent a chance to learn about novel hostparasite systems under new evolutionary selection regimes (Allendorf and Lundquist 2003; Sakai et al. 2001). In an emerging host-parasite system on Floreana that was likely established after 1960 (Kleindorfer and Sulloway 2016), and has been studied since 2004 (Kleindorfer et al. 2014), we find differences between habitats in numbers of adult flies and sex ratio in relation to proximity to the agricultural zone and across the breeding season. In neither sex did host nesting density predict number of flies caught, but we caught more males close to the agricultural zone at the onset of the breeding season. Intriguingly, the patterns we found are markedly different from those on Santa Cruz Island (Causton et al. 2019), where the number of flies caught was higher in the lowlands than in the highlands and a female-biased sex ratio at the onset of the host breeding season occurred. If the avian vampire fly populations are inhabiting different areas on different islands, this could pose additional challenges for biological control. Future work should extend trapping into the non-breeding season and assess long-term abundance changes in host and parasite abundances across years. Understanding of the movements and populations of both sexes of the avian vampire fly, such as the findings of this study, can inform island-specific targeted control, particularly relevant to control techniques that manipulate breeding behaviour such as the Sterile Insect Technique and pheromonebased mating disruption. This information, as well as the possible occurrence of lag effects of parasite abundance, would further inform the optimal timing and distribution of deploying control efforts. Further research into the spatial and temporal behaviour and ecology of the avian vampire fly in relation to island is critical to untangle the various drivers of adult populations in its invasive range.

 $\label{thm:continuous} \textbf{Supplementary Information} \ \ The \ online \ version \ contains \ supplementary \ material \ available \ at \ https://doi.org/10.1007/s00436-021-07350-1.$

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Author contribution Lauren K. Common and Sonia Kleindorfer designed the study and collected the data. Shane Sumasgutner created the maps and figures. Lauren K. Common analysed the data and wrote the manuscript. All authors discussed the results, edited the manuscript, and provided critical feedback.

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Data availability Data reported in this study will be uploaded to Dryad Digital Repository upon acceptance.

Declarations

Permits Permission to conduct this study was given by the Galápagos National Park and Charles Darwin Research Center, permit no. PC-02–20: Invasivos e invasivos: estableciendo la línea base para evaluar la iniciativa de control de roedores en Floreana sobre la densidad y comportamiento de anidación de pinzones de Darwin y el parasitismo por Philornis downsi, and Flinders University, permit no. E480/19.

Conflict of interest The authors declare no competing interests

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References

- Allendorf FW, Lundquist LL (2003) Introduction: Population biology, evolution, and control of invasive species. Conserv Biol 17(1):24–30. https://doi.org/10.1046/j.1523-1739.2003.02365.x
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixedeffects models using lme4. J Stat Softw 1(1):2015. https://doi.org/ 10.18637/iss.v067.i01
- Beirinckx K, Van Gossum H, J. Lajeunesse M, R. Forbes M, (2006) Sex biases in dispersal and philopatry: insights from a metaanalysis based on capture—mark–recapture studies of damselflies. Oikos 113(3):539–547. https://doi.org/10.1111/j.2006.0030-1299.
- Ben-Yosef M et al (2017) Host-specific associations affect the microbiome of *Philornis downsi*, an introduced parasite to the Galápagos Islands. Mol Ecol 26(18):4644–4656. https://doi.org/10.1111/mec.14219
- Bierzychudek P, Eckhart V (1988) Spatial segregation of the sexes of dioecious plants. Am Nat 132(1):34–43. https://doi.org/10.1086/ 284836

- Byers JE, Blakeslee AMH, Linder E, Cooper AB, Maguire TJ (2008) Controls of spatial variation in the prevalence of trematode parasites infecting a marine snail. Ecology 89(2):439–451. https://doi.org/10.1890/06-1036.1
- Carde RT, Minks AK (1995) Control of moth pests by mating disruption: successes and constraints. Annu Rev Entomol 40(1):559–585
- Cardon M, Loot G, Grenouillet G, Blanchet S (2011) Host characteristics and environmental factors differentially drive the burden and pathogenicity of an ectoparasite: a multilevel causal analysis. J Anim Ecol 80(3):657–667. https://doi.org/10.1111/j.1365-2656.
- Causton C, Cunninghame F, Tapia W (2013) Management of the avian parasite *Philornis downsi* in the Galapagos Islands: a collaborative and strategic action plan Galapagos Report 2011–2012. vol 2012. GNPS, GCREG, CDF and GC, Puerto Ayora, Galapagos, Ecuador, p 167–173
- Causton CE et al (2019) Population dynamics of an invasive bird parasite, *Philornis downsi* (Diptera: Muscidae), in the Galapagos Islands. PLoS ONE 14(10):e0224125. https://doi.org/10.1371/ journal.pone.0224125
- Causton CE, Peck SB, Sinclair BJ, Roque-Albelo L, Hodgson CJ, Landry B (2006) Alien insects: threats and implications for conservation of Galápagos Islands. Ann Entomol Soc Am 99(1):121–143. https://doi.org/10.1603/0013-8746(2006)099[0121:AITAIF]2.0.
- Choe JC, Crespi BJ (1997) The evolution of mating systems in insects and arachnids. Cambridge University Press Cambridge
- Conservancy G (2021) Galapagos Vital Signs: a satellite-based environmental monitoring system for the Galapagos Archipelago. https://galapagosvitalsigns.org Accessed 12 Jan 2021
- Dodson GN (1997) Resource defense mating system in antlered flies, Phytalmia spp. (Diptera: Tephritidae). Annals of the Entomological Society of America 90(4):496–504 doi:https://doi.org/10.1093/aesa/90.4.496
- Dudaniec RY, Carey AR, Svensson EI, Hansson B, Yong CJ, Lancaster LT (2021) Latitudinal clines in sexual selection, sexual size dimorphism, and sex-specific genetic dispersal during a poleward range expansion. J Anim Ecol 00:1–5. https://doi.org/10.1111/ 1365-2656 13488
- Dudaniec RY, Fessl B, Kleindorfer S (2007) Interannual and interspecific variation in intensity of the parasitic fly, *Philornis downsi*. Darwin's Finches Biological Conservation 139(3):325–332. https://doi.org/10.1016/j.biocon.2007.07.006
- Dudaniec RY, Gardner MG, Kleindorfer S (2010) Offspring genetic structure reveals mating and nest infestation behaviour of an invasive parasitic fly (*Philornis downsi*) of Galápagos birds. Biol Invasions 12(3):581–592. https://doi.org/10.1007/s10530-009-9464-x
- Dudaniec RY, Kleindorfer S (2006) Effects of the parasitic flies of the genus *Philornis* (Diptera: Muscidae) on birds. Emu - Austral Ornithology 106(1):13–20. https://doi.org/10.1071/MU04040
- Dunn AM, Hatcher MJ (2015) Parasites and biological invasions: parallels, interactions, and control. Trends Parasitol 31(5):189–199. https://doi.org/10.1016/j.pt.2014.12.003
- Dvorak M, Fessl B, Nemeth E, Kleindorfer S, Tebbich S (2012) Distribution and abundance of Darwin's finches and other land birds on Santa Cruz Island, Galápagos: evidence for declining populations.

 Oryx 46(1):78–86. https://doi.org/10.1017/S0030605311000597
- Dvorak M et al (2017) Conservation status of landbirds on Floreana: the smallest inhabited Galápagos Island. J Field Ornithol 88(2):132–145. https://doi.org/10.1111/jofo.12197
- Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. Science 197(4300):215–223
- Enkerlin WR et al (2017) The Moscamed Regional Programme: review of a success story of area-wide sterile insect technique application. Entomol Exp Appl 164(3):188–203. https://doi.org/10.1111/eea.12611



- Enriquez T, Colinet H (2017) Basal tolerance to heat and cold exposure of the spotted wing drosophila. Drosophila Suzukii Peerj 5:e3112. https://doi.org/10.7717/peerj.3112
- Escobar LE, Moen R, Craft ME, VanderWaal KL (2019) Mapping parasite transmission risk from white-tailed deer to a declining moose population. Eur J Wildl Res 65(4):60. https://doi.org/10. 1007/s10344-019-1297-z
- ESRI (2011) ArcGIS Desktop: Release 10. Environmental Systems Research Institute, Redlands, CA ESRI (2017) Data Galapagos: Web Map. Environmental Systems
- ESRI (2017) Data Galapagos: Web Map. Environmental Systems Research Institute. https://www.arcgis.com/home/item.html?id= ff95a67b2d8149ff9ab2c1feebca8b8c. Accessed 4 Dec 2020
- Fessl B, Couri MS, Tebbich S (2001) Philornis downsi Dodge & Aitken, new to the Galapagos Islands (Diptera, Muscidae). Studia Dipterologica 8(1):317–322
- Fessl B, Heimpel GE, Causton CE (2018) Invasion of an avian nest parasite, *Philornis downsi*, to the Galápagos Islands: colonization history, adaptations to novel ecosystems, and conservation challenges. In: Parker PG (ed) Disease Ecology: Galapagos Birds and their Parasites. Springer International Publishing, Cham, pp 213–266.
- Fessl B, Sinclair BJ, Kleindorfer S (2006) The life-cycle of *Philornis downsi* (Diptera: Muscidae) parasitizing Darwin's finches and its impacts on nestling survival. Parasitology 133(6):739–747. https://doi.org/10.1017/S0031182006001089
- Fessl B, Tebbich S (2002) Philornis downsi- a recently discovered parasite on the Galápagos archipelago – a threat for Darwin's finches? Ibis 144(3):445-451. https://doi.org/10.1046/j.1474-919X 2002 00076 x
- Fessl B et al (2010) How to save the rarest Darwin's finch from extinction: the mangrove finch on Isabela Island. Philosophical Transactions of the Royal Society b: Biological Sciences 365(1543):1019–1030. https://doi.org/10.1098/rstb.2009.0288
- Foster SE, Soluk DA (2006) Protecting more than the wetland: the importance of biased sex ratios and habitat segregation for conservation of the Hine's emerald dragonfly. Somatochlora Hineana Williamson Biological Conservation 127(2):158–166. https://doi.org/10.1016/j.biocon.2005.08.006
- Fox J (2003) Effect displays in R for generalised linear models. J Stat Softw 1(15):2003. https://doi.org/10.18637/jss.v008.i15
- Fox J, Weisberg S (2011) An R companion to applied regression. Sage Publications, California
- González C et al (2017) Entomological characterization of malaria in northern Colombia through vector and parasite species identification, and analyses of spatial distribution and infection rates. Malar J 16(1):431. https://doi.org/10.1186/s12936-017-2076-5
- Grueber CÉ, Nakagawa S, Laws RJ, Jamieson IG (2011) Multimodel inference in ecology and evolution: challenges and solutions. J Evol Biol 24(4):699–711. https://doi.org/10.1111/j.1420-9101. 2010.02210.x
- Hendrichs J, Katsoyannos BI, Papaj DR, Prokopy RJ (1991) Sex differences in movement between natural feeding and mating sites and tradeoffs between food consumption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Tephritidae). Oecologia 86(2):223–231. https://doi.org/10.1007/BF00317534
- Hendrichs J, Robinson AS, Cayol JP, Enkerlin W (2002) Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies. Florida Entomologist 85(1):1–13. https://doi.org/10.1653/ 0015-4040(2002)085[0001:MASITP]2.0.CO;2
- Hendrichs J, Vreysen M, Enkerlin W, Cayol J, Dyck V, Robinson A (2005) Strategic options in using sterile insects for area-wide integrated pest management. In: V.A. D, J. H, A. R (eds) Sterile Insect Technique. Springer, Dordrecht, p 841

- INEC (2015) Censo de Población y Vivienda Galápagos 2015. Instituto Nacional de Estadística y Censos, Ecuador
- Jose PA et al (2021) Shifting microbiomes complement life stage transitions and diet of the bird parasite *Philornis downsi* from the Galapagos Islands. Environ Microbiol. https://doi.org/10.1111/ 1462-2920.15435
- Kleindorfer S, Custance G, Peters Katharina J, Sulloway Frank J (2019) Introduced parasite changes host phenotype, mating signal and hybridization risk: *Philornis downsi* effects on Darwin's finch song. Proceedings of the Royal Society b: Biological Sciences 286(1904):20190461. https://doi.org/10.1098/rspb.2019.0461
- Kleindorfer S, Dudaniec RY (2016) Host-parasite ecology, behavior and genetics: a review of the introduced fly parasite *Philornis* downsi and its Darwin's finch hosts. BMC Zoology 1(1):1. https:// doi.org/10.1186/s40850-016-0003-9
- Kleindorfer S, Peters KJ, Custance G, Dudaniec RY, O'Connor JA (2014) Changes in *Philornis* infestation behavior threaten Darwin's finch survival. Current Zoology 60(4):542–550. https://doi. org/10.1093/czoolo/60.4.542
- Kleindorfer S, Peters KJ, Hohl L, Sulloway FJ (2016) Flight behaviour of an introduced parasite affects its Galapagos Island hosts: Philornis downsi and Darwin's finches. In: Weis DS (ed) Judith S. Biological Invasions and Animal Behaviour. Cambridge University Press, Cambridge, pp 158–179
- Kleindorfer S, Sulloway FJ (2016) Naris deformation in Darwin's finches: experimental and historical evidence for a post-1960s arrival of the parasite *Philornis downsi*. Global Ecology and Conservation 7:122–131. https://doi.org/10.1016/j.gecco.2016.05.006
- Lahuatte PF, Lincango MP, Heimpel GE, Causton CE (2016) Rearing larvae of the avian nest parasite, *Philornis downsi* (Diptera: Muscidae), on chicken blood-based diets. Journal of Insect Science 16(1) doi:https://doi.org/10.1093/jisesa/iew064
- Lawson LP et al (2017) Slow motion extinction: inbreeding, introgression, and loss in the critically endangered mangrove finch (Camarhynchus heliobates). Conserv Genet 18(1):159–170. https://doi.org/10.1007/s10592-016-0890-x
- Lincango P, Causton C (2009) Ensayos de atrayentes para la captura de la mosca parásito, *Philornis downsi* (Diptera: Muscidae) en las Islas Galápagos. Puerto Ayora, Galapagos, Ecuador: Charles Darwin Foundation
- Lincango P, Causton C, Cedeño D, Castañeda J, Hillstrom A, Freund D (2015) Interactions between the Avian Parasite, *Philornis downsi* (Diptera: Muscidae) and the Galapagos Flycatcher, *Myiarchus magnirostris* Gould (Passeriformes: Tyrannidae). J Wildl Dis 51(4):907–910. https://doi.org/10.7589/2015-01-025
- Lyons CL, Coetzee M, Terblanche JS, Chown SL (2014) Desiccation tolerance as a function of age, sex, humidity and temperature in adults of the African malaria vectors Anopheles arabiensis and Anopheles funestus. J Exp Biol 217(21):3823. https://doi.org/10. 1242/jeb.104638
- Mathieu-Bégné E, Loot G, Mazé-Guilmo E, Mullet V, Genthon C, Blanchet S (2020) Combining species distribution models and population genomics underlines the determinants of range limitation in an emerging parasite. Ecography 44(2):307–319. https:// doi.org/10.1111/ecog.05301
- Maxwell SM et al (2019) Seasonal spatial segregation in blue sharks (*Prionace glauca*) by sex and size class in the Northeast Pacific Ocean. Divers Distrib 25(8):1304–1317. https://doi.org/10.1111/ ddi.12941
- McNew SM, Clayton DH (2018) Alien invasion: Biology of *Philornis* flies highlighting *Philornis downsi*, an introduced parasite of Galápagos birds. Annu Rev Entomol 63(1):369–387. https://doi.org/10.1146/annurey-ento-020117-043103
- Midgarden D, Lira E, Silver M (2014) Spatial Analysis of tephritid fruit fly traps. In: Shelly T, Epsky N, Jang EB, Reyes-Flores J,



Appendix 5

Biological Invasions, pp.1-19.

Biol Invasions https://doi.org/10.1007/s10530-022-02935-y

ORIGINAL PAPER



Genetics reveals shifts in reproductive behaviour of the invasive bird parasite *Philornis downsi* collected from Darwin's finch nests

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Abstract Due to novel or dynamic fluctuations in environmental conditions and resources, host and parasite relationships can be subject to diverse selection pressures that may lead to significant changes during and after invasion of a parasite. Genomic analyses are useful for elucidating evolutionary processes in invasive parasites following their arrival to a new area and host. *Philornis downsi* (Diptera: Muscidae), the avian vampire fly, was introduced to the Galápagos Islands circa 1964 and has since spread across the archipelago, feeding on the blood of developing nestlings of endemic land birds. Since its discovery, there have been significant changes to the dynamics of *P. downsi* and its novel hosts, such as shifting

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mortality rates and changing oviposition behaviour, however no temporal genetic studies have been conducted. We collected P. downsi from nests and traps from a single island population over a 14-year period, and genotyped flies at 469 single nucleotide polymorphisms (SNPs) using restriction-site associated DNA sequencing (RADSeq). Despite significant genetic differentiation (FST) between years, there was no evidence for genetic clustering within or across four sampling years between 2006 and 2020, suggesting a lack of population isolation. Sibship reconstructions from P. downsi collected from 10 Darwin's finch nests sampled in 2020 showed evidence for shifts in reproductive behaviour compared to a similar genetic analysis conducted in 2004-2006. Compared with this previous study, females mated with fewer males, individual females oviposited fewer offspring per nest, but more unique females oviposited per nest. These findings are important to consider within reproductive control techniques, and have fitness implications for both parasite evolution and host fitness.

Keywords Reproductive behaviour · *Philornis* · Avian parasite · Darwin's finches · Genetics

Introduction

Biological invasions by pathogenic and parasitic species are an increasing threat to the health of humans, wildlife, and ecosystems (Fisher et al. 2012; Early



et al. 2016; Ricciardi et al. 2017; Jactel et al. 2020). Island populations are especially vulnerable to the impacts of invasive pathogens and parasites, given their isolation, susceptibility to disease and lack of evolved resistance (Wikelski et al. 2004; Russell et al. 2017; Brettell et al. 2021). Invasions can exert extreme selection pressures on both introduced and native species, as well as parasites and hosts (Sakai et al. 2001; Keller and Taylor 2008; Whitney and Gabler 2008; Le Roux 2021), which can influence both the success of invasions and the effectiveness of control methods (Leger and Espeland 2010; Chown et al. 2016; Mayer et al. 2021). This is particularly important for invasive parasites, which are affected by both novel environmental processes and novel hosts. Environmental variables within a parasite's invasive range may impact its growth and life cycle, and thereby influence how parasites affect their novel hosts (e.g., Cline et al. 2014; Liu et al. 2021). Novel hosts exert unique selection pressures on invasive parasites that may lead to altered host-parasite dynamics (Telfer and Bown 2012; McIntire and Juliano 2021) via shifts in parasite strategy and population genetic structure (Brown et al. 2009; Emde et al. 2014; Beaurepaire et al. 2019). Long-term studies of parasite invasions are critical for identifying evolutionary changes within introduced populations, which may be consequential for the conservation and survival of vulnerable host species.

Long-term studies of invasion dynamics are rare, particularly for invasive species with complex life cycles, such as pathogens and parasites (Miura et al. 2006; Feis et al. 2016). Genetic analyses are useful for elucidating the invasion history, contemporary dispersal, and evolutionary shifts within introduced parasite populations (Lawson Handley et al. 2011; Cristescu 2015; Kamenova et al. 2017). Understanding these genetic processes within invasive species can inform management by identifying dispersal routes between populations, the risk of subsequent invasions, or the evolution of resistance to control measures (Sakai et al. 2001; Gaskin et al. 2011). The efficacy of biological control methods often requires rigorous and contemporary information on mating behaviour, selection patterns and demography of the target population (Roderick and Navajas 2003; Lance and McInnis 2005). For example, the Sterile Insect Technique (SIT) is a method used to suppress and eradicate insect pests (e.g., Diptera:

Enkerlin et al. 2017; Lepidoptera: Dyck et al. 2021; Gato et al. 2021; Coleoptera: Himuro et al. 2022). SIT involves the release of large numbers of sterile adult males, and relies on low female remating frequency (i.e., polyandry) and low dispersal (Hendrichs et al. 2005; Lance and McInnis 2005). Dispersal rates may increase significantly during insect invasions and range expansions as selection can favour individuals with greater dispersal capacity (Travis and Dytham 2002; Lombaert et al. 2014; Dudaniec et al. 2022). Rates of female remating have been found to increase during range expansion in insects (Laugier et al. 2013; Crowther et al. 2019), potentially as a strategy to increase female fecundity and genetic diversity at colonised sites. Therefore, changes to parasite dispersal and reproductive behaviours can affect the feasibility of costly and time-consuming control measures. Changes to parasite reproductive behaviour may also affect parasite relatedness, and therefore host fitness (Buckling and Brockhurst 2008; Gleichsner et al. 2018). Kin selection predicts that high parasite relatedness leads to decreased competition between parasites and more prudent exploitation of the host, therefore higher host fitness and lower host mortality (Frank 1992; Buckling and Brockhurst 2008). Thus, understanding these changes in invasive systems is critical for both effective management, and measuring impacts on affected native host species.

The avian vampire fly, P. downsi (Diptera: Muscidae) (Dodge and Aitken, 1968), was discovered in the nests of Darwin's finches on the Galápagos Islands in 1997 (Fessl et al. 2001). It was accidentally introduced to the Galápagos archipelago circa 1964 from its native distribution on the South American mainland (Fessl et al. 2001, 2018; Causton et al. 2006). The free-living larvae of P. downsi reside in nests of many land bird species, feeding on the blood and tissue of developing nestlings (Fessl et al. 2006b). This novel parasitism has significant effects on avian hosts on the Galápagos, causing anaemia, mortality, and permanent physical deformations (Fessl et al. 2006a; Kleindorfer and Dudaniec 2016; Katsis et al. 2021). Philornis downsi has been detected on 15 of the 17 islands across the archipelago, infesting nearly all studied passerine species (Wiedenfeld et al. 2007; Fessl et al. 2018; McNew and Clayton 2018). Due to its severe mortality effects on hosts, particularly critically endangered Darwin's finch species (Lawson et al. 2017; Kleindorfer et al. 2021), control and



eradication of *P. downsi* on the Galápagos is a high priority.

Previous studies have found that P. downsi shows little evidence for genetic differentiation across five islands in the Galápagos archipelago (Santa Cruz, Floreana, Isabela, Santiago and San Cristobal) and across the lowland and highland habitats (Dudaniec et al. 2008a; Koop et al. 2020), indicative of moderate to high genetic dispersal. However, given strong selection pressures associated with invasion (Le Roux 2021), genetic drift in small founding populations (Polechová 2018), and interactions with multiple novel hosts (Telfer and Bown 2012; McIntire and Juliano 2021), P. downsi is expected to show evolutionary shifts since its introduction. Further, previous research detected morphological changes in P. downsi, with an ~11% decrease in female body length and a~26% decrease in female abdomen size between 2004-2016, the latter trait being strongly correlated with fecundity (Common et al. 2020). Similarly, host mortality rates and parasite intensity (number of parasites per nest) shifted between 2000 and 2014. increasing in some species while decreasing in others (Dudaniec et al. 2007; Cimadom et al. 2014; Kleindorfer and Dudaniec 2016). The oviposition behaviour of P. downsi also appears to be changing over recent decades. Previously, females oviposited primarily during late incubation, so their eggs hatched simultaneously with eggs of their host (O'Connor et al. 2010). In later years, larvae were detected in incubating nests of several host species, suggesting females are ovipositing earlier in the nesting cycle (Common et al. 2019). These changes in body size, intensity, and oviposition behaviour lend support that shifts in dispersal and reproductive behaviour may be occurring across time and could be measurable at the genetic level.

The mating and oviposition behaviour of *P. downsi* has not been measured genetically since Dudaniec et al. (2010), which used microsatellite data to explore *P. downsi* remating frequency and oviposition behaviour with data from 2004 to 2006. Via microsatellite-based sibship reconstructions (i.e., inference of full-and half-sibling relationships between individuals within a nest) and genetic relatedness, Dudaniec et al. (2010) found that one to six females infested a single nest, each contributing an average of ~5 offspring per nest. Multiple mating was common, with each female *P. downsi* mating with an average of 1.9 males

(Dudaniec et al. 2010). In this study, we analyse P. downsi collected from traps and the nests of the small tree finch (Camarhynchus parvulus), medium tree finch (C. pauper) and small ground finch (Geospiza fuliginosa) on Floreana Island (Galápagos) with four sampling years that span a 14-year period. This time period represents 64% of the period in which the fly has been documented in finch nests (Fessl et al. 2001). With genomic dataset with higher resolution than the previous microsatellite study, derived from restriction site-associated DNA sequencing (RADSeq), we aim to examine temporal shifts in genetic structure and reproductive behaviour in P. downsi. Specifically, we examine inter-annual variation in (i) genetic divergence, (ii) effective population size, and (iii) evidence for population bottlenecks. Within infra-populations (i.e., the parasites present within each host nest), we use sibship reconstructions to examine for temporal shifts in (iv) genetic relatedness, (v) the number of female flies ovipositing per nest, (vi) the number of offspring assigned per female, (vii) the number of male flies contributing to the offspring in each nest, and (viii) the number of males assigned to the offspring of each female, (i.e., an estimate of female remating frequency). We anticipate that this information will offer an updated and temporal insight into P. downsi evolution within its invasive Galápagos range, with implications for host fitness, and future control programs.

Materials and methods

Study species

Philornis downsi is a Dipteran ectoparasite that feeds on developing nestlings (Fessl and Tebbich 2002; Kleindorfer and Dudaniec 2016; Common et al. 2019). Adult *P. downsi* are vegetarian and non-parasitic, feeding on decaying vegetable matter and fruit (Fessl et al. 2018). Adult females lay their eggs in incubating or brooding nests of 150 known host bird species across 10 Orders in their native distribution across mainland South America, and their invasive range in the Galápagos Islands (Fessl et al. 2018; McNew and Clayton 2018). The eggs of *P. downsi* usually hatch concurrently with host species hatching, and first instar larvae move to the nares of the nestlings where they feed on blood and tissues



of the developing birds (Fessl et al. 2006b). Second and third instar larvae also feed within the nares, but commonly move to the base of the nest during the day, feeding both internally, within the nares and ear canals, and externally, piercing the skin of the nestling, at night (Fessl et al. 2006b; O'Connor et al. 2010). Larvae pupate in the bottom of the nest after 4–7 days of feeding, emerging as adults after 7–14 days (Kleindorfer et al. 2014; Lahuatte et al. 2016; Bulgarella et al. 2017).

Field sample collection

Philornis downsi adults, larvae and pupae were collected during the 2006, 2008, 2014 and 2020 Darwin's finch (Passeriformes: Thraupidae) breeding seasons (January-April) from nests of small ground finches (G. fuliginosa), small tree finches (C. parvulus) and medium tree finches (C. pauper) on Floreana Island. The study site was located in the highlands on Floreana (01°17'S, 090°27'W, 300-400 m asl), a humid Scalesia forest at the base of Cerro Pajas volcano. Nests were located and monitored from incubation to nest termination (fledging or nestling death) following well-established field protocols (Kleindorfer et al. 2014). Nests were monitored every three days during the egg phase and every two days during the feeding phase, with nest activity determined using a borescope. Within 24 h of nest termination, nests were dismantled to collect all P. downsi specimens residing within the base of the nest. All collected specimens were identified to age class (i.e., first-third instar larvae, pupae, adult, Common et al. 2019) under a Leica MS5 dissecting microscope, preserved in 90% ethanol and stored in a −20 °C freezer. The number of nests per year and the number of specimens collected from each nest are presented in Table S1.

To sample adult *P. downsi*, McPhail traps (BioQuip Products, California, USA) were deployed in two of the four sampling years, 2014 (February 18th–April 15th) and 2020 (January 19th–March 5th). McPhail traps were baited with 150 mL of fermented papaya sugar mixture (600 g ripe papaya, 75 g white sugar, 4 L water, blended and fermented for three days; Lincango and Causton 2009). In 2014, 28 traps were placed every 15 m along four 90 m transects at two to seven metres high (m above ground). Bait lure was replaced, and specimens were collected every seven

days, for a total of seven trapping events. In 2020, 32 traps were placed every 50 m in a 200 m×100 m lattice in two study plots, and along two 200 m transects in two separate plots (Common et al. 2022). Traps were hung at four and seven metres high, to capture both male and female *P. downsi*, as previous research found a difference in capture height between the sexes (Kleindorfer et al. 2016). Bait lure was replaced, and specimens were collected every five days, for a total of nine trapping events.

DNA extraction, sequencing, and filtering

DNA extraction was undertaken using whole specimens of 285 P. downsi individuals (larvae, pupae and adults) by Eurofins BioDiagnostics Inc. (Wisconsin, USA). Sample sizes for each sampling year were: 2006=27; 2008=40; 2014=43; 2020=175 (Table S2). The whole specimen was extracted for larvae (n=26) and pupae (n=199). For adult specimens, the head, thorax, and several legs were used for extraction (n=60). Extracted DNA concentrations were standardised to $10 \text{ ng/}\mu\text{l}$ and prepared in to paired-end RAD libraries with the $Sbf\ I$ restriction enzyme, similar to the method of Baird et al. (2008) and following a protocol performed by Floragenex, Inc. (Oregon, USA), as described in Text S1. Samples were sequenced on the Illumina HiSeq4000 platform.

A bioinformatics pipeline implemented by Floragenex Inc. (Oregon, USA) was used to process the raw sequencing data, call variants and filtering, as described in Text S1. A locus was retained if it was present in a minimum of 60% of individuals and had a minor allele frequency (MAF) of 0.02 and a minimum read depth>8. Individual missingness (i.e., the percentage of missing data per individual specimen) was calculated in veftools (Danecek et al. 2011). Due to poor DNA quality, missing data was of concern, therefore we subsampled and analysed the data for 10% and 30% missingness (Figure S1). These values for missing data were chosen to explore the effects of low and high missing data on our results, and to examine these patterns with a stringent (10%) data set and a dataset with more individuals (30%). Further filtering was conducted in Plink 1.9 (Chang et al. 2015) for each dataset to identify and remove loci deviating from Hardy-Weinberg equilibrium (HWE) at P < 0.01. To minimise the effects of linkage



disequilibrium (LD), we excluded one marker from each pair with $R^2 > 0.5$ using the window-based method.

Genetic variation and structure

To assess changes in genetic structure across the sampling period, all specimens collected from nests and traps were analysed (1) for all years pooled, and (2) separately per year (2006, 2008, 2014, 2020), and for each of the 10% and 30% missingness datasets. Samples from a given year are independent generations as previous studies found that *P. downsi* only survives to the next year's breeding season, but not subsequent breeding seasons, and our sampling periods are 2–6 years apart (Causton et al. 2019; Bulgarella et al. 2022).

To avoid potential bias in allele frequencies due to highly related individuals within nests, we calculated pairwise relatedness in vcftools (Danecek et al. 2011), which calculates relatedness using the method developed by Manichaikul et al. (2010). We subsequently removed one individual from each pair that had a relatedness value > 0.25 (i.e., full siblings) from the dataset. Trapping data represents a random sample of the adult fly population and therefore all trapping samples were included in the analysis, together with those collected from nests. The sample sizes for analysing genetic structure per year were: 10% missingness: 2006 = 15, 2008 = 14, 2014 = 19, 2020 = 73; total N = 121; 30% missingness: 2006 = 20, 2008 = 22, 2014 = 28, 2020 = 101; total N = 171.

Genetic diversity parameters were calculated per year using the R package *hierfstat* (Goudet 2005) including observed heterozygosity (H_o), expected heterozygosity (H_e), and the inbreeding coefficient ($F_{\rm IS}$). Allelic richness statistics were calculated per year using the R package *PopGenReport* (Adamack and Gruber 2014). Pairwise $F_{\rm ST}$ between years was calculated using the method developed by Weir, Cockerham (1984) implemented in *hierfstat*, with bootstrapped 95% confidence intervals and 10,000 permutations to determine significance. An analysis of molecular variance (AMOVA) was calculated to determine the variance between years in GenoDive 3.05 (Meirmans 2020).

We examined for genetic structure using two approaches: (1) pooling all samples across the four years, and (2) separating the samples by year to determine within-year substructure. Two methods to detect genetic structure were applied. We used discriminant analysis of principal components (DAPC; Jombart et al. 2010) implemented in the package adegenet (Jombart 2008). DAPC is a model-free approach that transforms genotypes into principal components (PC), applies a discriminant analysis to the number of PCs retained to optimize among-group variation and minimize within-group variation, calculating the optimum number of clusters using Bayesian Information Criterion. Finally, we used the software STRUCTURE 2.3.4 (Pritchard et al. 2000), which utilises an iterative Bayesian clustering method, to calculate allele frequencies and individual assignments to genetic clusters. In STRUCTURE, 10 runs with 20,000 Markov Chain Monte Carlo (MCMC) iterations after a 10,000-iteration burn-in period was conducted for each K value (all years combined: K = 1-10; per year analysis: K = 1-5). The number of distinct genetic clusters, K, was selected using STRU CTURESELECTOR (Li and Liu 2018), which implements two methods for K selection. The change in K (ΔK) approach compares the rate of change of the log probability to predict the optimal K (Evanno et al. 2005). STRUCTURESELECTOR also reports four estimators MEDMEDK, MEDMEAK, MAXMEDK, and MAXMEAK to determine the optimal K (Puechmaille 2016). These methods assign subpopulations to a cluster if the mean or median individual membership coefficient was above the threshold of 0.5.

Effective population size

The effective population size (Ne) of P. downsi on Floreana Island was estimated using the program COLONY 2.0.6.7 (Jones and Wang 2010). COLONY uses a maximum likelihood method on the frequency of full- and half-sibling assignments within pairs of randomly selected individuals to determine effective population size (Wang 2009). This method was selected as it is more flexible than other No estimation methods, and therefore more robust in handling violations of assumptions, such as non-random mating (Wang 2009). As COLONY assumes all individuals are from the same population within a single generation, the samples were split per year and analysed separately. Highly related individuals (r>0.25) were removed from the dataset for the purpose of N_e estimation, and the 10% and 30% missingness datasets



were further analysed separately. To explore the effect of genotyping error rate on N_e estimates (e.g., allelic dropouts and missing data), all datasets were analysed using three error rates: 1%, 5% and 10% (Wang 2019; Guppy et al. 2020).

Bottleneck detection

We used the program BOTTLENECK 1.2.02 (Pirv et al. 1999) to assess whether a recent population bottleneck has occurred. BOTTLENECK tests for heterozygosity excess compared to expectations under mutation-drift equilibrium (Cornuet and Luikart 1996) by comparing expected and observed heterozygosity and allele frequency per locus to determine if a locus is in heterozygosity excess or deficit, and whether this difference is significant. Data were analysed for each year (and for each of the 10% and 30% datasets) under two mutation models, the infinite allele model (IAM) and the stepwise mutation model (SMM) as these models are most suitable for biallelic SNP data (Cornuet and Luikart 1996; Kogura et al. 2011). The sign test with 1000 permutations was used to determine the significance of heterozygosity excess across loci. To detect a mode-shift in allele frequencies that may be indicative of a population bottleneck (Luikart et al. 1998), we examined the allele frequency distribution. An approximately L-shaped distribution is expected under mutation-drift equilibrium (Luikart et al. 1998).

Within-nest relatedness and sibship reconstruction

To estimate genetic relatedness and reconstruct sibships within each sampled nest, $P.\ downsi$ were analysed from seven small ground finch $(G.\ fuliginosa)$ and three small tree finch $(C.\ parvulus)$ nests collected in 2020. Parasite intensity in sampled nests was $41.7\pm7.6\ (N=10)$. Due to the small body size of first and second instar larvae, only third instar, pupae and adult specimens collected from nests were sequenced. Dudaniec et al. (2010) found relatedness did not vary with the number of individuals sampled from a nest and concluded a subsample of 10% of the infra-population was deemed sufficient to estimate in-nest relatedness. Thus, at least 10% of each infrapopulation was analysed (average 24%, range 13.3-36.0%) for a total of 98 specimens sampled from 10 nests.



Mean pairwise genetic relatedness was calculated between individuals within each nest in veftools using the method of Manichaikul et al. (2010). The program COLONY 2.0.6.7 (Jones and Wang 2010) was used to reconstruct sibship and parentage within each infrapopulation. COLONY uses a maximum likelihood method to infer parentage and sibship structure from multi-locus genotype data (Jones and Wang 2010). We ran COLONY across the two datasets: 10\% and 30\% missingness. Each nest was run using an error rate of 1%, 5% and 10% to explore the effect of genotyping error on number of putative maternal and paternal genotypes identified in each nest (Wang 2019; Guppy et al. 2020). Polyandry was selected for females, as studies on other Dipterans (Arnqvist and Nilsson 2000; Dunn et al. 2005), and on P. downsi specifically (Dudaniec et al. 2010), found evidence for multiple mating. As in Dudaniec et al. (2010), monogamy was selected for males because it is highly unlikely that females that mated with the same male are ovipositing in the same nest due to random mating and a large population size.

We explored the effects of individual missingness (10% and 30%) and error rate on the number of putative maternal and paternal genotypes inferred from COLONY using ANOVA in R 4.1.0 (R Core Development Team 2020). Furthermore, to test the assumption that 10% of the infrapopulation sampled is sufficient to assess relatedness, we used multiple linear regression in R to explore the effect of the percentage of the total infrapopulation genotyped and total infrapopulation size (parasite intensity) on the number of putative female and male genotypes, and on mean pairwise individual relatedness within nests.

Results

Data filtering

A total of 127,871 SNP variants were obtained from RAD sequencing. After removing SNPs due to low quality or missing data, 7021 SNPs remained. The total number of reads and RAD clusters per dataset are presented in Table S3. After removing SNPs with a MAF < 0.02, those in linkage disequilibrium and deviating from HWE, a total of 469 SNPs remained in the 10% missingness dataset (N=138) and 462 SNPs remained in the 30% missingness dataset (N=188)

(Table S4). For analyses that required only unrelated individuals (i.e., genetic structure), individuals with pairwise relatedness greater than 0.25 were removed, giving final sample sizes of: 10% = 121, 30% = 171.

Genetic diversity and structure

Across years combined, observed heterozygosity was lower than the expected heterozygosity ($H_0 = 0.290$, H_e=0.383, Table 1; Table S5 for results at 30% missingness) and the inbreeding coefficient (F_{IS}) was = 0. 243 (Table 1). Within years, H_0 ranged from 0.255 to 0.323 and F_{IS} from 0.112 to 0.352 $\,$ (Table 1). Mean allelic richness increased consistently from 2006 (1.91) to 2020 (2.00, Table 1). The study year 2020 was the only year with private alleles (i.e., unique alleles found only within this sampling year), with four alleles being unique to the 2020 specimens (Table 1). Pairwise FST between years ranged from 0.003 to 0.010. All pairwise F_{ST} values between years were significant to P < 0.05, suggesting some genetic differentiation between study years (Table S6a). However, analysis of molecular variance (AMOVA) comparing years showed that only 0.7% of the molecular variance was explained by year (mean $F_{ST} = 0.007 \pm 0.001$ standard deviation (SD), P < 0.001), with 12.2% of the variation explained among individuals (mean $F_{IS} = 0.123 \pm 0.017$ SD, P < 0.001), and 87.1% explained within individuals (mean $F_{IT} = 0.129 \pm 0.017$).

For the analysis of genetic structure pooling all years using DAPC, the lowest BIC value suggested an optimal K of 1 at 10% missingness, and K=2 at 30% missingness (Figure S2). The result of K=2 was not explained by year, sex or age and therefore is not biologically meaningful. The most likely number of clusters of all years pooled determined by the maximum

likelihood method of STRUCTURE was K=2 for 10% missingness and K=1 for 30% missingness, and the ΔK method of cluster selection suggested K=3for both datasets. However, the assignment probabilities of each individual to the second or third clusters were low (e.g., 10%: cluster $1 = 0.46 \pm 0.009$, cluster $2=0.46\pm0.009$; cluster $3=0.08\pm0.01$), suggesting little to no support for genetic clustering across years. DAPC analysis on data separated by year suggested an optimal K of 1 for each year, with the exception of 2020 using the 30% missingness dataset, which had an optimal K of 2 (Figure S3). The mean percentage of individual missingness was high in individuals assigned to cluster 2 compared to cluster 1 (e.g., 2020; cluster 1: $3.78\% \pm 0.40$, N=82; cluster 2: $21.15\% \pm 1.14$, N=18), which may partially explain the detection of two genetic clusters. Furthermore, the clusters did not follow any pattern of clustering by age, sex or host species. When analysing each year separately using STRUCTURE, K=1 was also deduced with the MedK method for each year. Across all analyses, the individuals assigned to the second or third clusters were not supported by assignment probabilities and did not show a pattern across year, host species, age or sex. Therefore, the P. downsi population on Floreana shows little to no evidence for restricted gene flow or genetic divergence across the sampling period.

Effective population size and bottlenecks

Estimates of the effective population size of *P. downsi* on Floreana Island were low and varied across years (Table S7). Estimated N_e using COLONY was highest in 2006 (10% missingness: N_e =420, 95% jack-knifed CI=115- ∞ , Table S7) and 2014 (10% missingness: N_e =342, 95% jack-knifed CI=133- ∞ ,

Table 1 Observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), allelic diversity and richness calculated for all years combined and separated by year on the 10% missingness dataset. Mean AR = Mean Allelic Richness

	*						
	N	H _o	H _e	F_{IS}	# of alleles	Mean AR	# Private alleles
All	121	0.290	0.383	0.243			
2006	15	0.323	0.363	0.112	1.913	2.06	0
2008	14	0.255	0.393	0.352	1.934	2.30	0
2014	19	0.278	0.394	0.294	1.966	2.35	0
2020	73	0.290	0.383	0.206	2.000	2.38	4



Table S7). Estimated N_e was lowest in 2020 (10% missingness: N_e =64, 95% jack-knifed CI=45–93, Table S7). However, many of the upper 95% jack-knifed confidence intervals were undefined, and so we interpret the N_e values as relative estimates across years and with significant caution. Evidence for population bottlenecks was supported by a mode-shift distortion of allele frequencies from the expected L-shaped distribution in all study years (Figure S4), detected by BOTTLENECK. There was a significant deviation from expected equilibrium heterozygosity using both the IAM and the SMM (Table S8, sign test: P<0.001) in all study years.

Infrapopulation genetic relatedness and sibship reconstruction

Mean individual pairwise relatedness varied between $P.\ downsi$ infrapopulations, ranging from -0.089 to 0.205 (10%: 0.079 ± 0.016 ; 30%: 0.027 ± 0.030). Relatedness did not vary with the percentage of the infrapopulation genotyped (Table S9, 10% range: 13.3-36.0% genotyped per nest; 0.001 ± 0.003 , P=0.709) or infrapopulation size in either dataset (Table S9, 10%: -0.001 ± 0.001 , P=0.344). Therefore, our genetic relatedness estimates were not affected by genotyping effort per infrapopulation.

The percentage of individual missingness (i.e., 10% or 30% dataset) did not significantly affect the number of putative parental genotypes identified in each nest (Table S10, putative maternal genotypes: $F_{1,50} = 1.63$, P = 0.405; putative paternal genotypes: $F_{1,50} = 10.21$, P = 0.182). There was also no effect of error rate on

Table 2 Results of sibship reconstruction analysis for all 2020 data with 10% individual missingness (N=10 nests). Data are presented as mean \pm SE; range. Value descriptions are as follows: # female infestations: mean number of reconstructed female genotypes per infrapopulation; # paternal genotypes: mean number of reconstructed male genotypes per infrapopulation.

number of parental genotypes (Table S10, putative maternal genotypes: $F_{1,50}$ =2.11, P=0.636; putative males: $F_{1,50}$ =13.78, P=0.299). The number of putative maternal or paternal genotypes did not differ with percentage of the infrapopulation genotyped or infrapopulation size (Table S11- S12). Thus, our genotyping effort was sufficient to characterise the sibship relationships within infrapopulations.

All results below are presented for the 10% missingness dataset at 5% error rate (Table 2, see Table S13 for results of 30% missingness dataset, which gave similar results). Multiple female infestations per nest were common, with an estimate of 4.88 ± 0.48 (range = 3-7) maternal genotypes estimated from each nest. Each of these maternal genotypes were associated with 14.3-33.3% of the infrapopulation. The number of P. downsi offspring assigned to each maternal genotype ranged between 1.00 and 3.00 (Table 2, mean = 1.89 ± 0.28). The number of paternal genotypes contributing to each infrapopulation was 6.13 ± 0.61 (range = 3–7). There was evidence for female multiple mating, with a mean of 1.27 ± 0.09 (range = 1.00-1.75) paternal genotypes per maternal genotype.

Discussion

Understanding how demographic and reproductive processes change throughout parasite invasion is critical for tracking novel host-parasite interactions and the success of control techniques, especially when native host species are at risk. We found evidence

lation; # males per female: mean number of males assigned to the offspring of each female per nest; # offspring per female: mean number of offspring assigned to each maternal genotype; % total offspring per female: mean percentage of the total offspring contributed by each female per infrapopulation

Error rate	# Female infestations	# Paternal genotypes	# Males per female	# Offspring per female	% Total offspring per female
1%	$5.13 \pm 0.52;$	$6.63 \pm 0.71;$	$1.30 \pm 0.12;$	$1.80 \pm 0.26;$	$21.07 \pm 2.29;$
	3-7	3-8	1.00-2.00	1.00-3.00	14.29-33.33
5%	$4.88 \pm 0.48;$ 3-7	$6.13 \pm 0.61;$ 3-8	1.27 ± 0.09 ; 1.00-1.75	$1.89 \pm 0.28;$ 1.00-3.00	$22.00 \pm 2.22;$ 14.29-33.33
10%	$4.75 \pm 0.53;$	$5.63 \pm 0.60;$	$1.21 \pm 0.10;$	$2.01 \pm 0.34;$	$23.04 \pm 2.63;$
	3–7	3-7	1.00-1.75	1.00-3.20	16.67-33.33



that female reproductive behaviour has shifted since the 2004-2006 genetic study conducted by Dudaniec et al. (2010). Compared to 2004-2006, the number of female infestations per nest increased by ~70% from 2004 to 2020 (Fig. 1). The number of paternal genotypes per nest remained stable (5.44 ± 0.45) in Dudaniec et al. 2010, compared to 6.13 ± 0.61 , Fig. 1), but the number of reported males per female (i.e., rate of multiple mating) decreased ~ 35%. Concordantly, the genetically assigned females in 2020 had fewer offspring assigned to each of them per nest, with a smaller percentage of the infrapopulation assigned to each maternal genotype comparing time periods (2004–2006: $43.6\% \pm 4.38$, from Dudaniec et al. 2010; 2020: 22.0% ± 2.22). Female P. downsi are investing fewer offspring per nest, but more females are ovipositing in each nest. These changed have occurred with no significant change

to parasite intensity within nests over the sampling period (2004–2006: 30.8 ± 16.5 , Dudaniec et al. 2010; 2021: 43.4 ± 8.3 ; Common et al. 2021). Our study did not detect any genetic clustering of P. downsi on Floreana Island across our four sampling years between 2006 and 2020, however F_{ST} indicated some genetic differentiation between years. Although there was no compelling evidence for neutral genetic structure, the changes in reproductive behaviour we report suggest that P. downsi may be under selection pressures that could lead to adaptation to its novel environment and hosts.

Shifts in reproductive behaviour

Sibship reconstructions of *P. downsi* offspring collected from nests revealed temporal shifts in female reproductive behaviour over a 14-year period.

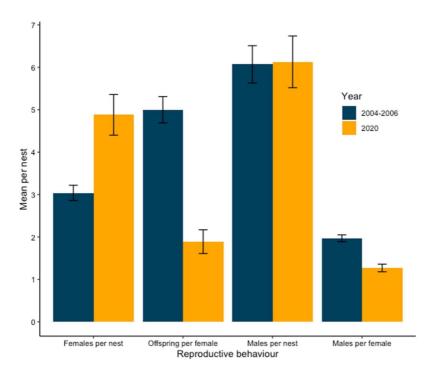


Fig. 1 Summary of COLONY results, comparing the results found by Dudaniec et al. (2010) from *P. downsi* collected in Darwin's finch nests between 2004 and 2006 (microsatellite data, with typing errors (5%); N nests = 57; N indi-

viduals=1020), to *P. downsi* collected from Darwin's finch nests in 2020 (SNP data, 10% missingness, 5% error dataset; N nests=10; N individuals=77). Data are presented as mean \pm standard error



Between 2004 and 2006, Dudaniec et al. (2010) found that on average, approximately three females oviposited in a single nest, each mating with an approximate average of two males and contributing an average of five offspring per nest. Compared to the previous microsatellite data that used the same analytical approach (Dudaniec et al. 2010), we found evidence that P. downsi females are mating with fewer males on average, more females are ovipositing per nest, and each female is contributing fewer offspring per nest (Fig. 1). Although the number of loci used in this study was low (469 loci at 10% missingness and 462 loci at 30% missingness), it is sufficient for reconstructing family structure as ~ 10 SNPs is equivalent to one microsatellite (Wang and Santure 2009). Thus, this genetic dataset has much higher resolution than the previous 2010 study (Dudaniec et al. 2010), which used eight microsatellites (Dudaniec et al. 2008b). The two different datasets may have differences in data resolution and therefore this may account for some of the differences in the results. However, studies evaluating the performance of SNPs versus microsatellites found high power to resolve relatedness and parentage when using 100-500 SNPs, and results between the two types of data were highly congruent (Flanagan and Jones 2019; Premachandra et al. 2019; Weng et al. 2021). Despite the congruence of the two datasets, we note that there may be inherent variation in their results due to the differences in genetic approach, thus care should be taken when comparing them. The maximum likelihood method implemented in COLONY (Jones and Wang 2010) is robust even with high inbreeding coefficients (~0.4; Wang and Santure 2009). It is also important to note that we were unable to genotype smaller first and second instar larvae collected from the nests, and therefore we may have missed offspring from females ovipositing later in the nesting cycle. This methodology was also used by Dudaniec et al. (2010), with only a small proportion (1%) of genotyped specimens being second instar. Hence, our estimates of the number of females per nest may be underestimated but are comparable to Dudaniec et al. (2010).

Mating of females to multiple males (i.e., polyandry) is common in insects and generally beneficial to female fecundity (Arnqvist and Nilsson 2000; Dunn et al. 2005; Abraham et al. 2011). However, increased multiple mating can decrease female longevity in insects, due to the increased cost of both mating and

egg production (Arnqvist and Nilsson 2000; Kawagoe et al. 2001; Gotoh and Tsuchiya 2008). Given that females must survive during the arid, non-breeding period on the Galápagos (Causton et al. 2019; Bulgarella et al. 2022), selection for increased female longevity may be driving the observed decrease in multiple mating. The costs and benefits of polyandry in *P. downsi* are currently not understood. Research into the fitness of invasive species under different rates of multiple mating may further our understanding of the functional significance of changes to mating behaviour.

There are several potential explanations for, and fitness consequences of, changing oviposition behaviour in P. downsi. Female body size, in particular abdomen size, has decreased on Floreana Island between 2004 and 2016 (Common et al. 2020). Female body and abdomen size are correlated with fecundity (i.e., number of eggs laid: Pincheira-Donoso and Hunt 2017; Common et al. 2020). Smallerbodied females may have fewer eggs to lay, and therefore may lay fewer eggs overall or per nest. Because of this, more females may be able to oviposit per nest, without increasing intensity. Female insects and parasites have been known to adjust their clutch size depending on conspecific density, competition, or host quality (Van Alphen and Visser 1990; Damman 1991; Visser and Rosenheim 1998; Díaz-Fleischer and Aluja 2003; Aluja et al. 2019). A decrease in the number of eggs laid per nest may also be due to smaller host size (Díaz-Fleischer and Aluja 2003; Dudaniec et al. 2007). Nestlings are dying younger, and younger nestlings are smaller than older nestlings (G. fuliginosa and C. parvulus nestling body mass increases approximately 1 g per day: Kleindorfer et al. unpublished data) meaning P. downsi larvae are feeding on smaller hosts than in earlier years (Kleindorfer et al. 2014). Earlier age of host death also creates an unreliable resource, as host death, often due to P. downsi parasitism, could occur before larvae are ready to pupate. The unreliability of host resources could contribute to declining parasite clutch sizes, as females oviposit fewer eggs in more nests as a form of spatial bet hedging (Hopper 1999; McLaughlin and Wasserberg 2021). Due to small sample sizes of our two host species, we were unable to analyse P. downsi reproductive behaviour between species, which is important for continued monitoring of host-parasite dynamics. Exploration of P. downsi clutch size on



larger-bodied hosts, such as the Galápagos Mockingbird (*Mimus parvulus*), which is better able to tolerate *P. downsi* parasitism (Knutie et al. 2016), may reveal differences in oviposition behaviour between different host species.

The changes in oviposition behaviour we report may also have implications for avian host fitness, as their nests are forced to sustain increasingly unrelated cohorts of parasites in their nest. When the most optimal parasite strategy requires prudent exploitation of the host (i.e., when parasite transmission requires a living host or requires host resources to reach maturity), increasing parasite relatedness is predicted to decrease virulence (i.e., damage to the host, Buckling and Brockhurst 2008). This is because cooperation between highly related individuals results in indirect fitness benefits for the parasite, i.e., lowered virulence ensures the host survives, allowing for parasites to reach maturity at greater rates, therefore maximising inclusive fitness (Buckling and Brockhurst 2008). Alternatively, where cohorts are highly unrelated, more virulent parasites have a competitive advantage, selecting for increased host exploitation to increase individual parasite development and fitness (Frank 1992; Buckling and Brockhurst 2008). Increased parasite relatedness has further been found to increase parasite transmission, decrease host reproduction and survival (Davies et al. 2002; Gleichsner et al. 2018). Darwin's finches may therefore be facing increased exploitation by P. downsi as infrapopulation relatedness decreases, potentially increasing nestling mortality and driving decreasing host age at death (Kleindorfer et al. 2014).

Genetic diversity and bottlenecks

Genetic drift or bottleneck events after species' introductions can decrease genetic variation, with a strong negative effect on allelic richness, particularly shortly after the event occurs (Dlugosch and Parker 2008; Santos et al. 2012). Allelic richness increased in *P. downsi* across years from 2.06 to 2.38. Allelic richness and heterozygosity in invasive populations increase and stabilize over large time scales, with high gene flow, and with multiple introductions (Dlugosch and Parker 2008; Greenbaum et al. 2014). Observed heterozygosity in *P. downsi* was lower compared to Dudaniec et al. (2008a, b) from 2004 to 2006, but consistent with Koop et al. (2020) from 2015 to 2017.

The decrease in heterozygosity we detect across time, particularly in earlier years (2006–2014) may be due to genetic drift after introduction or bottleneck effects. Multiple introductions of *P. downsi* to the archipelago from the mainland have not been investigated, so historical introductions may have occurred. As no *P. downsi* have been detected on the Galápagos prior to 1964, there may have been enough generations after invasion to recover from founder effects.

We found evidence for a recent bottleneck in all years, consistent with past studies (Dudaniec et al. 2008a). With low rainfall and hence a paucity of active hosts, coupled with shorter male survivorship (188 days) compared with females (265 days; Causton et al. 2019), the annual bottleneck on Floreana Island may be associated with high male (and likely female) mortality at the end of the year, prior to the onset of the next fly breeding season. Therefore, large variation in annual P. downsi populations are expected, not only because of the initial colonisation bottleneck, but also given annual reductions in population size after the host breeding season ends (Causton et al. 2019). Only relatively small numbers of adults survive to the next breeding season (Causton et al. 2019; Bulgarella et al. 2022), and therefore only a small proportion of the census population contributes to the next generation.

We report a high inbreeding coefficient ($F_{\rm IS}$) in *P. downsi* across years of 0.243. Studies have found that $F_{\rm IS}$ can be correlated with the proportion of missing data, and when data were not missing at random (Marandel et al. 2020). Therefore, our results may be biased towards a higher $F_{\rm IS}$; although values of our 10% dataset are in line with those found on Floreana by Koop et al. (2020; $F_{\rm IS}$ =0.19). Although high, our reported $F_{\rm IS}$ was also consistent with estimates for *P. downsi* on other islands, on the South American mainland (Koop et al. 2020), and with other invasive insect populations (Karsten et al. 2013; Kirk et al. 2013; Andersen and Mills 2018; Do et al. 2022).

Lack of genetic structure

Our study found no evidence for genetic clustering in the P. downsi Floreana Island population within and across years, suggesting significant gene flow that is consistent across time, and thus, suggests the island is not genetically isolated. Genetic differentiation (F_{ST}) was lower in this study than previously found in



P. downsi populations (Dudaniec et al. 2008a; Koop et al. 2020). However, this is not unexpected as previous studies compared genetic differentiation across islands and habitats, whereas this study compared within a single island and habitat across time. Despite low F_{ST} values, pairwise comparisons between years were all significant, indicating subtle genetic differentiation across the years examined. This differentiation may be affected by the annual population bottlenecks we detected and as suggested by other studies of P. downsi (Dudaniec et al. 2008a; Causton et al. 2019). Philornis downsi shows high dispersal capability between habitats and islands (Dudaniec et al. 2008a; Fessl et al. 2018; Koop et al. 2020), and combined with frequent tourist movement (Toral-Granda et al. 2017) and strong air currents (Peck 1994) between islands, it is likely that the island populations of P. downsi are well-connected. The lack of genetic structure detected in this study could support this, however more study is required to fully understand P. downsi movement and gene flow across the entire archipelago, and how these drive or restrict genetic differentiation of separate island populations.

High gene flow may swamp selection and therefore local adaptation to specific islands or host species (Tigano and Friesen 2016; Jacob et al. 2017), but testing for selection was beyond the scope of our data resolution. It is possible that P. downsi may still be adapting to the habitat and hosts of the Galápagos Islands, given high genetic differentiation and low gene flow reported between the archipelago and mainland source populations in Ecuador (Koop et al. 2020). Evidence for gene flow between islands in more recent studies (Koop et al. 2020), and the lack of genetic structure we report on Floreana suggests that P. downsi on this island is no longer showing signs of genetic isolation from other islands, as concluded by Dudaniec et al. (2008a) who examined gene flow between three islands.

Effective population size

It is important to note the infinite values and upper bounds in our estimates of effective population size, which indicate that values of $N_{\rm e}$ could be much higher than reported (Wang 2009; Do et al. 2014). We emphasize that these results should be interpreted with caution. The small $N_{\rm e}$ values may be an artefact of our sampling design or poor data resolution.

Particularly in the 2020 study year, a higher proportion of specimens were collected from nests. Although highly related individuals were removed before analysis, it is possible that this sampling design artificially inflated sibship frequencies and hence underestimated the effective population size. Larger numbers of individuals collected from traps with increased number of loci analysed could refine the estimation of effective population size.

The 'relative' effective population size of P. downsi on Floreana Island showed considerable variation between years. N_e was highest in 2006 and 2014 (but values for 2008 could not be obtained for the 10% missingness dataset), and lowest in 2020. Notably, this temporal decrease matches a decrease in mean in-nest P. downsi intensities (Common et al. 2021) and daily catch rates of adult P. downsi across the sampling years (2014: 0.35 ± 0.03 ; Kleindorfer et al. unpublished data; 2020: 0.28 ± 0.02; Common et al. 2022). The estimated values for effective population size were significantly smaller than expected, given the widespread distribution and high in-nest intensity of P. downsi. However, there are many reasons why Ne may be lower than expected. Positive selection (Charlesworth 2009), individual variation in reproductive success (Hedrick 2005), and temporal variation in population size (Hedrick 2009) can all result in smaller N_a in relation to census size, with evidence for all these factors present in *P. downsi*.

Missing data

Due to high rates of missing data in our dataset, we considered the effects of individual missingness on our results. To maximise the number of individuals included in the dataset, we analysed the data with a maximum of 30% missingness, and to maximise the confidence in our data, we analysed the data with a maximum of 10% missingness. Overall, we found results between the 10% and 30% individual missingness data sets were congruent. As stated previously, the inbreeding coefficient, F_{IS}, was also higher in the 30% missingness dataset, likely because F_{IS} is correlated with missingness where missing data is nonrandom (Marandel et al. 2020). Although there were differences in cluster assignments between the two datasets, the number of genetic clusters remained at one for both rates of missingness. We anticipate that missingness in the data likely drove the detection of



 $K\!=\!2$ or 3, and therefore it is important to understand how missingness is distributed across individuals within each cluster, as this may bias results and lead to falsely identifying genetic clusters (Yi and Latch 2022). For the in-nest relatedness and sib-ship reconstruction analyses, we did not find an effect of missingness on the number of putative maternal or paternal genotypes. Therefore, although 30% missingness was sufficient in our study to explore genetic structure and relatedness, researchers must be aware of the biases associated with high missingness datasets. Where missing data is a problem, we recommend a more conservative value of 10% individual missingness to ensure high confidence of results."

Management implications

The lack of genetic structure and divergence in P. downsi between years suggests a lack of isolation and frequent gene flow from other islands to Floreana, and possibly throughout the archipelago. Therefore, a targeted, island-specific control method for P. downsi may not be effective in the face of ongoing colonisations. Archipelago-wide strategies, such as increasing quarantine restrictions on boats moving between islands, may be more effective in the longer term. More information on gene flow and migration between human inhabited and uninhabited islands, and Galápagos and the mainland, is needed to fully determine the efficacy of targeted control methods. Changing reproductive behaviours may affect some control methods as female remating is a key factor in the success of SIT. The decrease in remating frequency reported in this study may decrease the risk of females mating with a viable male, and therefore increase SIT success (Hendrichs et al. 2005; Lance and McInnis 2005). Another potential control technique is the utilisation of synthetic gene drives; selfish transgenic elements that bias heredity with the aim to suppress or eradicate populations, such as pests or invasive species (Sinkins and Gould 2006; Bunting et al. 2022). Determining the efficacy of synthetic gene drives requires detailed information of both the genetic structure and life history of target populations (James et al. 2020). The lack of population isolation on Floreana suggests that use of a selfsustaining gene drive on this island could spread throughout the archipelago. Our study, and other

recent genetic and ecological studies on *P. downsi* provide the knowledge base upon which potential control methods can be explored.

Conclusions

While some previous studies have examined spatial genetic variation in P. downsi (Dudaniec et al. 2008a; Koop et al. 2020), here we investigated temporal changes in genetic variation and reproductive behaviours and provided insight into the evolutionary dynamics of this invasive host-parasite system. For the Floreana Island population of P. downsi sampled between 2006 and 2020, we characterised genetic processes and changes approximately 25 years after the fly's discovery in Darwin's finch nests. There was evidence for subtle genetic differentiation between years, however there was limited to no evidence for genetic clustering. If gene flow was restricted to Floreana and the P. downsi population was isolated, we would expect to see genetic differentiation across the 14-year sampling period, particularly given the population bottlenecks we observe, the low estimated effective population size and previous studies indicating hostparasite co-evolution over this period. Changes to reproductive behaviour are concordant with previous morphological shifts in P. downsi, and may suggest host-parasite co-evolutionary interactions or environment adaptations that require further study. The inclusion of multiple locations and comparison to the mainland for exploring signatures of selection would provide a more comprehensive idea of how P. downsi is responding to novel habitats and hosts during its invasion. Decreased relatedness among parasites in nests could be leading to increased host exploitation, and therefore increasing rates of mortality in threatened Darwin's finch populations. Historical and contemporary estimates of genetic migration rates could further resolve the parasite's colonisation history and gene flow across the archipelago. This information could then be used to predict the success of targeted management strategies and how control will affect gene flow and adaptation. Continued research into temporal genetic changes in parasitic populations is imperative for planning adaptive and effective control strategies, and hence the conservation of vulnerable host species.



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Author contributions All authors contributed to the study design and collected the samples. Analysis and the first draft of the manuscript was completed by L.K.C. All authors commented on and edited previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Genetic datasets (filtered vcf files) and sample information used within this study can be accessed from Figshare: https://figshare.com/s/fd7a7d058daf20a498fa.

Declarations

Conflict of interest The authors declare no financial or non-financial competing interests.

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References

- Abraham S, Goane L, Rull J, Cladera J, Willink E, Vera MT (2011) Multiple mating in Anastrepha fraterculus females and its relationship with fecundity and fertility. Entomol Exp Appl 141:15–24. https://doi.org/10.1111/j. 1570-7458.2011.01160.x
- Adamack AT, Gruber B (2014) PopGenReport: simplifying basic population genetic analyses in R. Methods Ecol Evol 5:384–387. https://doi.org/10.1111/2041-210X. 12158
- Aluja M, Birke A, Díaz-Fleischer F, Rull J (2019) Phenotypic plasticity in clutch size regulation among populations of a potential invasive fruit fly from environments that vary in host heterogeneity and isolation. Bull Entomol 109:169–177. https://doi.org/10.1017/S00074853180003 29
- Andersen JC, Mills NJ (2018) Comparative genetics of invasive populations of walnut aphid, Chromaphis juglandicola, and its introduced parasitoid, Trioxys pallidus, in California. Ecol Evol 8:801–811. https://doi.org/10.1002/ece3.3667
- Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Anim Behav 60:145–164. https://doi.org/10.1006/anbe.2000. 1446
- Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE 3:e3376. https:// doi.org/10.1371/journal.pone.0003376
- Beaurepaire AL, Moro A, Mondet F, Le Conte Y, Neumann P, Locke B (2019) Population genetics of ectoparasitic mites suggest arms race with honeybee hosts. Sci Rep 9:11355. https://doi.org/10.1038/s41598-019-47801-5
- Brettell LE, Martin SJ, Riegler M, Cook JM (2021) Vulnerability of island insect pollinator communities to pathogens. J Invertebr Pathol 186:107670. https://doi.org/10.1016/j.jip.2021.107670
- Brown CR, Padhi A, Moore AT, Brown MB, Foster JE, Pfeffer M, O'Brien VA, Komar N (2009) Ecological divergence of two sympatric lineages of Buggy Creek virus, an arbovirus associated with birds. Ecology 90:3168–3179. https://doi.org/10.1890/08-1731.1
- Buckling A, Brockhurst MA (2008) Kin selection and the evolution of virulence. Heredity 100:484–488. https://doi.org/10.1038/sj.hdy.6801093
- Bulgarella M, Quiroga MA, Boulton RA, Ramírez IE, Moon RD, Causton CE, Heimpel GE (2017) Life cycle and host specificity of the parasitoid Conura annulifera (Hymenoptera: Chalcididae), a potential biological control agent of Philornis downsi (Diptera: Muscidae) in the Galápagos islands. Ann Entomol Soc Am 110:317–328. https://doi.org/10.1093/aesa/saw102
- Bulgarella M, Lincango MP, Lahuatte PF, Oliver JD, Cahuana A, Ramírez IE, Sage R, Colwitz AJ, Freund DA, Miksanek JR, Moon RD, Causton CE, Heimpel GE (2022) Persistence of the invasive bird-parasitic fly *Phi*lornis downsi over the host interbreeding period in the



- Galapagos islands. Sci Rep 12:2325. https://doi.org/10. 1038/s41598-022-06208-5
- Bunting MD, Pfitzner C, Gierus L, White M, Piltz S, Thomas PQ (2022) Generation of gene drive mice for invasive pest population suppression. Methods Mol Biol 2495:203–230. https://doi.org/10.1007/978-1-0716-2301-5 11
- Causton CE, Peck SB, Sinclair BJ, Roque-Albelo L, Hodgson CJ, Landry B (2006) Alien insects: threats and implications for conservation of Galápagos islands. Ann Entomol Soc Am 99:121–143. https://doi.org/10.1603/0013-8746(2006)0990121:AITAIFI2.0.CO:2
- Causton CE, Moon RD, Cimadom A, Boulton RA, Cedeño D, Lincango MP, Tebbich S, Ulloa A (2019) Population dynamics of an invasive bird parasite, *Philornis downsi* (Diptera: Muscidae), in the Galapagos Islands. PLoS ONE 14:e0224125. https://doi.org/10.1371/journal.pone. 0224125
- Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience. https://doi.org/10.1186/s13742-015-0047-8
- Charlesworth B (2009) Effective population size and patterns of molecular evolution and variation. Nat Rev Genet 10:195–205. https://doi.org/10.1038/nrg2526
- Chown SL, Hodgins KA, Griffin PC (2016) Biological invasions, climate change, and genomics. Crop Breeding. Apple Academic Press, 59–114
- Cimadom A, Ulloa A, Meidl P, Zöttl M, Zöttl E, Fessl B, Nemeth E, Dvorak M, Cunninghame F, Tebbich S (2014) Invasive parasites, habitat change and heavy rainfall reduce breeding success in Darwin's finches. PLoS ONE 9:e107518. https://doi.org/10.1371/journal.pone.0107518
- Cline TJ, Kitchell JF, Bennington V, McKinley GA, Moody EK, Weidel BC (2014) Climate impacts on landlocked sea lamprey: implications for host-parasite interactions and invasive species management. Ecosphere. https://doi. org/10.1890/ES14-00059.1
- Common LK, Dudaniec RY, Colombelli-Négrel D, Kleindorfer S (2019) Taxonomic shifts in *Philornis* larval behaviour and rapid changes in *Philornis downsi* Dodge & Aitken (Diptera: Muscidae): An invasive avian parasite on the Galápagos Islands. In: Sarwar M (ed) Life cycle and development of Diptera. IntechOpen, England
- Common LK, O'Connor JA, Dudaniec RY, Peters KJ, Klein-dorfer S (2020) Evidence for rapid downward fecundity selection in an ectoparasite (*Philornis downsi*) with earlier host mortality in Darwin's finches. J Evol Biol 33:524–533. https://doi.org/10.1111/jeb.13588
- Common LK, Sumasgutner P, Dudaniec RY, Colombelli-Négrel D, Kleindorfer S (2021) Avian vampire fly (*Philornis downsi*) mortality differs across Darwin's finch host species. Sci Rep 11:15832. https://doi.org/10.1038/s41598-021-94996-7
- Common LK, Sumasgutner P, Sumasgutner SC, Colombelli-Négrel D, Dudaniec RY, Kleindorfer S (2022) Temporal and spatial variation in sex-specific abundance of the avian vampire fly (*Philornis downsi*). Parasitol Res 121:63–74. https://doi.org/10.1007/s00436-021-07350-1
- Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks

- from allele frequency data. Genetics 144:2001–2014. https://doi.org/10.1093/genetics/144.4.2001
- Cristescu ME (2015) Genetic reconstructions of invasion history. Mol Ecol 24:2212–2225. https://doi.org/10.1111/mec.13117
- Crowther LP, Wright DJ, Richardson DS, Carvell C, Bourke AFG (2019) Spatial ecology of a range-expanding bumble bee pollinator. Ecol Evol 9:986–997. https://doi.org/ 10.1002/ece3.4722
- Damman H (1991) Oviposition behaviour and clutch size in a group-feeding Pyralid moth, *Omphalocera munroei*. J Anim Ecol 60:193–204. https://doi.org/10.2307/5454
- Danecek P, Auton A, Abecasis G, Albers CA, Banks E, Depristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R, Genomes Project Analysis G (2011) The variant call format and VCFtools. Bioinformatics 27:2156-2158. https://doi.org/10.1093/ bioinformatics/btr330
- Davies CM, Fairbrother E, Webster JP (2002) Mixed strain schistosome infections of snails and the evolution of parasite virulence. Parasitology 124:31–38. https://doi.org/ 10.1017/S0031182001008873
- Díaz-Fleischer F, Aluja M (2003) Influence of conspecific presence, experience, and host quality on oviposition behavior and clutch size determination in *Anastrepha ludens* (Diptera: Tephritidae). J Insect Behav 16:537– 554. https://doi.org/10.1023/A:1027307424150
- Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x
- Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014) NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol Ecol Resour 14:209–214. https://doi.org/10.1111/1755-0998.12157
- Do Y, Park W-B, Park J-K, Kim CJ, Choi MB (2022) Genetic and morphological variation of Vespa velutina nigrithorax which is an invasive species in a mountainous area. Sci Rep 12:4737. https://doi.org/10.1038/ s41598-022-08756-2
- Dudaniec RY, Fessl B, Kleindorfer S (2007) Interannual and interspecific variation in intensity of the parasitic fly, *Philornis downsi*, in Darwin's finches. Biol Conserv 139:325–332. https://doi.org/10.1016/j.biocon.2007.07. 006
- Dudaniec RY, Gardner MG, Donnellan S, Kleindorfer S (2008a) Genetic variation in the invasive avian parasite, *Philornis downsi* (Diptera, Muscidae) on the Galápagos archipelago. BMC Ecol 8:13. https://doi.org/10.1186/1472-6785-8-13
- Dudaniec RY, Gardner MG, Kleindorfer S (2008b) Isolation, characterization and multiplex polymerase chain reaction of novel microsatellite loci for the avian parasite *Philornis downsi* (Diptera: Muscidae). Mol Ecol Resour 8:142–144. https://doi.org/10.1111/j.1471-8286.2007. 01900.x
- Dudaniec RY, Gardner MG, Kleindorfer S (2010) Offspring genetic structure reveals mating and nest infestation behaviour of an invasive parasitic fly (*Philornis downsi*)



- of Galápagos birds. Biol Invasions 12:581–592. https://doi.org/10.1007/s10530-009-9464-x
- Dudaniec RY, Carey AR, Svensson EI, Hansson B, Yong CJ, Lancaster LT (2022) Latitudinal clines in sexual selection, sexual size dimorphism, and sex-specific genetic dispersal during a poleward range expansion. J Anim Ecol 91:1104–1118. https://doi.org/10.1111/1365-2656. 13488
- Dunn DW, Sumner JP, Goulson D (2005) The benefits of multiple mating to female seaweed flies, *Coelopa frigida* (Diptera: Coelpidae). Behav Ecol Sociobiol 58:128–135. https://doi.org/10.1007/s00265-005-0922-x
- Dyck VA, Hendrichs J, Robinson AS (2021) Sterile insect technique: principles and practice in area-wide integrated pest management. Taylor & Francis, Oxfordshire United Kingdom
- Early R, Bradley BA, Dukes JS, Lawler JJ, Olden JD, Blumenthal DM, Gonzalez P, Grosholz ED, Ibañez I, Miller LP, Sorte CJB, Tatem AJ (2016) Global threats from invasive alien species in the twenty-first century and national response capacities. Nat Commun 7:12485. https://doi. org/10.1038/ncomms12485
- Emde S, Rueckert S, Kochmann J, Knopf K, Sures B, Klimpel S (2014) Nematode eel parasite found inside acanthocephalan cysts - a "Trojan horse" strategy? Parasit Vectors 7:504. https://doi.org/10.1186/s13071-014-0504-8
- Enkerlin WR, Gutiérrez Ruelas JM, Pantaleon R, Soto Litera C, Villaseñor Cortés A, Zavala López JL, Orozco Dávila D, Montoya Gerardo P, Silva Villarreal L, Cotoc Roldán E, Hernández López F, Arenas Castillo A, Castellanos Dominguez D, Valle Mora A, Rendón Arana P, Cáceres Barrios C, Midgarden D, Villatoro Villatoro C, Lira Prera E, Zelaya Estradé O, Castañeda Aldana R, López Culajay J, Ramírez y Ramírez F, Liedo Fernández P, Ortíz Moreno G, Reyes Flores J, Hendrichs J (2017) The Moscamed regional programme: review of a success story of area-wide sterile insect technique application. Entomol Exp Appl 164:188–203. https://doi.org/10.1111/eea.12611
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x
- Feis ME, Goedknegt MA, Thieltges DW, Buschbaum C, Wegner KM (2016) Biological invasions and host–parasite coevolution: different coevolutionary trajectories along separate parasite invasion fronts. Zoology 119:366–374. https://doi.org/10.1016/j.zool.2016.05.012
- Fessl B, Tebbich S (2002) *Philornis downsi* a recently discovered parasite on the Galápagos archipelago— a threat for Darwin's finches? Ibis 144:445–451. https://doi.org/10.1046/j.1474-919X.2002.00076.x
- Fessl B, Couri MS, Tebbich S (2001) Philornis downsi Dodge & Aitken, new to the Galapagos islands (Diptera, Muscidae). Stud Dipterol 8:317–322
- Fessl B, Kleindorfer S, Tebbich S (2006a) An experimental study on the effects of an introduced parasite in Darwin's finches. Biol Conserv 127:55–61. https://doi.org/10. 1016/j.biocon.2005.07.013
- Fessl B, Sinclair BJ, Kleindorfer S (2006b) The life-cycle of *Philornis downsi* (Diptera: Muscidae) parasitizing

- Darwin's finches and its impacts on nestling survival. Parasitology 133:739–747. https://doi.org/10.1017/ S0031182006001089
- Fessl B, Heimpel GE, Causton CE (2018) Invasion of an avian nest parasite, *Philornis downsi*, to the Galápagos Islands: Colonization history, adaptations to novel ecosystems, and conservation challenges. In: Parker PG (ed) Disease ecology: galapagos birds and their parasites. Springer International Publishing, Cham, pp 213–266
- Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194. https://doi.org/10.1038/nature10947
- Flanagan SP, Jones AG (2019) The future of parentage analysis: from microsatellites to SNPs and beyond. Mol Ecol 28:544–567. https://doi.org/10.1111/mec.14988
- Frank SA (1992) A kin selection model for the evolution of virulence. Proc R Soc Lond Ser B: Biol Sci 250:195–197. https://doi.org/10.1098/rspb.1992.0149
- Gaskin JF, Bon M-C, Cock MJW, Cristofaro M, Biase AD, De Clerck-Floate R, Ellison CA, Hinz HL, Hufbauer RA, Julien MH, Sforza R (2011) Applying molecularbased approaches to classical biological control of weeds. Biol Control 58:1–21. https://doi.org/10.1016/j. biocontrol.2011.03.015
- Gato R, Menéndez Z, Prieto E, Argilés R, Rodríguez M, Baldoquín W, Hernández Y, Pérez D, Anaya J, Fuentes I, Lorenzo C (2021) Sterile insect technique: successful suppression of an Aedes aegypti field population in Cuba. Insects. 12(5):469
- Gleichsner AM, Reinhart K, Minchella DJ (2018) The influence of related and unrelated co-infections on parasite dynamics and virulence. Oecologia 186:555–564. https://doi.org/10.1007/s00442-017-4035-9
- Gotoh T, Tsuchiya A (2008) Effect of multiple mating on reproduction and longevity of the phytoseiid mite Neoseiulus californicus. Exp Appl Acarol 44:185. https:// doi.org/10.1007/s10493-008-9143-0
- Goudet J (2005) hierfstat, a package for r to compute and test hierarchical F-statistics. Mol Ecol Notes 5:184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x
- Greenbaum G, Templeton AR, Zarmi Y, Bar-David S (2014) Allelic richness following population founding events—a stochastic modeling framework incorporating gene flow and genetic drift. PLoS ONE 9:e115203. https://doi.org/10.1371/journal.pone.0115203
- Guppy JL, Jones DB, Kjeldsen SR, Le Port A, Khatkar MS, Wade NM, Sellars MJ, Steinig EJ, Raadsma HW, Jerry DR, Zenger KR (2020) Development and validation of a RAD-Seq target-capture based genotyping assay for routine application in advanced black tiger shrimp (*Penaeus monodon*) breeding programs. BMC Genom 21:541. https://doi.org/10.1186/s12864-020-06960-w
- Hedrick P (2005) Large variance in reproductive success and the Ne/N ratio. Evolution 59:1596–1599. https://doi. org/10.1111/j.0014-3820.2005.tb01809.x
- Hedrick PW (2009) Genetics of populations. Jones & Bartlett Publishers, Massachusetts
- Hendrichs J, Vreysen M, Enkerlin W, Cayol J, Dyck V, Robinson A (2005) Strategic options in using sterile insects for area-wide integrated pest management. In: Dyck

- VA, Hendrichs J, Robinson AS (eds) Sterile insect technique. Springer, Dordrecht, p 841
- Himuro C, Kohama T, Matsuyama T, Sadoyama Y, Kawamura F, Honma A, Ikegawa Y, Haraguchi D (2022) First case of successful eradication of the sweet potato weevil, Cylas formicarius (Fabricius), using the sterile insect technique. PLoS ONE 17:e0267728. https://doi.org/10.1371/journal.pone.0267728
- Hopper KR (1999) Risk-spreading and bet-hedging in insect population biology. Annu Rev Entomol 44:535–560. https://doi.org/10.1146/annurev.ento.44.1.535
- Jacob S, Legrand D, Chaine AS, Bonte D, Schtickzelle N, Huet M, Clobert J (2017) Gene flow favours local adaptation under habitat choice in ciliate microcosms. Nat Ecol Evol 1:1407–1410. https://doi.org/10.1038/ s41559-017-0269-5
- Jactel H, Desprez-Loustau M-L, Battisti A, Brockerhoff E, Santini A, Stenlid J, Björkman C, Branco M, Dehnen-Schmutz K, Douma JC (2020) Pathologists and entomologists must join forces against forest pest and pathogen invasions. NeoBiota 58:107. https://doi.org/10. 3897/neobiota.58.54389
- James SL, Marshall JM, Christophides GK, Okumu FO, Nolan T (2020) Toward the definition of efficacy and safety criteria for advancing gene drive-modified mosquitoes to field testing. Vector-Borne and Zoonotic Diseases 20:237–251. https://doi.org/10.1089/vbz.2019.2606
- Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403– 1405. https://doi.org/10.1093/bioinformatics/btn129
- Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94. https://doi.org/10.1186/1471-2156-11-94
- Jones OR, Wang J (2010) COLONY: A program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555. https://doi.org/10.1111/j. 1755-0998.2009.02787.x
- Kamenova S, Bartley TJ, Bohan DA, Boutain JR, Colautti RI, Domaizon I, Fontaine C, Lemainque A, Le Viol I, Mollot G, Perga ME, Ravigné V, Massol F (2017) Chapter three—invasions toolkit: current methods for tracking the spread and impact of invasive species. In: Bohan DA, Dumbrell AJ, Massol F (eds) Advances in ecological research. Academic Press, pp 85–182
- Karsten M, van Vuuren BJ, Barnaud A, Terblanche JS (2013) Population genetics of Ceratitis capitata in South Africa: implications for dispersal and pest management. PLoS ONE 8:e54281. https://doi.org/10.1371/journal.pone. 0054281
- Katsis AC, Colombelli-Négrel D, Common LK, O'connor JA, Dudaniec RY, García-Loor J, Kleindorfer S (2021) Nestling behaviour predicts naris deformation in Darwin's finches parasitized by the avian vampire fly. Biol J Linn Soc. https://doi.org/10.1093/biolinnean/blab092
- Kawagoe T, Suzuki N, Matsumoto K (2001) Multiple mating reduces longevity of females of the windmill butterfly Atrophaneura alcinous. Ecol Entomol 26:258–262. https://doi.org/10.1046/j.1365-2311.2001.00326.x
- Keller SR, Taylor DR (2008) History, chance and adaptation during biological invasion: separating stochastic

- phenotypic evolution from response to selection. Ecol Lett 11:852–866. https://doi.org/10.1111/j.1461-0248. 2008.01188.x
- Kirk H, Dorn S, Mazzi D (2013) Worldwide population genetic structure of the oriental fruit moth (*Grapholita molesta*), a globally invasive pest. BMC Ecol 13:12. https://doi. org/10.1186/1472-6785-13-12
- Kleindorfer S, Dudaniec RY (2016) Host-parasite ecology, behavior and genetics: a review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. BMC Zool 1:1. https://doi.org/10.1186/s40850-016-0003-9
- Kleindorfer S, Peters KJ, Custance G, Dudaniec RY, O'Connor JA (2014) Changes in *Philornis* infestation behavior threaten Darwin's finch survival. Curr Zool 60:542–550. https://doi.org/10.1093/czoolo/60.4.542
- Kleindorfer S, Peters KJ, Hohl L, Sulloway FJ (2016) Flight behaviour of an introduced parasite affects its Galapagos island hosts: *Philornis downsi* and Darwin's finches. In: Weis JS, Sol D (eds) Biological invasions and animal behaviour. Cambridge University Press, Cambridge, pp 158–79
- Kleindorfer S, Common LK, Sumasgutner P (2021) Nesting success and nesting height in the critically endangered medium tree finch (*Camarhynchus pauper*). Birds 2(4):427–444
- Knutie SA, Owen JP, McNew SM, Bartlow AW, Arriero E, Herman JM, DiBlasi E, Thompson M, Koop JA, Clayton DH (2016) Galápagos mockingbirds tolerate introduced parasites that affect Darwin's finches. Ecology. 97(4):940–50
- Kogura Y, Seeb JE, Azuma N, Kudo H, Abe S, Kaeriyama M (2011) The genetic population structure of lacustrine sockeye salmon, Oncorhynchus nerka, in Japan as the endangered species. Environ Biol Fishes 92:539–550. https://doi.org/10.1007/s10641-011-9876-1
- Koop JAH, Causton CE, Bulgarella M, Cooper E, Heimpel GE (2020) Population structure of a nest parasite of Darwin's finches within its native and invasive ranges. Conserv Genet. https://doi.org/10.1007/s10592-020-01315-0
- Lahuatte PF, Lincango MP, Heimpel GE, Causton CE (2016) Rearing larvae of the avian nest parasite, *Philornis downsi* (Diptera: Muscidae), on chicken blood-based diets. J Insect Sci 16(1):84
- Lance D, McInnis D (2005) Biological basis of the sterile insect technique. Sterile Insect Technique. Springer, pp. 69–94
- Laugier GJM, Le Moguédec G, Tayeh A, Loiseau A, Osawa N, Estoup A, Facon B (2013) Increase in male reproductive success and female reproductive investment in invasive populations of the Harlequin Ladybird *Harmonia axy*ridis. PLoS ONE 8:e77083. https://doi.org/10.1371/journ al.pone.0077083
- Lawson Handley LJ, Estoup A, Evans DM, Thomas CE, Lombaert E, Facon B, Aebi A, Roy HE (2011) Ecological genetics of invasive alien species. Biocontrol 56:409–428. https://doi.org/10.1007/s10526-011-9386-2
- Lawson LP, Fessl B, Hernán Vargas F, Farrington HL, Francesca Cunninghame H, Mueller JC, Nemeth E, Christian Sevilla P, Petren K (2017) Slow motion extinction: inbreeding, introgression, and loss in the critically endangered mangrove finch (Camarhynchus heliobates).



- Conserv Genet 18:159–170. https://doi.org/10.1007/s10592-016-0890-x
- Le Roux J (2021) The Evolutionary Ecology of Invasive Species. Elsevier Science, Amsterdam
- Leger EA, Espeland EK (2010) PERSPECTIVE: coevolution between native and invasive plant competitors: implications for invasive species management. Evol Appl 3:169– 178. https://doi.org/10.1111/j.1752-4571.2009.00105.x
- Li Y-L, Liu J-X (2018) StructureSelector: a web-based software to select and visualize the optimal number of clusters using multiple methods. Mol Ecol Resour 18:176– 177. https://doi.org/10.1111/1755-0998.12719
- Lincango P, Causton C (2009) Ensayos de atrayentes para la captura de la mosca parásito, *Philornis downsi* (Diptera: Muscidae) en las Islas Galápagos. Puerto Ayora, Galapagos, Ecuador: Charles Darwin Foundation
- Liu Y, Henkel J, Beaurepaire A, Evans JD, Neumann P, Huang Q (2021) Comparative genomics suggests local adaptations in the invasive small hive beetle. Ecol Evol 11:15780–15791. https://doi.org/10.1002/ece3.8242
- Lombaert E, Estoup A, Facon B, Joubard B, Grégoire JC, Jannin A, Blin A, Guillemaud T (2014) Rapid increase in dispersal during range expansion in the invasive ladybird Harmonia axyridis. J Evol Biol 27:508–517. https://doi.org/10.1111/jeb.12316
- Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Hered 89:238– 247. https://doi.org/10.1093/jhered/89.3.238
- Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen W-M (2010) Robust relationship inference in genome-wide association studies. Bioinformatics 26:2867–2873. https://doi.org/10.1093/bioinformatics/ bto559
- Marandel F, Charrier G, Lamy J-B, Le Cam S, Lorance P, Trenkel VM (2020) Estimating effective population size using RADseq: effects of SNP selection and sample size. Ecol Evol 10:1929–1937. https://doi.org/10.1002/ece3. 6016
- Mayer M, Shine R, Brown GP (2021) Rapid divergence of parasite infectivity and host resistance during a biological invasion. Biol J Linn Soc 132:861–871. https://doi.org/ 10.1093/biolinnean/blaa229
- McIntire KM, Juliano SA (2021) Detrimental effects of a failed infection by a co-invasive parasite on a native congeneric parasite and its native host. Biol Invasions 23:1637– 1648. https://doi.org/10.1007/s10530-021-02464-0
- McLaughlin LG, Wasserberg G (2021) Spatial bet hedging in sand fly oviposition: factors affecting skip oviposition in *Phlebotomus papatasi* sand flies. Vector-Borne Zoonotic Dis 21:280–288. https://doi.org/10.1089/vbz.2020.2737
- McNew SM, Clayton DH (2018) Alien invasion: biology of *Philornis* flies highlighting *Philornis downsi*, an introduced parasite of Galápagos birds. Annu Rev Entomol 63:369–387. https://doi.org/10.1146/annur ev-ento-020117-043103
- Meirmans PG (2020) genodive version 3.0: easy-to-use soft-ware for the analysis of genetic data of diploids and polyploids. Mol Ecol Resour 20:1126–1131. https://doi.org/10.1111/1755-0998.13145

- Miura O, Torchin ME, Kuris AM, Hechinger RF, Chiba S (2006) Introduced cryptic species of parasites exhibit different invasion pathways. Proc Natl Acad Sci USA 103:19818. https://doi.org/10.1073/pnas.0609603103
- O'Connor JA, Robertson J, Kleindorfer S (2010) Video analysis of host–parasite interactions in nests of Darwin's finches. Oryx 44:588–594. https://doi.org/10.1017/S0030605310000086
- Peck SB (1994) Aerial dispersal of insects between and to islands in the Galápagos Archipelago. Ecuador Ann Entomol Soc Am 87:218–224. https://doi.org/10.1093/aesa/87.2.218
- Pincheira-Donoso D, Hunt J (2017) Fecundity selection theory: concepts and evidence. Biol Rev 92:341–356. https://doi. org/10.1111/brv.12232
- Piry S, Luikart G, Cornuet JM (1999) Computer note. BOT-TLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. J Hered 90:502–503. https://doi.org/10.1093/jhered/ 90.4.502
- Polechová J (2018) Is the sky the limit? On the expansion threshold of a species' range. PLoS Biol 16:e2005372. https://doi.org/10.1371/journal.pbio.2005372
- Premachandra HKA, Nguyen NH, Knibb W (2019) Effectiveness of SNPs for parentage and sibship assessment in polygamous yellowtail kingfish Seriola lalandi. Aquaculture 499:24–31. https://doi.org/10.1016/j.aquaculture. 2018.09.022
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945. https://doi.org/10.1093/genetics/155.2.945
- Puechmaille SJ (2016) The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Mol Ecol Resour 16:608–627. https://doi.org/ 10.1111/1755-0998.12512
- R Core Development Team (2020) R: A language and environment for statistical computing. R version 4.0.0. R Foundation for Statistical Computing, Vienna, Austria
- Ricciardi A, Blackburn TM, Carlton JT, Dick JTA, Hulme PE, Iacarella JC, Jeschke JM, Liebhold AM, Lockwood JL, MacIsaac HJ, Pyšek P, Richardson DM, Ruiz GM, Simberloff D, Sutherland WJ, Wardle DA, Aldridge DC (2017) Invasion science: a horizon scan of emerging challenges and opportunities. Trends Ecol Evol 32:464– 474. https://doi.org/10.1016/j.tree.2017.03.007
- Roderick GK, Navajas M (2003) Genes in new environments: genetics and evolution in biological control. Nat Rev Genet 4:889–899. https://doi.org/10.1038/nrg1201
- Russell JC, Meyer J-Y, Holmes ND, Pagad S (2017) Invasive alien species on islands: impacts, distribution, interactions and management. Environ Conserv 44:359–370. https://doi.org/10.1017/S0376892917000297
- Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332. https://doi.org/ 10.1146/annurev.ecolsys.32.081501.114037
- Santos J, Pascual M, Simões P, Fragata I, Lima M, Kellen B, Santos M, Marques A, Rose MR. Matos M (2012) From

- nature to the laboratory: the impact of founder effects on adaptation. J Evol Biol 25:2607–2622. https://doi.org/10.1111/jeb.12008
- Sinkins SP, Gould F (2006) Gene drive systems for insect disease vectors. Nat Rev Genet 7:427–435. https://doi.org/10.1038/nrg1870
- Telfer S, Bown K (2012) The effects of invasion on parasite dynamics and communities. Funct Ecol 26:1288–1299. https://doi.org/10.1111/j.1365-2435.2012.02049.x
- Tigano A, Friesen VL (2016) Genomics of local adaptation with gene flow. Mol Ecol 25:2144–2164. https://doi.org/ 10.1111/mec.13606
- Toral-Granda MV, Causton CE, Jäger H, Trueman M, Izurieta JC, Araujo E, Cruz M, Zander KK, Izurieta A, Garnett ST (2017) Alien species pathways to the Galapagos islands. Ecuador PLOS One 12:e0184379. https://doi.org/10.1371/journal.pone.0184379
- Travis JM, Dytham C (2002) Dispersal evolution during invasions. Evol Ecol Res 4:1119–1129
- Van Alphen JJ, Visser ME (1990) Superparasitism as an adaptive strategy for insect parasitoids. Annu Rev Entomol 35:59–79. https://doi.org/10.1146/annurev.en.35.010190. 000423
- Visser ME, Rosenheim JA (1998) The influence of competition between foragers on clutch size decisions in insect Parasitoids. Biol Control 11:169–174. https://doi.org/10. 1006/bcon.1997.0589
- Wang J (2009) A new method for estimating effective population sizes from a single sample of multilocus genotypes. Mol Ecol 18:2148–2164. https://doi.org/10.1111/j.1365-294X.2009.04175.x
- Wang J (2019) Pedigree reconstruction from poor quality genotype data. Heredity 122:719–728. https://doi.org/10.1038/s41437-018-0178-7
- Wang J, Santure AW (2009) Parentage and sibship inference from multilocus genotype data under polygamy. Genetics

- 181:1579–1594. https://doi.org/10.1534/genetics.108.
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358– 1370. https://doi.org/10.2307/2408641
- Weng Z, Yang Y, Wang X, Wu L, Hua S, Zhang H, Meng Z (2021) Parentage analysis in giant grouper (*Epinephelus lanceolatus*) using microsatellite and SNP markers from genotyping-by-sequencing data. Genes 12(7):1042
- Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, 'invasive traits' and recipient communities: challenges for predicting invasive potential. Divers Distrib 14:569–580. https://doi.org/10.1111/j.1472-4642.2008. 00473.x
- Wiedenfeld DA, Jiménez GU, Fessl B, Kleindorfer S, Carlos Valarezo J (2007) Distribution of the introduced parasitic fly *Philornis downsi* (Diptera, Muscidae) in the Galápagos islands. Pac Conserv Biol 13:14–19. https://doi.org/ 10.1071/PC070014
- Wikelski M, Foufopoulos J, Vargas H, Snell H (2004) Galápagos Birds and Diseases: Invasive Pathogens as Threats for Island Species. Ecology and Society 9. http://www.jstor.org/stable/26267654
- Yi X, Latch EK (2022) Nonrandom missing data can bias principal component analysis inference of population genetic structure. Mol Ecol Resour 22:602–611. https://doi.org/ 10.1111/1755-0998.13498

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References

Abraham S, Goane L, Rull J, Cladera J, Willink E, Vera MT (2011) Multiple mating in *Anastrepha fraterculus* females and its relationship with fecundity and fertility. Entomologia Experimentalis et Applicata 141:15-24.

Adamack AT, Gruber B (2014) PopGenReport: simplifying basic population genetic analyses in R. Methods in Ecology and Evolution 5:384-387.

Aldrich JM (1923) The Genus *Philornis*— a Bird-Infesting Group of Anthomyiidæ. Annals of the Entomological Society of America 16:304-309.

Alizon S, Michalakis Y (2015) Adaptive virulence evolution: the good old fitness-based approach. Trends in Ecology & Evolution 30:248-254.

Allendorf FW, Lundquist LL (2003) Introduction: Population biology, evolution, and control of invasive species. Conservation Biology 17:24-30.

Aluja M, Birke A, Díaz-Fleischer F, Rull J (2019) Phenotypic plasticity in clutch size regulation among populations of a potential invasive fruit fly from environments that vary in host heterogeneity and isolation. Bulletin of Entomological Research 109:169-177.

Andersen JC, Mills NJ (2018) Comparative genetics of invasive populations of walnut aphid, *Chromaphis juglandicola*, and its introduced parasitoid, *Trioxys pallidus*, in California. Ecology and Evolution 8:801-811.

Andersen NM (1994) The evolution of sexual size dimorphism and mating systems in water striders (Hemiptera: Gerridae): A phylogenetic approach. Écoscience 1:208-214.

Arendt WJ (1985a) *Philornis* Ectoparasitism of Pearly-eyed Thrashers. I. Impact on Growth and Development of Nestlings. The Auk 102:270-280.

Arendt WJ (1985b) *Philornis* Ectoparasitism of Pearly-eyed Thrashers. II. Effects on Adults and Reproduction. The Auk 102:281-292.

Armbruster P, Hutchinson RA (2002) Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). Journal of Medical Entomology 39:699-704.

Arnold ML, Martin NH (2010) Hybrid fitness across time and habitats. Trends in Ecology & Evolution 25:530-536.

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Animal Behaviour 60:145-164.

Atkinson CT, Saili KS, Utzurrum RB, Jarvi SI (2013) Experimental Evidence for Evolved Tolerance to Avian Malaria in a Wild Population of Low Elevation Hawai'i 'Amakihi (Hemignathus virens). EcoHealth 10:366-375.

Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA (2008) Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. PLOS ONE 3:e3376.

Ballesteros-Mejia L, Angulo E, Diagne C, Cooke B, Nuñez M, A, Courchamp F (2021) Economic costs of biological invasions in Ecuador: the importance of the Galapagos Islands. NeoBiota 67:375 - 400.

Bateman AJ (1948) Intra-sexual selection in Drosophila. Heredity 2:349-368.

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models using lme4. Journal of Statistical Software; Vol 1, Issue 1 (2015)

Beadell JS, Ishtiaq F, Covas R, Melo M, Warren BH, Atkinson CT, Bensch S, Graves GR, Jhala YV, Peirce MA, Rahmani AR, Fonseca DM, Fleischer RC (2006) Global phylogeographic limits of Hawaii's avian malaria. Proceedings of the Royal Society B: Biological Sciences 273:2935-2944.

Beaurepaire AL, Moro A, Mondet F, Le Conte Y, Neumann P, Locke B (2019) Population genetics of ectoparasitic mites suggest arms race with honeybee hosts. Scientific Reports 9:11355.

Becker T (1907) Katalog der Paläarktischen dipteren. Wesselényi, Budapest;

Begon M, Harper JL, Townsend CR (1986) Ecology. Individuals, populations and communities. Blackwell scientific publications

Beirinckx K, Van Gossum H, J. Lajeunesse M, R. Forbes M (2006) Sex biases in dispersal and philopatry: insights from a meta-analysis based on capture—mark—recapture studies of damselflies. Oikos 113:539-547.

Bellard C, Cassey P, Blackburn TM (2016) Alien species as a driver of recent extinctions. Biology Letters 12:20150623.

Ben-Yosef M, Zaada DSY, Dudaniec RY, Pasternak Z, Jurkevitch E, Smith RJ, Causton CE, Lincango MP, Tobe SS, Mitchell JG, Kleindorfer S, Yuval B (2017) Host-specific associations affect the microbiome of *Philornis downsi*, an introduced parasite to the Galápagos Islands. Molecular Ecology 26:4644-4656.

Bezemer TM, Harvey JA, Cronin JT (2014) Response of native insect communities to invasive plants. Annual Review of Entomology 59:119.
263

Bier E (2022) Gene drives gaining speed. Nature Reviews Genetics 23:5-22.

Bierzychudek P, Eckhart V (1988) Spatial Segregation of the Sexes of Dioecious Plants. The American Naturalist 132:34-43.

Blackmore MS, Lord CC (2000) The relationship between size and fecundity in *Aedes albopictus*. Journal of Vector Ecology 25:212-217.

Blanckenhorn WU (2000) The evolution of body size: What keeps organisms small? The Quarterly Review of Biology 75:385-407.

Blay S, Yuval B (1999) Oviposition and fertility in the Mediterranean Fruit Fly (Diptera: Tephritidae): Effects of male and female body size and the availability of sperm. Annals of the Entomological Society of America 92:278-284.

Braña F (1996) Sexual dimorphism in Lacertid lizards: Male head increase vs female abdomen increase? Oikos 75:511-523.

Brettell LE, Martin SJ, Riegler M, Cook JM (2021) Vulnerability of island insect pollinator communities to pathogens. Journal of Invertebrate Pathology 186:107670.

Briegel H (1990) Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. Journal of Medical Entomology 27:839-850.

Brockhurst MA, Koskella B (2013) Experimental coevolution of species interactions. Trends in Ecology & Evolution 28:367-375.

Brown CR, Padhi A, Moore AT, Brown MB, Foster JE, Pfeffer M, O'Brien VA, Komar N (2009) Ecological divergence of two sympatric lineages of Buggy Creek virus, an arbovirus associated with birds. Ecology 90:3168-3179.

Buckling A, Brockhurst MA (2008) Kin selection and the evolution of virulence. Heredity 100:484-488.

Buckling A, Rainey PB (2002) Antagonistic coevolution between a bacterium and a bacteriophage. Proceedings of the Royal Society of London. Series B: Biological Sciences 269:931-936.

Bulgarella M, Heimpel GE (2015) Host range and community structure of avian nest parasites in the genus *Philornis* (Diptera: Muscidae) on the island of Trinidad. Ecology and Evolution 5:3695-3703.

Bulgarella M, Lincango MP, Lahuatte PF, Oliver JD, Cahuana A, Ramírez IE, Sage R, Colwitz AJ, Freund DA, Miksanek JR, Moon RD, Causton CE, Heimpel GE (2022) Persistence of the invasive bird-parasitic fly *Philornis downsi* over the host interbreeding period in the Galapagos Islands. Scientific Reports 12:2325.

Bulgarella M, Quiroga MA, Boulton RA, Ramírez IE, Moon RD, Causton CE, Heimpel GE (2017) Life cycle and host specificity of the parasitoid *Conura annulifera* (Hymenoptera: Chalcididae), a potential biological control agent of *Philornis downsi* (Diptera: Muscidae) in the Galápagos Islands. Annals of the Entomological Society of America 110:317-328.

Bulgarella M, Quiroga MA, Brito vera GA, Dregni JS, Cunninghame F, Mosquera Muñoz DA, Monje LD, Causton CE, Heimpel GE (2015) Philornis downsi (Diptera: Muscidae), an Avian Nest Parasite Invasive to the Galápagos Islands, in Mainland Ecuador. Annals of the Entomological Society of America 108:242-250.

Bulgarella M, Quiroga MA, Heimpel GE (2019) Additive negative effects of Philornis nest parasitism on small and declining Neotropical bird populations. Bird Conservation International 29:339-360.

Bunting MD, Pfitzner C, Gierus L, White M, Piltz S, Thomas PQ (2022) Generation of Gene Drive Mice for Invasive Pest Population Suppression. Methods Mol Biol 2495:203-230.

Burdon JJ, Thrall PH, Ericson L (2013) Genes, communities & invasive species: understanding the ecological and evolutionary dynamics of host–pathogen interactions. Current Opinion in Plant Biology 16:400-405.

Burnham KP, Anderson DR (1998) Practical Use of the Information-Theoretic Approach. In: Burnham KP and Anderson DR (eds) Model Selection and Inference: A Practical Information-Theoretic Approach. Springer New York, New York, NY, pp. 75-117

Butlin RK, Day TH (1984) The effect of larval competition on development time and adult size in the seaweed fly, *Coelopa frigida*. Oecologia 63:122-127.

Byers JE, Blakeslee AMH, Linder E, Cooper AB, Maguire TJ (2008) Controls of spatial variation in the prevalence of trematode parasites infecting a marine snail. Ecology 89:439-451.

Calder WA (1996) Size, function, and life history. Courier Corporation, Mineola, New York

Carde RT, Minks AK (1995) Control of moth pests by mating disruption: successes and constraints. Annual Review of Entomology 40:559-585.

Cardon M, Loot G, Grenouillet G, Blanchet S (2011) Host characteristics and environmental factors differentially drive the burden and pathogenicity of an ectoparasite: A multilevel causal analysis. Journal of Animal Ecology 80:657-667.

265

Carlson DA, Mayer MS, Silhacek DL, James JD, Beroza M, Bierl BA (1971) Sex Attractant Pheromone of the House Fly: Isolation, Identification and Synthesis. Science 174:76-78.

Causton C, Cunninghame F, Tapia W (2013) Management of the avian parasite *Philornis downsi* in the Galapagos Islands: A collaborative and strategic action plan. Galapagos Report 2011-2012. GNPS, GCREG, CDF and GC, Puerto Ayora, Galapagos, Ecuador, pp. 167-173

Causton CE, Moon RD, Cimadom A, Boulton RA, Cedeño D, Lincango MP, Tebbich S, Ulloa A (2019) Population dynamics of an invasive bird parasite, *Philornis downsi* (Diptera: Muscidae), in the Galapagos Islands. PLOS ONE 14:e0224125.

Causton CE, Peck SB, Sinclair BJ, Roque-Albelo L, Hodgson CJ, Landry B (2006) Alien insects: Threats and implications for conservation of Galápagos Islands. Annals of the Entomological Society of America 99:121-143.

Cha DH, Mieles AE, Lahuatte PF, Cahuana A, Lincango MP, Causton CE, Tebbich S, Cimadom A, Teale SA (2016) Identification and Optimization of Microbial Attractants for *Philornis downsi*, an Invasive Fly Parasitic on Galapagos Birds. Journal of Chemical Ecology 42:1101-1111.

Chalkowski K, Lepczyk CA, Zohdy S (2018) Parasite Ecology of Invasive Species: Conceptual Framework and New Hypotheses. Trends in Parasitology 34:655-663.

Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience 4:s13742-015-0047-8.

Chapman D, Purse BV, Roy HE, Bullock JM (2017) Global trade networks determine the distribution of invasive non-native species. Global Ecology and Biogeography 26:907-917.

Chapman JW, Dumbauld BR, Itani G, Markham JC (2012) An introduced Asian parasite threatens northeastern Pacific estuarine ecosystems. Biological Invasions 14:1221-1236.

Chapman JW, Knapp JJ, Howse PE, Goulson D (1998) An evaluation of (*Z*)-9-tricosene and food odours for attracting house flies, *Musca domestica*, to baited targets in deep-pit poultry units. Entomologia Experimentalis et Applicata 89:183-192.

Charles H, Dukes JS (2007) Impacts of Invasive Species on Ecosystem Services. In: Nentwig W (ed) Biological Invasions. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 217-237

Charlesworth B (2009) Effective population size and patterns of molecular evolution and variation. Nature Reviews Genetics 10:195-205.

Choe JC, Crespi BJ (1997) The evolution of mating systems in insects and arachnids. Cambridge University Press Cambridge

Chown SL, Hodgins KA, Griffin PC (2016) Biological invasions, climate change, and genomics. Crop Breeding. Apple Academic Press, pp. 59-114

Cimadom A, Causton C, Cha DH, Damiens D, Fessl B, Hood-Nowotny R, Lincango P, Mieles AE, Nemeth E, Semler EM, Teale SA, Tebbich S (2016) Darwin's finches treat their feathers with a natural repellent. Scientific Reports 6:34559.

Cimadom A, Jäger H, Schulze CH, Hood-Nowotny R, Wappl C, Tebbich S (2019) Weed management increases the detrimental effect of an invasive parasite on arboreal Darwin's finches. Biological Conservation 233:93-101.

Cimadom A, Ulloa A, Meidl P, Zöttl M, Zöttl E, Fessl B, Nemeth E, Dvorak M, Cunninghame F, Tebbich S (2014) Invasive parasites, habitat change and heavy rainfall reduce breeding success in Darwin's Finches. PLOS ONE 9:e107518.

Clark NJ, Clegg SM (2015) The influence of vagrant hosts and weather patterns on the colonization and persistence of blood parasites in an island bird. Journal of Biogeography 42:641-651.

Clavero M, García-Berthou E (2005) Invasive species are a leading cause of animal extinctions. Trends in Ecology & Evolution 20:110.

Cline TJ, Kitchell JF, Bennington V, McKinley GA, Moody EK, Weidel BC (2014) Climate impacts on landlocked sea lamprey: Implications for host-parasite interactions and invasive species management. Ecosphere 5:art68.

Collignon R, Boroczky K, Mieles A, Causton C, Lincango M, Teale S (2014) Cuticular lipids and mate attraction in the avian parasite *Philornis downsi* (Diptera: Muscidae). International Society of Chemical Ecology. Poster July:8-12.

Collignon RM (2011) Semiochemicals of *Philornis downsi* (Dipter: Muscidae), a parasite of passerine birds of the Galapagos Islands. State University of New York College of Environmental Science and Forestry

Common LK, Dudaniec RY, Colombelli-Négrel D, Kleindorfer S (2019) Taxonomic shifts in *Philornis* larval behaviour and rapid changes in *Philornis downsi* Dodge & Aitken (Diptera: Muscidae): An invasive avian parasite on the Galápagos Islands. In: Sarwar M (ed) Life Cycle and Development of Diptera. IntechOpen,

Common LK, Kleindorfer S, Colombelli-Négrel D, Dudaniec RY (2022a) Genetics reveals shifts in reproductive behaviour of the invasive bird parasite *Philornis downsi* collected from Darwin's finch nests. Biological Invasions

Common LK, O'Connor JA, Dudaniec RY, Peters KJ, Kleindorfer S (2020) Evidence for rapid downward fecundity selection in an ectoparasite (*Philornis downsi*) with earlier host mortality in Darwin's finches. Journal of Evolutionary Biology 33:524-533.

Common LK, Sumasgutner P, Dudaniec RY, Colombelli-Négrel D, Kleindorfer S (2021) Avian vampire fly (*Philornis downsi*) mortality differs across Darwin's finch host species. Scientific Reports 11:15832.

Common LK, Sumasgutner P, Sumasgutner SC, Colombelli-Négrel D, Dudaniec RY, Kleindorfer S (2022b) Temporal and spatial variation in sex-specific abundance of the avian vampire fly (*Philornis downsi*). Parasitology Research 121:63-74.

Cornuet JM, Luikart G (1996) Description and Power Analysis of Two Tests for Detecting Recent Population Bottlenecks From Allele Frequency Data. Genetics 144:2001-2014.

Coulson SJ, Bale JS (1990) Characterisation and limitations of the rapid cold-hardening response in the housefly *Musca domestica* (Diptera: Muscidae). Journal of Insect Physiology 36:207-211.

Couri M (1984) Notes and descriptions of Philornis flies (Diptera, Muscidae, Cyrtoneurininae). Revista Brasileira de Entomologia 28:473-490.

Couri M (1985) Considerações sobre as relações ecológicas das larvas de *Philornis* Meinert, 1890 (Diptera, Muscidae) com aves. Revista Brasileira de Entomologia 29:17-20.

Couri M (1999) Myiasis caused by obligatory parasites. Ia. *Philornis* Meinert (Muscidae). Myiasis in man and animals in the Neotropical region. Editora Pleiade, Sao Paulo, Brazil:44-70.

Couri M, Carvalho Cd (2003) Systematic relations among *Philornis* Meinert, *Passeromyia* Rodhain & Villeneuve and allied genera (Diptera, Muscidae). Brazilian Journal of Biology 63:223-232.

Couri M, Rabuffetti F, Reboreda J (2005) New data on *Philornis seguyi* Garcia (1952)(Diptera, Muscidae). Brazilian Journal of Biology 65:631-637.

Couri MS, Antoniazzi LR, Beldomenico P, Quiroga M (2009) Argentine *Philornis* Meinert species (Diptera: Muscidae) with synonymic notes. Zootaxa 2261:77132.

Couri MS, Barbosa L, Marini MÂ, Duca C, Pujol-Luz JR (2018) A new host for *Philornis torquans* (Diptera, Muscidae) from the Brazilian Cerrado. Papéis Avulsos de Zoologia 58

Couri MS, De Carvalho CJB, LÖWenberg-Neto P (2007a) Phylogeny of *Philornis* Meinert species (Diptera: Muscidae). Zootaxa 1530:23.

Couri MS, Murphy TG, Hoebeke R (2007b) *Philornis fasciventris* (Wulp)(Diptera: Muscidae): description of the male, larva and puparium, with notes on biology and host association. Neotropical Entomology 36:889-893.

Cox Robert M, Calsbeek R (2009) Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. The American Naturalist 173:176-187.

Cox RM, Skelly SL, John-Alder HB (2003) A comparative test of adaptive hypothesis for sexual size dimorphism in lizards. Evolution 57:1653-1669.

Cristescu ME (2015) Genetic reconstructions of invasion history. Molecular Ecology 24:2212-2225.

Crooks JA, Soulé ME, Sandlund O (1999) Lag times in population explosions of invasive species: Causes and implications. Invasive species and biodiversity management 103:125.

Crowther LP, Wright DJ, Richardson DS, Carvell C, Bourke AFG (2019) Spatial ecology of a range-expanding bumble bee pollinator. Ecology and Evolution 9:986-997.

Crystal-Ornelas R, Hudgins EJ, Cuthbert RN, Haubrock PJ, Fantle-Lepczyk J, Angulo E, Kramer AM, Ballesteros-Mejia L, Leroy B, Leung B, López-López E, Diagne C, Courchamp F (2021) Economic costs of biological invasions within North America. NeoBiota 67:485-510.

Curry RL (1986) Whatever happened to the Floreana mockingbird? Noticias de Galapagos 43:13-15.

Damman H (1991) Oviposition Behaviour and Clutch Size in a Group-Feeding Pyralid Moth, *Omphalocera munroei*. Journal of Animal Ecology 60:193-204.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R, Genomes Project Analysis G (2011) The variant call format and VCFtools. Bioinformatics 27:2156-2158.

Darwin C (1896) The descent of man and selection in relation to sex. D. Appleton Daszak P, Cunningham AA, Hyatt AD (2000) Emerging Infectious Diseases of Wildlife-- Threats to Biodiversity and Human Health. Science 287:443-449.

Davies CM, Fairbrother E, Webster JP (2002) Mixed strain schistosome infections of snails and the evolution of parasite virulence. Parasitology 124:31-38.

Davies N (2010) Cuckoos, Cowbirds and Other Cheats. Bloomsbury Publishing

De la Peña MR, Beldoménico PM, Antoniazzi L (2003) Pichones de aves parasitados por larvas de *Philornis* sp.(Diptera: Muscidae) en un sector de la provincia biogeográfica del Espinal de Santa Fe, Argentina. Revista FAVE—Ciencias Veterinarias 2:141-146.

De León LF, Sharpe DMT, Gotanda KM, Raeymaekers JAM, Chaves JA, Hendry AP, Podos J (2019) Urbanization erodes niche segregation in Darwin's finches. Evolutionary Applications 12:1329-1343.

Decaestecker E, Gaba S, Raeymaekers JAM, Stoks R, Van Kerckhoven L, Ebert D, De Meester L (2007) Host–parasite 'Red Queen' dynamics archived in pond sediment. Nature 450:870-873.

Díaz-Fleischer F, Aluja M (2003) Influence of Conspecific Presence, Experience, and Host Quality on Oviposition Behavior and Clutch Size Determination in *Anastrepha ludens* (Diptera: Tephritidae). Journal of Insect Behavior 16:537-554.

Dick CW, Patterson BD (2007) Against all odds: Explaining high host specificity in dispersal-prone parasites. International Journal for Parasitology 37:871-876.

Dlugosch KM, Parker IM (2008) Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17:431-449.

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014) NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources 14:209-214.

Do Y, Park W-B, Park J-K, Kim CJ, Choi MB (2022) Genetic and morphological variation of *Vespa velutina nigrithorax* which is an invasive species in a mountainous area. Scientific Reports 12:4737.

Dodge HR (1963) A New *Philornis* with Coprophagous Larva, and Some Related Species (Diptera: Muscidae). Journal of the Kansas Entomological Society 36:239-247.

Dodge HR, Aitken THG (1968) *Philornis* Flies from Trinidad (Diptera: Muscidae). Journal of the Kansas Entomological Society 41:134-154.

Dodson GN (1997) Resource Defense Mating System in Antlered Flies, *Phytalmia* spp. (Diptera: Tephritidae). Annals of the Entomological Society of America 90:496-504.

Doherty KM (2012) Chemical attractants of *Philornis downsi* [Diptera: Muscidae], an invasive parasite of birds in the Galapagos Islands. State

University of New York College of Environmental Science and Forestry.

Dudaniec RY, Carey AR, Svensson EI, Hansson B, Yong CJ, Lancaster LT (2021) Latitudinal clines in sexual selection, sexual size dimorphism, and sex-specific genetic dispersal during a poleward range expansion. Journal of Animal Ecology 00:1-5.

Dudaniec RY, Fessl B, Kleindorfer S (2007) Interannual and interspecific variation in intensity of the parasitic fly, *Philornis downsi*, in Darwin's finches. Biological Conservation 139:325-332.

Dudaniec RY, Gardner MG, Donnellan S, Kleindorfer S (2008a) Genetic variation in the invasive avian parasite, *Philornis downsi* (Diptera, Muscidae) on the Galápagos archipelago. BMC Ecology 8:13.

Dudaniec RY, Gardner MG, Kleindorfer S (2008b) Isolation, characterization and multiplex polymerase chain reaction of novel microsatellite loci for the avian parasite *Philornis downsi* (Diptera: Muscidae). Molecular Ecology Resources 8:142-144.

Dudaniec RY, Gardner MG, Kleindorfer S (2010) Offspring genetic structure reveals mating and nest infestation behaviour of an invasive parasitic fly (*Philornis downsi*) of Galápagos birds. Biological Invasions 12:581-592.

Dudaniec RY, Kleindorfer S (2006) Effects of the parasitic flies of the genus *Philornis* (Diptera: Muscidae) on birds. Emu - Austral Ornithology 106:13-20.

Dudaniec RY, Kleindorfer S, Fessl B (2006) Effects of the introduced ectoparasite *Philornis downsi* on haemoglobin level and nestling survival in Darwin's Small Ground Finch (*Geospiza fuliginosa*). Austral Ecology 31:88-94.

Dueñas M-A, Hemming DJ, Roberts A, Diaz-Soltero H (2021) The threat of invasive species to IUCN-listed critically endangered species: A systematic review. Global Ecology and Conservation 26:e01476.

Duffy MA, Sivars-Becker L (2007) Rapid evolution and ecological host–parasite dynamics. Ecology Letters 10:44-53.

Dunn AM, Hatcher MJ (2015) Parasites and biological invasions: parallels, interactions, and control. Trends in Parasitology 31:189-199.

Dunn AM, Torchin ME, Hatcher MJ, Kotanen PM, Blumenthal DM, Byers JE, Coon CAC, Frankel VM, Holt RD, Hufbauer RA, Kanarek AR, Schierenbeck KA, Wolfe LM, Perkins SE (2012) Indirect effects of parasites in invasions. Functional Ecology 26:1262-1274.

Dunn DW, Sumner JP, Goulson D (2005) The benefits of multiple mating to female seaweed flies, *Coelopa frigida* (Diptera: Coelpidae). Behavioral Ecology and Sociobiology 58:128-135.

Dvorak M, Fessl B, Nemeth E, Kleindorfer S, Tebbich S (2012) Distribution and abundance of Darwin's finches and other land birds on Santa Cruz Island, Galápagos: Evidence for declining populations. Oryx 46:78-86.

Dvorak M, Nemeth E, Wendelin B, Fessl B (2021) More extinctions on the Galápagos Islands? An unsuccessful search for 4 landbirds on Floreana. The Wilson Journal of Ornithology 133:514-518.

Dvorak M, Nemeth E, Wendelin B, Herrera P, Mosquera D, Anchundia D, Sevilla C, Tebbich S, Fessl B (2017) Conservation status of landbirds on Floreana: The smallest inhabited Galápagos Island. Journal of Field Ornithology 88:132-145.

Dyck VA, Hendrichs J, Robinson AS (2021) Sterile insect technique: principles and practice in area-wide integrated pest management. Taylor & Francis

Early R, Bradley BA, Dukes JS, Lawler JJ, Olden JD, Blumenthal DM, Gonzalez P, Grosholz ED, Ibañez I, Miller LP, Sorte CJB, Tatem AJ (2016) Global threats from invasive alien species in the twenty-first century and national response capacities. Nature Communications 7:12485.

Ebert D, Fields PD (2020) Host–parasite co-evolution and its genomic signature. Nature Reviews Genetics 21:754-768.

Edworthy AB, Langmore NE, Heinsohn R (2019) Native fly parasites are the principal cause of nestling mortality in endangered Tasmanian pardalotes. Animal Conservation 22:96-103.

Egas M, Dieckmann U, Sabelis Maurice W (2004) Evolution restricts the coexistence of specialists and generalists: The role of trade-off structure. The American Naturalist 163:518-531.

Emde S, Rueckert S, Kochmann J, Knopf K, Sures B, Klimpel S (2014) Nematode eel parasite found inside acanthocephalan cysts - a "Trojan horse" strategy? Parasites & Vectors 7:504.

Emlen ST, Oring LW (1977) Ecology, Sexual Selection, and the Evolution of Mating Systems. Science 197:215-223.

Enkerlin WR, Gutiérrez Ruelas JM, Pantaleon R, Soto Litera C, Villaseñor Cortés A, Zavala López JL, Orozco Dávila D, Montoya Gerardo P, Silva Villarreal L, Cotoc Roldán E, Hernández López F, Arenas Castillo A, Castellanos Dominguez D, Valle Mora A, Rendón Arana P, Cáceres Barrios C, Midgarden D, Villatoro Villatoro C, Lira Prera E, Zelaya Estradé O, Castañeda Aldana R, López Culajay J, Ramírez y Ramírez F, Liedo Fernández P, Ortíz Moreno G, Reyes Flores J, Hendrichs J (2017) The Moscamed Regional Programme: Review of a success story of area-wide sterile insect technique application. Entomologia Experimentalis et Applicata 164:188-203.

Enriquez T, Colinet H (2017) Basal tolerance to heat and cold exposure of the spotted wing drosophila, *Drosophila suzukii*. PeerJ 5:e3112.

Escobar LE, Moen R, Craft ME, VanderWaal KL (2019) Mapping parasite transmission risk from white-tailed deer to a declining moose population. European Journal of Wildlife Research 65:60.

Esperk T (2006) Larval instar as a key element of insect growth schedules. Tartu University Press

ESRI (2011) ArcGIS Desktop: Release 10. Environmental Systems Research Institute, Redlands, CA,

ESRI (2017) Data Galapagos: Web Map. In: Environmental Systems Research Institute. https://www.arcgis.com/home/item.html?id=ff95a67b2d8149ff9ab2c1feebca8b8c

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14:2611-2620.

Feis ME, Goedknegt MA, Thieltges DW, Buschbaum C, Wegner KM (2016) Biological invasions and host–parasite coevolution: Different coevolutionary trajectories along separate parasite invasion fronts. Zoology 119:366-374.

Fessl B, Anchundia D, Carrión J, Cimadom A, Cotin J, Cunninghame F, Dvorak M, Mosquera D, Nemeth E, Sevilla C (2015) Galápagos landbirds (passerines, cuckoos, and doves): Status, threats, and knowledge gaps. Galapagos Report 2016

Fessl B, Couri MS, Tebbich S (2001) *Philornis downsi* Dodge & Aitken, new to the Galapagos Islands (Diptera, Muscidae). Studia Dipterologica 8:317-22.

Fessl B, Heimpel GE, Causton CE (2018) Invasion of an avian nest parasite, *Philornis downsi*, to the Galápagos Islands: Colonization history, adaptations to novel ecosystems, and conservation challenges. In: Parker PG (ed) Disease Ecology: Galapagos Birds and their Parasites. Springer International Publishing, Cham, pp. 213-266

Fessl B, Kleindorfer S, Tebbich S (2006a) An experimental study on the effects of an introduced parasite in Darwin's finches. Biological Conservation 127:55-61.

Fessl B, Sinclair BJ, Kleindorfer S (2006b) The life-cycle of *Philornis downsi* (Diptera: Muscidae) parasitizing Darwin's finches and its impacts on nestling survival. Parasitology 133:739-747.

Fessl B, Tebbich S (2002) *Philornis downsi*— a recently discovered parasite on the Galápagos archipelago — a threat for Darwin's finches? Ibis 144:445-451.

Fessl B, Young GH, Young RP, Rodríguez-Matamoros J, Dvorak M, Tebbich S, Fa JE (2010) How to save the rarest Darwin's finch from extinction: The mangrove finch on Isabela Island. Philosophical Transactions of the Royal Society B: Biological Sciences 365:1019-1030.

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186-194.

Flanagan SP, Jones AG (2019) The future of parentage analysis: From microsatellites to SNPs and beyond. Molecular Ecology 28:544-567.

Floate KD, Whitham TG (1993) The "Hybrid Bridge" Hypothesis: Host shifting via plant hybrid swarms. The American Naturalist 141:651-662.

Foster SE, Soluk DA (2006) Protecting more than the wetland: The importance of biased sex ratios and habitat segregation for conservation of the Hine's emerald dragonfly, *Somatochlora hineana* Williamson. Biological Conservation 127:158-166.

Fowler K, Partridge L (1989) A cost of mating in female fruitflies. Nature 338:760-761.

Fox J (2003) Effect displays in R for Generalised Linear Models. Journal of Statistical Software; Vol 1, Issue 15 (2003)

Fox J, Weisberg S (2011) An R companion to applied regression. Sage Publications, California

Fraik AK, Margres MJ, Epstein B, Barbosa S, Jones M, Hendricks S, Schönfeld B, Stahlke AR, Veillet A, Hamede R, McCallum H, Lopez-Contreras E, Kallinen SJ, Hohenlohe PA, Kelley JL, Storfer A (2020) Disease swamps molecular signatures of genetic-environmental associations to abiotic factors in Tasmanian devil (*Sarcophilus harrisii*) populations. Evolution 74:1392-1408.

Frank SA (1992) A kin selection model for the evolution of virulence. Proceedings of the Royal Society of London. Series B: Biological Sciences 250:195-197.

Frank SA (1994) Kin selection and virulence in the evolution of protocells and parasites. Proceedings of the Royal Society of London. Series B: Biological Sciences 258:153-161.

Frank SA, Schmid-Hempel P (2008) Mechanisms of pathogenesis and the evolution of parasite virulence. Journal of Evolutionary Biology 21:396-404.

Frankham R (1997) Do island populations have less genetic variation than mainland populations? Heredity 78:311-327.

Frankino WA, Juliano SA (1999) Costs of reproduction and geographic variation in the reproductive tactics of the mosquito *Aedes triseriatus*. Oecologia 120:59-68.

Fredensborg BL, Poulin R (2005) Larval helminths in intermediate hosts: Does competition early in life determine the fitness of adult parasites? International Journal for Parasitology 35:1061-1070.

Fritz RS, Moulia C, Newcombe G (1999) Resistance of hybrid plants and animals to herbivores, pathogens, and parasites. Annual Review of Ecology and Systematics 30:565-591.

Fry JD (1996) The evolution of host specialization: Are trade-offs overrated? The American Naturalist 148:S84-S107.

GalápagosConservancy (2021) Galapagos Vital Signs: a satellite-based environmental monitoring system for the Galapagos Archipelago. In. https://galapagosvitalsigns.org

Accessed: 12 Jan 2021

Galligan TH, Kleindorfer S (2009) Naris and beak malformation caused by the parasitic fly, *Philornis downsi* (Diptera: Muscidae), in Darwin's small ground finch, *Geospiza fuliginosa* (Passeriformes: Emberizidae). Biological Journal of the Linnean Society 98:577-585.

Gandon S (2002) Local adaptation and the geometry of host–parasite coevolution. Ecology Letters 5:246-256.

Gandon S, Buckling A, Decaestecker E, Day T (2008) Host–parasite coevolution and patterns of adaptation across time and space. Journal of Evolutionary Biology 21:1861-1866.

Gaskin JF, Bon M-C, Cock MJW, Cristofaro M, Biase AD, De Clerck-Floate R, Ellison CA, Hinz HL, Hufbauer RA, Julien MH, Sforza R (2011) Applying molecular-based approaches to classical biological control of weeds. Biological Control 58:1-21.

Gato R, Menéndez Z, Prieto E, Argilés R, Rodríguez M, Baldoquín W, Hernández Y, Pérez D, Anaya J, Fuentes I, Lorenzo C, González K, Campo Y, Bouyer J (2021) Sterile Insect Technique: Successful Suppression of an *Aedes aegypti* Field Population in Cuba. Insects 12

Gauld ID, Fitton MG (1987) Sexual dimorphism in Ichneumonidae: A response to Hurlbutt. Biological Journal of the Linnean Society 31:291-300.

Gião JZ, Godoy WAC (2006) Seasonal population dynamics in *Lucilia eximia* (Wiedemann) (Diptera: Calliphoridae). Neotropical Entomology 35:753-756.

Gibson AK, Refrégier G, Hood ME, Giraud T (2014) Performance of a hybrid fungal pathogen on pure-species and hybrid host plants. International Journal of Plant Sciences 175:724-730.

Gleichsner AM, Reinhart K, Minchella DJ (2018) The influence of related and unrelated co-infections on parasite dynamics and virulence. Oecologia 186:555-564.

González C, Molina AG, León C, Salcedo N, Rondón S, Paz A, Atencia MC, Tovar C, Ortiz M (2017) Entomological characterization of malaria in northern Colombia through vector and parasite species identification, and analyses of spatial distribution and infection rates. Malaria Journal 16:431.

Gotoh T, Tsuchiya A (2008) Effect of multiple mating on reproduction and longevity of the phytoseiid mite *Neoseiulus californicus*. Experimental and Applied Acarology 44:185.

Goudet J (2005) hierfstat, a package for r to compute and test hierarchical F-statistics. Molecular Ecology Notes 5:184-186.

Grant PR (1986) Ecology and Evolution of Darwin's Finches (Princeton Science Library Edition). Princeton University Press

Grant PR, Grant BR (2002) Unpredictable evolution in a 30-year study of Darwin's Finches. Science 296:707.

Grant PR, Grant BR (2016) Introgressive hybridization and natural selection in Darwin's finches. Biological Journal of the Linnean Society 117:812-822.

Greenbaum G, Templeton AR, Zarmi Y, Bar-David S (2014) Allelic Richness following Population Founding Events – A Stochastic Modeling Framework Incorporating Gene Flow and Genetic Drift. PLOS ONE 9:e115203.

Grueber CE, Nakagawa S, Laws RJ, Jamieson IG (2011) Multimodel inference in ecology and evolution: Challenges and solutions. Journal of Evolutionary Biology 24:699-711.

Guppy JL, Jones DB, Kjeldsen SR, Le Port A, Khatkar MS, Wade NM, Sellars MJ, Steinig EJ, Raadsma HW, Jerry DR, Zenger KR (2020) Development and validation of a RAD-Seq target-capture based genotyping assay for routine application in advanced black tiger shrimp (*Penaeus monodon*) breeding programs. BMC Genomics 21:541.

Haaland TR, Wright J, Ratikainen II (2020) Generalists versus specialists in fluctuating environments: A bet-hedging perspective. Oikos 129:879-890.

Harrington LC, Edman JD, Scott TW (2001) Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? Journal of Medical Entomology 38:411-422.

Haseyama KLF, Wiegmann BM, Almeida EAB, de Carvalho CJB (2015) Say goodbye to tribes in the new house fly classification: A new molecular phylogenetic analysis and an updated biogeographical narrative for the Muscidae (Diptera). Molecular Phylogenetics and Evolution 89:1-12.

Hatcher MJ, Dick JTA, Dunn AM (2012) Disease emergence and invasions. Functional Ecology 26:1275-1287.

Head G (1995) Selection on the fecundity and variation in the degree of sexual size dimorphism among spider species (Class Araneae). Evolution 49:776-781.

Hedrick P (2005) Large Variance in Reproductive Success and the Ne/N Ratio. Evolution 59:1596-1599.

Hedrick PW (2009) Genetics of populations. Jones & Bartlett Publishers

Hedrick PW, Kim TJ, Parker KM (2001) Parasite resistance and genetic variation in the endangered Gila topminnow. Animal Conservation 4:103-109.

Heger T, Jeschke JM (2014) The enemy release hypothesis as a hierarchy of hypotheses. Oikos 123:741-750.

Heimpel GE, Hillstrom A, Freund D, Knutie SA, Clayton DH (2017) Invasive Parasites and the Fate of Darwin's Finches in the Galapagos Islands: The Case of the Vegetarian Finch (*Platyspiza crassirostris*). The Wilson Journal of Ornithology 129:345-349.

Hendrichs J, Katsoyannos BI, Papaj DR, Prokopy RJ (1991) Sex differences in movement between natural feeding and mating sites and tradeoffs between food consumption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Tephritidae). Oecologia 86:223-231.

Hendrichs J, Robinson AS, Cayol JP, Enkerlin W (2002) Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: The importance of mating behavior studies. Florida Entomologist 85:1-13.

Hendrichs J, Vreysen M, Enkerlin W, Cayol J, Dyck V, Robinson A (2005) Strategic options in using sterile insects for area-wide integrated pest management. In: V.A. D, J. H and A. R (eds) Sterile Insect Technique. Springer, Dordrecht, pp. 841

Herrera JM, Bermúdez SE (2012) Myiasis by *Philornis* spp.(Diptera: Muscidae) in *Dendroica castanea* (Aves: Parulidae) in Panama. Revista Mexicana de Biodiversidad 83:854-855.

Himuro C, Kohama T, Matsuyama T, Sadoyama Y, Kawamura F, Honma A, Ikegawa Y, Haraguchi D (2022) First case of successful eradication of the sweet potato weevil, *Cylas formicarius* (Fabricius), using the sterile insect technique. PLOS ONE 17:e0267728.

Hoddle MS, Ramírez CC, Hoddle CD, Loayza J, Lincango MP, Van Driesche RG, Causton CE (2013) Post release evaluation of Rodolia cardinalis (Coleoptera: Coccinellidae) for control of Icerya purchasi (Hemiptera: Monophlebidae) in the Galápagos Islands. Biological Control 67:262-274.

Hoffmann BD, Broadhurst LM (2016) The economic cost of managing invasive species in Australia. NeoBiota 31:1-18.

Honěk A (1993) Intraspecific variation in body size and fecundity in insects: A general relationship. Oikos 66:483-492.

Hopper KR (1999) Risk-spreading and bet-hedging in insect population biology. Annual Review of Entomology 44:535-560.

Huber SK (2008) Effects of the introduced parasite *Philornis downsi* on nestling growth and mortality in the medium ground finch (*Geospiza fortis*). Biological Conservation 141:601-609.

Huber SK, Owen JP, Koop JAH, King MO, Grant PR, Grant BR, Clayton DH (2010) Ecoimmunity in Darwin's Finches: Invasive parasites trigger acquired immunity in the Medium Ground Finch (*Geospiza fortis*). PLOS ONE 5:e8605.

Huey RB, Gilchrist GW, Hendry AP (2005) Using invasive species to study evolution. Species invasions: Insights to ecology, evolution and biogeography:139-164.

Hulme PE (2009) Trade, transport and trouble: Managing invasive species pathways in an era of globalization. Journal of Applied Ecology 46:10-18.

Hulme PE (2014) Invasive species challenge the global response to emerging diseases. Trends in Parasitology 30:267-270.

Hurlbutt B (2008) Sexual size dimorphism in parasitoid wasps. Biological Journal of the Linnean Society 30:63-89.

Hutchinson GE (1957) Concluding Remarks. Cold Spring Harbor Symposia on Quantitative Biology. pp. 415-427

INEC (2015) Censo de Población y Vivienda Galápagos 2015. Instituto Nacional de Estadística y Censos, Ecuador,

Jacob S, Legrand D, Chaine AS, Bonte D, Schtickzelle N, Huet M, Clobert J (2017) Gene flow favours local adaptation under habitat choice in ciliate microcosms. Nature Ecology & Evolution 1:1407-1410.

Jactel H, Desprez-Loustau M-L, Battisti A, Brockerhoff E, Santini A, Stenlid J, Björkman C, Branco M, Dehnen-Schmutz K, Douma JC (2020) Pathologists and entomologists must join forces against forest pest and pathogen invasions. NeoBiota 58:107.

Jaenike J (1990) Host specialization in phytophagous insects. Annual Review of Ecology and Systematics 21:243-273.

James SL, Marshall JM, Christophides GK, Okumu FO, Nolan T (2020) Toward the Definition of Efficacy and Safety Criteria for Advancing Gene Drive-Modified Mosquitoes to Field Testing. Vector-Borne and Zoonotic Diseases 20:237-251.

Jardine SL, Sanchirico JN (2018) Estimating the cost of invasive species control. Journal of Environmental Economics and Management 87:242-257.

Jiang Y, Lei C-l, Niu C-y, Fang Y-l, Xiao C, Zhang Z-n (2002) Semiochemicals from ovaries of gravid females attract ovipositing female houseflies, Musca domestica. Journal of Insect Physiology 48:945-950.

Jiménez-Uzcátegui G, Llerena W, Milstead WB, Lomas EE, Wiedenfeld DA (2011) Is the population of the Floreana mockingbird Mimus trifasciatus declining. Cotinga 33:1-7.

Jiménez-Uzcátegui G, Milstead B, Márquez C, Zabala J, Buitrón P, Llerena A, Salazar S, Fessl B (2006) Galapagos vertebrates: endangered status and conservation actions. Galapagos Report 2007:104-110.

Johnson FA, Smith BJ, Bonneau M, Martin J, Romagosa C, Mazzotti F, Waddle H, Reed RN, Eckles JK, Vitt LJ (2017) Expert Elicitation, Uncertainty, and the Value of Information in Controlling Invasive Species. Ecological Economics 137:83-90.

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405.

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:94.

Jones OR, Wang J (2010) COLONY: A program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551-5.

Jose PA, Ben-Yosef M, Lahuatte P, Causton CE, Heimpel GE, Jurkevitch E, Yuval B (2021) Shifting microbiomes complement life stage transitions and diet of the bird parasite *Philornis downsi* from the Galapagos Islands. Environmental Microbiology

Kamenova S, Bartley TJ, Bohan DA, Boutain JR, Colautti RI, Domaizon I, Fontaine C, Lemainque A, Le Viol I, Mollot G, Perga ME, Ravigné V, Massol F (2017) Chapter Three - Invasions Toolkit: Current Methods for Tracking the Spread and Impact of Invasive Species. In: Bohan DA, Dumbrell AJ and Massol F (eds) Advances in Ecological Research. Academic Press, pp. 85-182

Karsten M, van Vuuren BJ, Barnaud A, Terblanche JS (2013) Population Genetics of *Ceratitis capitata* in South Africa: Implications for Dispersal and Pest Management. PLOS ONE 8:e54281.

Katsis AC, Colombelli-Négrel D, Common LK, O'connor JA, Dudaniec RY, García-Loor J, Kleindorfer S (2021) Nestling behaviour predicts naris deformation in Darwin's finches parasitized by the avian vampire fly. Biological Journal of the Linnean Society

Kawagoe T, Suzuki N, Matsumoto K (2001) Multiple mating reduces longevity of females of the windmill butterfly *Atrophaneura alcinous*. Ecological Entomology 26:258-262.

Kawecki Tadeusz J (1998) Red Queen meets Santa Rosalia: Arms races and the evolution of host specialization in organisms with parasitic lifestyles. The American Naturalist 152:635-651.

Keller SR, Taylor DR (2008) History, chance and adaptation during biological invasion: Separating stochastic phenotypic evolution from response to selection. Ecology Letters 11:852-866.

Kelly DW, Paterson RA, Townsend CR, Poulin R, Tompkins DM (2009) Parasite spillback: A neglected concept in invasion ecology? Ecology 90:2047-2056.

Keogh CL, Miura O, Nishimura T, Byers JE (2017) The double edge to parasite escape: invasive host is less infected but more infectable. Ecology 98:2241-2247.

Kier G, Kreft H, Lee TM, Jetz W, Ibisch PL, Nowicki C, Mutke J, Barthlott W (2009) A global assessment of endemism and species richness across island and mainland regions. Proceedings of the National Academy of Sciences 106:9322-9327.

Kinsella J, Winegarner C (1974) Notes on the life history of *Neomusca porteri* (Dodge), parasitic on nestlings of the great crested flycatcher in Florida. Journal of Medical Entomology 11:633.

Kirk H, Dorn S, Mazzi D (2013) Worldwide population genetic structure of the oriental fruit moth (*Grapholita molesta*), a globally invasive pest. BMC Ecology 13:12.

Kleindorfer S (2007a) The ecology of clutch size variation in Darwin's Small Ground Finch *Geospiza fuliginosa*: comparison between lowland and highland habitats. Ibis 149:730-741.

Kleindorfer S (2007b) Nesting success in Darwin's small tree finch, *Camarhynchus parvulus*: evidence of female preference for older males and more concealed nests. Animal Behaviour 74:795-804.

Kleindorfer S, Colombelli-Négrel D, Common LK, O'Connor Jody A, Peters KJ, Katsis AC, Dudaniec Rachael Y, Sulloway FJ, Adreani NM (2022) Functional traits and foraging behaviour: Avian vampire fly larvae change the beak and fitness of their Darwin's finch hosts. Functional Ecology 36:1806-1817.

Kleindorfer S, Common LK, O'Connor JA, Garcia-Loor J, Katsis AC, Dudaniec RY, Colombelli-Négrel D, Adreani NM (2021a) Female in-nest attendance predicts the number of 281

ectoparasites in Darwin's finch species. Proceedings of the Royal Society B: Biological Sciences 288:20211668.

Kleindorfer S, Common LK, Sumasgutner P (2021b) Nesting Success and Nesting Height in the Critically Endangered Medium Tree Finch (*Camarhynchus pauper*). Birds 2:427-444.

Kleindorfer S, Custance G, Peters Katharina J, Sulloway Frank J (2019a) Introduced parasite changes host phenotype, mating signal and hybridization risk: *Philornis downsi* effects on Darwin's finch song. Proceedings of the Royal Society B: Biological Sciences 286:20190461.

Kleindorfer S, Dudaniec RY (2009) Love thy neighbour? Social nesting pattern, host mass and nest size affect ectoparasite intensity in Darwin's tree finches. Behavioral Ecology and Sociobiology 63:731-739.

Kleindorfer S, Dudaniec RY (2016) Host-parasite ecology, behavior and genetics: A review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. BMC Zoology 1:1.

Kleindorfer S, Dudaniec RY (2020) Hybridization fluctuates with rainfall in Darwin's tree finches. Biological Journal of the Linnean Society 130:79-88.

Kleindorfer S, Fessl B, Peters K, Anchundia D (2019b) Field Guide. Resident land birds of Galapagos. Charles Darwin Foundation, Ecuador

Kleindorfer S, O'Connor JA, Dudaniec RY, Myers SA, Robertson J, Sulloway FJ (2014a) Species collapse via hybridization in Darwin's Tree Finches. The American Naturalist 183:325-341.

Kleindorfer S, Peters KJ, Custance G, Dudaniec RY, O'Connor JA (2014b) Changes in *Philornis* infestation behavior threaten Darwin's finch survival. Current Zoology 60:542-550.

Kleindorfer S, Peters KJ, Hohl L, Sulloway FJ (2016) Flight behaviour of an introduced parasite affects its Galapagos Island hosts: *Philornis downsi* and Darwin's finches. In: Judith S. Weis DS (ed) Biological Invasions and Animal Behaviour. Cambridge University Press, Cambridge, pp. 158-79

Kleindorfer S, Sulloway FJ (2016) Naris deformation in Darwin's finches: Experimental and historical evidence for a post-1960s arrival of the parasite *Philornis downsi*. Global Ecology and Conservation 7:122-131.

Kleindorfer SM, Chapman TW, Winkler H, Sulloway F (2006) Adaptive divergence in contiguous populations of Darwin's Small Ground Finch (*Geospiza fuliginosa*). Evolutionary Ecology Research 82:357-372.

Knutie SA (2018) Relationships among introduced parasites, host defenses, and gut microbiota of Galapagos birds. Ecosphere 9:e02286.

Knutie SA, Chaves JA, Gotanda KM (2019) Human activity can influence the gut microbiota of Darwin's finches in the Galapagos Islands. Molecular Ecology 28:2441-2450.

Knutie SA, Koop JAH, French SS, Clayton DH (2013) Experimental test of the effect of introduced hematophagous flies on corticosterone levels of breeding Darwin's finches.

General and Comparative Endocrinology 193:68-71.

Knutie SA, McNew SM, Bartlow AW, Vargas DA, Clayton DH (2014) Darwin's finches combat introduced nest parasites with fumigated cotton. Current Biology 24:R355-R356.

Knutie SA, Owen JP, McNew SM, Bartlow AW, Arriero E, Herman JM, DiBlasi E, Thompson M, Koop JAH, Clayton DH (2016) Galápagos mockingbirds tolerate introduced parasites that affect Darwin's finches. Ecology 0

Kogura Y, Seeb JE, Azuma N, Kudo H, Abe S, Kaeriyama M (2011) The genetic population structure of lacustrine sockeye salmon, *Oncorhynchus nerka*, in Japan as the endangered species. Environmental Biology of Fishes 92:539-550.

Kolluru GR, Zuk M (2001) Parasitism patterns and the size-fecundity relationship in the acoustically orienting dipteran parasitoid *Ormia ochracea*. Canadian Journal of Zoology 79:973-979.

Koop JA, Le Bohec C, Clayton DH (2013a) Dry year does not reduce invasive parasitic fly prevalence or abundance in Darwin's finch nests. Reports in Parasitology 3

Koop JAH, Causton CE, Bulgarella M, Cooper E, Heimpel GE (2020) Population structure of a nest parasite of Darwin's finches within its native and invasive ranges.

Conservation Genetics

Koop JAH, Huber SK, Laverty SM, Clayton DH (2011) Experimental demonstration of the fitness consequences of an introduced parasite of Darwin's Finches. PLOS ONE 6:e19706.

Koop JAH, Owen JP, Knutie SA, Aguilar MA, Clayton DH (2013b) Experimental demonstration of a parasite-induced immune response in wild birds: Darwin's finches and introduced nest flies. Ecology and Evolution 3:2514-2523.
283

Krasnov BR (2008) Functional and evolutionary ecology of fleas: A model for ecological parasitology. Cambridge University Press

Krasnov BR, Mouillot D, Shenbrot GI, Khokhlova IS, Poulin R (2004) Geographical variation in host specificity of fleas (Siphonaptera) parasitic on small mammals: The influence of phylogeny and local environmental conditions. Ecography 27:787-797.

Kurtz J, Schulenburg H, Reusch TB (2016) Host–parasite coevolution-rapid reciprocal adaptation and its genetic basis. Zoology 119:241-243.

Kutty SN, Pont AC, Meier R, Pape T (2014) Complete tribal sampling reveals basal split in Muscidae (Diptera), confirms saprophagy as ancestral feeding mode, and reveals an evolutionary correlation between instar numbers and carnivory. Molecular Phylogenetics and Evolution 78:349-364.

Lahuatte PF, Lincango MP, Heimpel GE, Causton CE (2016) Rearing larvae of the avian nest parasite, *Philornis downsi* (Diptera: Muscidae), on chicken blood-based diets. Journal of Insect Science 16

Lamichhaney S, Berglund J, Almén MS, Maqbool K, Grabherr M, Martinez-Barrio A, Promerová M, Rubin C-J, Wang C, Zamani N, Grant BR, Grant PR, Webster MT, Andersson L (2015) Evolution of Darwin's finches and their beaks revealed by genome sequencing. Nature 518:371-375.

Lamichhaney S, Han F, Webster MT, Andersson L, Grant BR, Grant PR (2018) Rapid hybrid speciation in Darwin's finches. Science 359:224-228.

Lance D, McInnis D (2005) Biological basis of the sterile insect technique. Sterile Insect Technique. Springer, pp. 69-94

Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biology 10:R25.

LaPointe DA, Goff ML, Atkinson CT (2005) Comparative susceptibility of introduced forest-dwelling mosquitos in Hawai'i to avian malaria, *Plasmodium relictum*. Journal of Parasitology 91:843-849.

Larson ER, Graham BM, Achury R, Coon JJ, Daniels MK, Gambrell DK, Jonasen KL, King GD, LaRacuente N, Perrin-Stowe TIN, Reed EM, Rice CJ, Ruzi SA, Thairu MW, Wilson JC, Suarez AV (2020) From eDNA to citizen science: emerging tools for the early detection of invasive species. Frontiers in Ecology and the Environment 18:194-202.

Laugier GJM, Le Moguédec G, Tayeh A, Loiseau A, Osawa N, Estoup A, Facon B (2013) Increase in Male Reproductive Success and Female Reproductive Investment in Invasive Populations of the Harlequin Ladybird *Harmonia axyridis*. PLOS ONE 8:e77083.

Lawson Handley LJ, Estoup A, Evans DM, Thomas CE, Lombaert E, Facon B, Aebi A, Roy HE (2011) Ecological genetics of invasive alien species. BioControl 56:409-428.

Lawson LP, Fessl B, Hernán Vargas F, Farrington HL, Francesca Cunninghame H, Mueller JC, Nemeth E, Christian Sevilla P, Petren K (2017) Slow motion extinction: inbreeding, introgression, and loss in the critically endangered mangrove finch (*Camarhynchus heliobates*). Conservation Genetics 18:159-170.

Le Brun N, Renaud F, Berrebi P, Lambert A (1992) Hybrid zones and host-parasite relationships: Effect on the evolution of parasitic specificity. Evolution 46:56-61.

Le Gros A, Stracey CM, Robinson SK (2011) Associations Between Northern Mockingbirds and the Parasite Philornis porteri in Relation to Urbanization. The Wilson Journal of Ornithology 123:788-796.

Le Roux J (2021) The Evolutionary Ecology of Invasive Species. Elsevier Science Lee CE (2002) Evolutionary genetics of invasive species. Trends in Ecology & Evolution 17:386-391.

Leger EA, Espeland EK (2010) PERSPECTIVE: Coevolution between native and invasive plant competitors: implications for invasive species management. Evolutionary Applications 3:169-178.

Lehmann T, Dalton R, Kim EH, Dahl E, Diabate A, Dabire R, Dujardin JP (2006) Genetic contribution to variation in larval development time, adult size, and longevity of starved adults of *Anopheles gambiae*. Infection, Genetics and Evolution 6:410-416.

Leibold MA (1995) The niche concept revisited: Mechanistic models and community context. Ecology 76:1371-1382.

Leite GA, Matsui QY, Couri MS, Monteiro AR (2009) New association between *Philornis* Meinert (Diptera: Muscidae) and Falconidae (Aves: Falconiformes). Neotropical Entomology 38:686-687.

Lethier H, Bueno P (2018) Report of the IUCN reactive monitoring mission to Galápagos Islands (Ecuador), 21-25 August 2017. IUCN,

Leuba C, Tebbich S, Nemeth E, Anchundia D, Heyer E, Mosquera DA, Richner H, Rojas Allieri ML, Sevilla C, Fessl B (2020) Effect of an introduced parasite in natural and

anthropogenic habitats on the breeding success of the endemic little vermilion flycatcher Pyrocephalus nanus in the Galápagos. Journal of Avian Biology 51

Lewontin RC, Birch LC (1966) Hybridization as a Source of Variation for Adaptation to New Environments. Evolution 20:315-336.

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25:1754-1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Genome Project Data Processing S (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078-2079.

Li Y-L, Liu J-X (2018) StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods. Molecular Ecology Resources 18:176-177.

Lieske R, Zwick P (2008) Effects of intraspecific competition on the life cycle of the stonefly, *Nemurella pictetii* (Plecoptera: Nemouridae). BMC Ecology 8:5.

Lincango P, Causton C (2008) Crianza en cautiverio de *Philornis downsi*, en las Islas Galápagos. Informe interno. Charles Darwin Fourndation

Lincango P, Causton C (2009) Ensayos de atrayentes para la captura de la mosca parásito, *Philornis downsi* (Diptera: Muscidae) en las Islas Galápagos. Puerto Ayora, Galapagos, Ecuador: Charles Darwin Foundation

Lincango P, Causton C, Cedeño D, Castañeda J, Hillstrom A, Freund D (2015) Interactions between the Avian Parasite, *Philornis downsi* (Diptera: Muscidae) and the Galapagos Flycatcher, *Myiarchus magnirostris* Gould (Passeriformes: Tyrannidae). Journal of Wildlife Diseases 51:907-910.

Liu Y, Henkel J, Beaurepaire A, Evans JD, Neumann P, Huang Q (2021) Comparative genomics suggests local adaptations in the invasive small hive beetle. Ecology and Evolution 11:15780-15791.

Llopis-Belenguer C, Blasco-Costa I, Balbuena JA, Sarabeev V, Stouffer DB (2020) Native and invasive hosts play different roles in host–parasite networks. Ecography 43:559-568.

Lombaert E, Estoup A, Facon B, Joubard B, Grégoire JC, Jannin A, Blin A, Guillemaud T (2014) Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*. Journal of Evolutionary Biology 27:508-517.

Loo WT, Dudaniec RY, Kleindorfer S, Cavanaugh CM (2019a) An inter-island comparison of Darwin's finches reveals the impact of habitat, host phylogeny, and island on the gut microbiome. PLOS ONE 14:e0226432.

Loo WT, García-Loor J, Dudaniec RY, Kleindorfer S, Cavanaugh CM (2019b) Host phylogeny, diet, and habitat differentiate the gut microbiomes of Darwin's finches on Santa Cruz Island. Scientific Reports 9:18781.

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89:238-247.

Lyimo IN, Ferguson HM (2009) Ecological and evolutionary determinants of host species choice in mosquito vectors. Trends in Parasitology 25:189-196.

Lymbery AJ, Morine M, Kanani HG, Beatty SJ, Morgan DL (2014) Co-invaders: The effects of alien parasites on native hosts. International Journal for Parasitology: Parasites and Wildlife 3:171-177.

Lyons CL, Coetzee M, Terblanche JS, Chown SL (2014) Desiccation tolerance as a function of age, sex, humidity and temperature in adults of the African malaria vectors *Anopheles arabiensis* and *Anopheles funestus*. The Journal of Experimental Biology 217:3823.

Mackenzie A (1996) A trade-off for host plant utilization in the black bean aphid, *Aphis fabae*. Evolution 50:155-162.

Macquart J (1854) Notice sur une nouvelle espèce d'Aricie, diptère de la tribu des Anthomyzides. Annales de la Societ Entomologique de France 1:657-660.

Malloch J (1921) Notes on some of van der Wulp's species of North American Anthomyiidae (Diptera). Entomological News 32:40-45.

Malmqvist B, Adler PH, Strasevicius D (2004) Testing hypotheses on egg number and size in black flies (Diptera: Simuliidae). Journal of Vector Ecology 29:248-256.

Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen W-M (2010) Robust relationship inference in genome-wide association studies. Bioinformatics 26:2867-2873.

Marandel F, Charrier G, Lamy J-B, Le Cam S, Lorance P, Trenkel VM (2020) Estimating effective population size using RADseq: Effects of SNP selection and sample size. Ecology and Evolution 10:1929-1937.

Marbuah G, Gren I-M, McKie B (2014) Economics of Harmful Invasive Species: A Review. Diversity 6:500-523.

Märkle H, John S, Cornille A, Fields PD, Tellier A (2021) Novel genomic approaches to study antagonistic coevolution between hosts and parasites. Molecular Ecology 30:3660-3676.

Marzal A, García-Longoria L, Cárdenas Callirgos JM, Sehgal RNM (2015) Invasive avian malaria as an emerging parasitic disease in native birds of Peru. Biological Invasions 17:39-45.

Marzal A, Ricklefs RE, Valkiūnas G, Albayrak T, Arriero E, Bonneaud C, Czirják GA, Ewen J, Hellgren O, Hořáková D, Iezhova TA, Jensen H, Križanauskienė A, Lima MR, de Lope F, Magnussen E, Martin LB, Møller AP, Palinauskas V, Pap PL, Pérez-Tris J, Sehgal RNM, Soler M, Szöllősi E, Westerdahl H, Zetindjiev P, Bensch S (2011) Diversity, Loss, and Gain of Malaria Parasites in a Globally Invasive Bird. PLOS ONE 6:e21905.

Mathieu-Bégné E, Loot G, Mazé-Guilmo E, Mullet V, Genthon C, Blanchet S (2020) Combining species distribution models and population genomics underlines the determinants of range limitation in an emerging parasite. Ecography 44:307-319.

Maxwell SM, Scales KL, Bograd SJ, Briscoe DK, Dewar H, Hazen EL, Lewison RL, Welch H, Crowder LB (2019) Seasonal spatial segregation in blue sharks (*Prionace glauca*) by sex and size class in the Northeast Pacific Ocean. Diversity and Distributions 25:1304-1317.

Mayer M, Shine R, Brown GP (2021) Rapid divergence of parasite infectivity and host resistance during a biological invasion. Biological Journal of the Linnean Society 132:861-871.

McCann S, Day JF, Allan S, Lord CC (2009) Age modifies the effect of body size on fecundity in *Culex quinquefasciatus* Say (Diptera: Culicidae). Journal of Vector Ecology 34:174-181.

McCosker JE, Rosenblatt RH (2010) The fishes of the Galápagos Archipelago: an update. Proceedings of the California Academy of Sciences 61:167.

McIntire KM, Juliano SA (2021) Detrimental effects of a failed infection by a coinvasive parasite on a native congeneric parasite and its native host. Biological Invasions 23:1637-1648.

McLaughlin LG, Wasserberg G (2021) Spatial Bet Hedging in Sand Fly Oviposition: Factors Affecting Skip Oviposition in *Phlebotomus papatasi* Sand Flies. Vector-Borne and Zoonotic Diseases 21:280-288.

McNew SM, Clayton DH (2018) Alien invasion: Biology of *Philornis* flies highlighting *Philornis downsi*, an introduced parasite of Galápagos birds. Annual Review of Entomology 63:369-387.

McNew SM, Goodman GB, Yépez R J, Clayton DH (2020) Parasitism by an invasive nest fly reduces future reproduction in Galápagos mockingbirds. Oecologia 192:363-374.

McNew SM, Knutie SA, Goodman GB, Theodosopoulos A, Saulsberry A, Yépez R J, Bush SE, Clayton DH (2019) Annual environmental variation influences host tolerance to parasites. Proceedings of the Royal Society B: Biological Sciences 286:20190049.

Meeus I, Brown MJF, De Graaf DC, Smagghe GUY (2011) Effects of Invasive Parasites on Bumble Bee Declines. Conservation Biology 25:662-671.

Meier R, Kotrba M, Ferrar P (1999) Ovoviviparity and viviparity in the Diptera. Biological Reviews 74:199-258.

Meinert F (1889) Philornis molesta in paa Fugle snyltende Tachinarie

Meirmans PG (2020) genodive version 3.0: Easy-to-use software for the analysis of genetic data of diploids and polyploids. Molecular Ecology Resources 20:1126-1131.

Mendonça EdC, Couri MS (1999) New associations between *Philornis* Meinert (Diptera, Muscidae) and Thamnophilidae (Aves, Passeriformes). Revista Brasileira de Zoologia 16:1223-1225.

Midgarden D, Lira E, Silver M (2014) Spatial Analysis of Tephritid Fruit Fly Traps. In: Shelly T, Epsky N, Jang EB, Reyes-Flores J and Vargas R (eds) Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies: Lures, Area-Wide Programs, and Trade Implications. Springer Netherlands, Dordrecht, pp. 277-320

Miller TEX, Inouye BD (2013) Sex and stochasticity affect range expansion of experimental invasions. Ecology Letters 16:354-361.

Millien V (2006) Morphological Evolution Is Accelerated among Island Mammals. PLOS Biology 4:e321.

Miura O, Torchin ME, Kuris AM, Hechinger RF, Chiba S (2006) Introduced cryptic species of parasites exhibit different invasion pathways. Proceedings of the National Academy of Sciences 103:19818.

Mollot G, Pantel JH, Romanuk TN (2017) The Effects of Invasive Species on the Decline in Species Richness: A Global Meta-Analysis. In: Bohan DA, Dumbrell AJ and Massol F (eds) Advances in Ecological Research. Academic Press, pp. 61-83

Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences 98:5446-5451.

Mosquera D, Fessl B, Anchundia D, Heyer E, Leuba C, Nemeth E, Rojas ML, Sevilla C, Tebbich S (2022) The invasive parasitic fly *Philornis downsi* is threatening Little Vermilion Flycatchers on the Galapagos Islands. Avian Conservation and Ecology 17

Moulia C, Brun NL, Loubes C, Marin R, Renaud F (1995) Hybrid vigour against parasites in interspecific crosses between two mice species. Heredity 74:48-52.

Nijhout HF, Callier V (2015) Developmental mechanisms of body size and wing-body scaling in insects. Annual Review of Entomology 60:141-156.

NOAA (2016) World Vector Shorelines. In: NOAA Shoreline Website. https://shoreline.noaa.gov/data/datasheets/wvs.html

Nores AI (1995) Botfly Ectoparasitism of the Brown Cacholote and the Firewood-Gatherer. The Wilson Bulletin 107:734-738.

North AR, Burt A, Godfray HCJ (2019) Modelling the potential of genetic control of malaria mosquitoes at national scale. BMC Biology 17:26.

Nunney L (1996) The response to selection for fast larval development in *Drosophila melanogaster* and its effect on adult weight: An example of a fitness trade-off. Evolution 50:1193-1204.

O'Connor JA, Dudaniec RY, Kleindorfer S (2010) Parasite infestation and predation in Darwin's small ground finch: contrasting two elevational habitats between islands. Journal of Tropical Ecology 26:285-292.

O'Connor JA, Robertson J, Kleindorfer S (2014) Darwin's finch begging intensity does not honestly signal need in parasitised nests. Ethology 120:228-237.

O'Connor JA, Robertson J, Kleindorfer S (2010a) Video analysis of host–parasite interactions in nests of Darwin's finches. Oryx 44:588-594.

O'Connor JA, Sulloway FJ, Robertson J, Kleindorfer S (2010b) *Philornis downsi* parasitism is the primary cause of nestling mortality in the critically endangered Darwin's medium tree finch (*Camarhynchus pauper*). Biodiversity and Conservation 19:853-866.

Olson DH, Aanensen DM, Ronnenberg KL, Powell CI, Walker SF, Bielby J, Garner TWJ, Weaver G, The Bd Mapping G, Fisher MC (2013) Mapping the Global Emergence of *Batrachochytrium dendrobatidis*, the Amphibian Chytrid Fungus. PLOS ONE 8:e56802.

Olsson M, Shine R, Wapstra E, Ujvari B, Madsen T (2002) Sexual dimorphism in lizard body shape: The roles of sexual selection and fecundity selection. Evolution 56:1538-1542.

Oniki Y (1983) Notes on fly (Muscidae) parasitism of nestlings of South American birds. Gerfaut 73:281-286.

Oorebeek M, Kleindorfer S (2008) Climate or host availability: what determines the seasonal abundance of ticks? Parasitology Research 103:871.

Orozco F, Bell AE (1974) Reciprocal recurrent selection compared to within-strain selection for increasing rate of egg lay of *Tribolium* under optimal and stress conditions. Genetics 77:143.

Packer JG, Meyerson LA, Richardson DM, Brundu G, Allen WJ, Bhattarai GP, Brix H, Canavan S, Castiglione S, Cicatelli A, Čuda J, Cronin JT, Eller F, Guarino F, Guo W-H, Guo W-Y, Guo X, Hierro JL, Lambertini C, Liu J, Lozano V, Mozdzer TJ, Skálová H, Villarreal D, Wang R-Q, Pyšek P (2017) Global networks for invasion science: benefits, challenges and guidelines. Biological Invasions 19:1081-1096.

Papadopoulos NT, Katsoyannos BI, Nestle D (2003) Spatial Autocorrelation Analysis of a *Ceratitis capitata* (Diptera: Tephritidae) Adult Population in a Mixed Deciduous Fruit Orchard in Northern Greece. Environmental Entomology 32:319-326.

Papathanos PA, Windbichler N, Akbari OS (2014) Sex ratio manipulation for insect population control. Transgenic insects: techniques and applications 83:100.

Papkou A, Guzella T, Yang W, Koepper S, Pees B, Schalkowski R, Barg M-C, Rosenstiel PC, Teotónio H, Schulenburg H (2019) The genomic basis of Red Queen dynamics during rapid reciprocal host–pathogen coevolution. Proceedings of the National Academy of Sciences 116:923-928.

Parker G (1978) Evolution of competitive mate searching. Annual Review of Entomology 23:173-196.

Parker TH, Wilkin TA, Barr IR, Sheldon BC, Rowe L, Griffith SC (2011) Fecundity selection on ornamental plumage colour differs between ages and sexes and varies over small spatial scales. Journal of Evolutionary Biology 24:1584-1597.

Paterson RA, Townsend CR, Poulin R, Tompkins DM (2011) Introduced brown trout alter native acanthocephalan infections in native fish. Journal of Animal Ecology 80:990-998.

Paterson S, Vogwill T, Buckling A, Benmayor R, Spiers AJ, Thomson NR, Quail M, Smith F, Walker D, Libberton B, Fenton A, Hall N, Brockhurst MA (2010) Antagonistic coevolution accelerates molecular evolution. Nature 464:275-278.

Patitucci LD, Quiroga M, Couri MS, Saravia-Pietropaolo MJ (2017) Oviposition in the bird parasitic fly *Philornis torquans* (Nielsen, 1913) (Diptera: Muscidae) and eggs' adaptations to dry environments. Zoologischer Anzeiger 267:15-20.

Peck SB (1994) Aerial Dispersal of Insects Between and to Islands in the Galápagos Archipelago, Ecuador. Annals of the Entomological Society of America 87:218-224.

Peck SB (2001) Smaller orders of insects of the Galápagos Islands, Ecuador: evolution, ecology, and diversity. NRC Research Press

Peck SB (2008) The Beetles of the Galápagos Islands, Ecuador: Evolution, ecology and diversity (Insecta: Coleoptera). Journal of Insect Conservation 12:729-730.

Peckarsky BL, Cowan CA (1991) Consequences of larval intraspecific competition to stonefly growth and fecundity. Oecologia 88:277-288.

Pelletier F, Coltman DW (2018) Will human influences on evolutionary dynamics in the wild pervade the Anthropocene? BMC Biology 16:7.

Penczykowski RM, Laine A-L, Koskella B (2016) Understanding the ecology and evolution of host–parasite interactions across scales. Evolutionary Applications 9:37-52.

Perry BW, Schield DR, Castoe TA (2018) Evolution: Plasticity versus selection, or plasticity and selection? Current Biology 28:R1104-R1106.

Peters KJ, Evans C, Aguirre JD, Kleindorfer S (2019) Genetic admixture predicts parasite intensity: Evidence for increased hybrid performance in Darwin's tree finches. Royal Society Open Science 6:181616.

Peters KJ, Myers SA, Dudaniec RY, O'Connor JA, Kleindorfer S (2017) Females drive asymmetrical introgression from rare to common species in Darwin's tree finches. Journal of Evolutionary Biology 30:1940-1952.

Petren K, Grant PR, Grant BR, Clack AA, Lescano NV (2010) Multilocus genotypes from Charles Darwin's finches: biodiversity lost since the voyage of the Beagle.

Philosophical Transactions of the Royal Society B: Biological Sciences 365:1009-1018.

Phillips BL, Shine R (2004) Adapting to an invasive species: Toxic cane toads induce morphological change in Australian snakes. Proceedings of the National Academy of Sciences 101:17150-17155.

Pike CL, Ramirez IE, Anchundia DJ, Fessl B, Heimpel GE, Causton CE (2021) Behavior of the Avian Parasite *Philornis downsi* (Diptera: Muscidae) in and Near Host Nests in the Galapagos Islands. Journal of Insect Behavior 34:296-311.

Pincheira-Donoso D, Hunt J (2017) Fecundity selection theory: Concepts and evidence. Biological Reviews 92:341-356.

Piry S, Luikart G, Cornuet JM (1999) Computer note. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. Journal of Heredity 90:502-503.

Polechová J (2018) Is the sky the limit? On the expansion threshold of a species' range. PLOS Biology 16:e2005372.

Pont AC, AC P (1974) A revision of the genus *Passeromyia* Rodhain & Villeneuve (Diptera: Muscidae). Bulletin of the British Museum (Natural History) Entomology 30:341-372.

Poulin R (2011) Evolutionary ecology of parasites. Princeton University Press Poulin R, Keeney DB (2008) Host specificity under molecular and experimental scrutiny. Trends in Parasitology 24:24-28.

Poulin R, Paterson RA, Townsend CR, Tompkins DM, Kelly DW (2011) Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. Freshwater Biology 56:676-688.

Poullain V, Gandon S, Brockhurst MA, Buckling A, Hochberg ME (2008) The evolution of specificity in evolving and coevolving antagonistic interactions between bacteria and its phage. Evolution 62:1-11.

Premachandra HKA, Nguyen NH, Knibb W (2019) Effectiveness of SNPs for parentage and sibship assessment in polygamous yellowtail kingfish *Seriola lalandi*. Aquaculture 499:24-31.

Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ (2008) Adaptive evolution in invasive species. Trends in Plant Science 13:288-294.

Preston-Mafham K (2001) Resource defence mating system in two flies from Sulawesi: *Gymnonerius fuscus* Wiedemann and *Telostylinus* sp. near *duplicatus* Wiedemann (Diptera: Neriidae). Journal of Natural History 35:149-156.

Preziosi RF, Fairbairn DJ (1996) Sexual size dimorphism and selection in the wild in the waterstrider *Aquarius remigis*: Body size, components of body size and male mating success. Journal of Evolutionary Biology 9:317-336.

Preziosi RF, Fairbairn DJ, Roff DA, Brennan JM (1996) Body size and fecundity in the waterstrider *Aquarius remigis*: a test of Darwin's fecundity advantage hypothesis.

Oecologia 108:424-431.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. Genetics 155:945.

Proudfoot GA, Teel PD, Mohr RM (2006) Ferruginous Pygmy-Owl (*Glaucidium brasilianum*) and Eastern Screech-Owl (*Megascopes asio*): New Hosts for *Philornis mimicola* (Diptera: Muscidae) and *Ornithodoros concanensis* (Acari: Argasidae). Journal of Wildlife Diseases 42:873-876.

Puechmaille SJ (2016) The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Molecular Ecology Resources 16:608-627.

Quintero-Fong L, Toledo J, Ruiz-Montoya L, Rendón P, Orozco-Dávila D, Valle-Mora J, Liedo P (2018) Demography of a genetic sexing strain of *Anastrepha ludens* (Diptera: Tephritidae): Effects of selection based on mating performance. Agricultural and Forest Entomology 20:1-8.

Quiroga MA, Monje LD, Arrabal JP, Beldomenico PM (2016) New molecular data on subcutaneous *Philornis* (Diptera: Muscidae) from southern South America suggests the existence of a species complex. Revista Mexicana de Biodiversidad 87:1383-1386.

Quiroga MA, Reboreda JC (2012) Lethal and Sublethal Effects of Botfly (*Philornis seguyi*) Parasitism on House Wren Nestlings. The Condor 114:197-202.

Quiroga MA, Reboreda JC (2013) Sexual differences in life history traits of *Philornis seguyi* (Diptera: Muscidae) parasitizing house wrens (*Troglodytes aedon*). Annals of the Entomological Society of America 106:222-227.

R Core Development Team (2020) R: A language and environment for statistical computing. R version 4.0.0. R Foundation for Statistical Computing, Vienna, Austria,

Raban RR, Marshall JM, Akbari OS (2020) Progress towards engineering gene drives for population control. Journal of Experimental Biology 223:jeb208181.

Rabuffetti FL, Reboreda JC (2007) Early Infestation by Bot Flies (*Philornis Seguyi*) Decreases Chick Survival and Nesting Success in Chalk-Browed Mockingbirds (*Mimus Saturninus*). The Auk 124:898-906.

Raghavan RK, Barker SC, Cobos ME, Barker D, Teo EJM, Foley DH, Nakao R, Lawrence K, Heath ACG, Peterson AT (2019) Potential Spatial Distribution of the Newly 294

Introduced Long-horned Tick, *Haemaphysalis longicornis* in North America. Scientific Reports 9:498.

Ramirez IE (2018) *Philornis downsi:* Interactions with its Host in the Introduced Range and its Parasitoids in its Native Range. University of Minnesota, Ann Arbor, pp. 72

Ramirez IE, Causton CE, Gutierrez GA, Mosquera Denis A, Piedrahita P, Heimpel GE (2022) Specificity within bird–parasite–parasitoid food webs: A novel approach for evaluating potential biological control agents of the avian vampire fly. Journal of Applied Ecology 59:2189-2198.

Reeve JP, Fairbairn DJ (1999) Change in sexual size dimorphism as a correlated response to selection on fecundity. Heredity 83:697-706.

Reichard M, Polačik M, Tarkan AS, Spence R, Gaygusuz Ö, Ercan E, Ondračková M, Smith C (2010) The bitterling–mussel coevolutionary relationship in areas of recent and ancient sympatry. Evolution 64:3047-3056.

Reigada C, Godoy WAC (2005) Seasonal fecundity and body size in *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). Neotropical Entomology 34:163-168.

Riback T, Godoy W (2008a) Fecundity, body size and population dynamics of *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae). Brazilian Journal of Biology 68:123-128.

Riback TIS, Godoy WAC (2008b) Fecundity, body size and population dynamics of *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae). Brazilian Journal of Biology 68:123-128.

Ricciardi A, Blackburn TM, Carlton JT, Dick JTA, Hulme PE, Iacarella JC, Jeschke JM, Liebhold AM, Lockwood JL, MacIsaac HJ, Pyšek P, Richardson DM, Ruiz GM, Simberloff D, Sutherland WJ, Wardle DA, Aldridge DC (2017) Invasion Science: A Horizon Scan of Emerging Challenges and Opportunities. Trends in Ecology & Evolution 32:464-474.

Roderick GK, Navajas M (2003) Genes in new environments: genetics and evolution in biological control. Nature Reviews Genetics 4:889-899.

Roff D (2001) Evolution of Life History. In: Levin SA (ed) Encyclopedia of Biodiversity (Second Edition). Academic Press, Waltham, pp. 631-641

Romey WL, Wallace AC (2007) Sex and the selfish herd: Sexual segregation within nonmating whirligig groups. Behavioral Ecology 18:910-915.

Romine M, Knutie SA, Crow CM, Vaziri GJ, Chaves J, Koop JAH, Lamichhaney S (2021) The genome sequence of the avian vampire fly (*Philornis downsi*), an invasive nest parasite of Darwin's finches in Galápagos. bioRxiv:2021.06.09.447800.

Roswell M, Dushoff J, Winfree R (2019) Male and female bees show large differences in floral preference. PLOS ONE 14:e0214909.

Ruberanziza E, Owada K, Clark NJ, Umulisa I, Ortu G, Lancaster W, Munyaneza T, Mbituyumuremyi A, Bayisenge U, Fenwick A (2019) Mapping soil-transmitted helminth parasite infection in Rwanda: Estimating endemicity and identifying at-risk populations. Tropical Medicine and Infectious Disease 4:93.

Ruckstuhl K, Neuhaus P (2006) Sexual segregation in vertebrates. Cambridge University Press, Cambridge

Russell JC, Meyer J-Y, Holmes ND, Pagad S (2017) Invasive alien species on islands: impacts, distribution, interactions and management. Environmental Conservation 44:359-370.

Sabrosky CW, Bennett GF, Whitworth TL (1989) Bird blow flies (Protocalliphora) in North America (Diptera: Calliphoridae) with notes on Palearctic species. Smithsonian Institution Press

Sage R, Boulton RA, Lahuatte PF, Causton CE, Cloutier R, Heimpel GE (2018) Environmentally cued hatching in the bird-parasitic nest fly *Philornis downsi*. Entomologia Experimentalis et Applicata 166:752-760.

Saino N, Ambrosini R, Caprioli M, Liechti F, Romano A, Rubolini D, Scandolara C (2017) Wing morphology, winter ecology, and fecundity selection: Evidence for sex-dependence in barn swallows (*Hirundo rustica*). Oecologia 184:799-812.

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annual Review of Ecology and Systematics 32:305-332.

Samuel MD, Woodworth BL, Atkinson CT, Hart PJ, LaPointe DA (2015) Avian malaria in Hawaiian forest birds: infection and population impacts across species and elevations. Ecosphere 6:art104.

Santos J, Pascual M, Simões P, Fragata I, Lima M, Kellen B, Santos M, Marques A, Rose MR, Matos M (2012) From nature to the laboratory: the impact of founder effects on adaptation. Journal of Evolutionary Biology 25:2607-2622.

Sarfati M, Krasnov BR, Ghazaryan L, Khokhlova IS, Fielden LJ, Degen AA (2005) Energy costs of blood digestion in a host-specific haematophagous parasite. Journal of Experimental Biology 208:2489.

Sarkar D (2008) Lattice: Multivariate data visualization with R. Springer Science & Business Media

Sato A, Tichy H, O'HUigin C, Grant PR, Grant BR, Klein J (2001) On the Origin of Darwin's Finches. Molecular Biology and Evolution 18:299-311.

Savage J, Vockeroth J (2010) Muscidae (house flies, stable flies). Manual of Central American Diptera 2:1281-1295.

Scharf I, Meiri S (2013) Sexual dimorphism of heads and abdomens: Different approaches to 'being large' in female and male lizards. Biological Journal of the Linnean Society 110:665-673.

Schatz AM, Park AW (2021) Host and parasite traits predict cross-species parasite acquisition by introduced mammals. Proceedings of the Royal Society B: Biological Sciences 288:20210341.

Schirmel J, Bundschuh M, Entling MH, Kowarik I, Buchholz S (2016) Impacts of invasive plants on resident animals across ecosystems, taxa, and feeding types: a global assessment. Global Change Biology 22:594-603.

Schmid-Hempel P (2021) Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics. Oxford University Press

Schulte RD, Makus C, Hasert B, Michiels NK, Schulenburg H (2010) Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. Proceedings of the National Academy of Sciences 107:7359-7364.

Sciarretta A, Tabilio MR, Lampazzi E, Ceccaroli C, Colacci M, Trematerra P (2018) Analysis of the Mediterranean fruit fly [*Ceratitis capitata* (Wiedemann)] spatio-temporal distribution in relation to sex and female mating status for precision IPM. PLOS ONE 13:e0195097.

Segura LN, Reboreda JC (2011) Botfly Parasitism Effects on Nestling Growth and Mortality of Red-Crested Cardinals. The Wilson Journal of Ornithology 123:107-115.

Sexton JP, Montiel J, Shay JE, Stephens MR, Slatyer RA (2017) Evolution of ecological niche breadth. Annual Review of Ecology, Evolution, and Systematics 48:183-206.

Shackleton RT, Bertzky B, Wood LE, Bunbury N, Jäger H, van Merm R, Sevilla C, Smith K, Wilson JRU, Witt ABR, Richardson DM (2020) Biological invasions in World Heritage Sites: current status and a proposed monitoring and reporting framework. Biodiversity and Conservation 29:3327-3347.

Shiao S-F, Yeh T-C (2008) Larval competition of *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae): Behavior and ecological studies of two blow fly species of forensic significance. Journal of Medical Entomology 45:785-799.

Shine R (1989) Ecological causes for the evolution of sexual dimorphism: A review of the evidence. The Quarterly Review of Biology 64:419-461.

Shine R, Brown GP, Phillips BL (2011) An evolutionary process that assembles phenotypes through space rather than through time. Proceedings of the National Academy of Sciences 108:5708-5711.

Shingleton A, W., Mirth C, K., Bates P, W. (2008) Developmental model of static allometry in holometabolous insects. Proceedings of the Royal Society B: Biological Sciences 275:1875-1885.

Silvestri L, Antoniazzi LR, Couri MS, Monje LD, Beldomenico PM (2011) First record of the avian ectoparasite *Philornis downsi* Dodge & Aitken, 1968 (Diptera: Muscidae) in Argentina. Systematic Parasitology 80:137.

Simberloff D (2003) Eradication—preventing invasions at the outset. Weed Science 51:247-253.

Simberloff D (2012) Risks of biological control for conservation purposes. BioControl 57:263-276.

Simpson A, Jarnevich C, Madsen J, Westbrooks R, Fournier C, Mehrhoff L, Browne M, Graham J, Sellers E (2009) Invasive species information networks: collaboration at multiple scales for prevention, early detection, and rapid response to invasive alien species. Biodiversity 10:5-13.

Singh D, Bala M (2009) The effect of starvation on the larval behavior of two forensically important species of blow flies (Diptera: Calliphoridae). Forensic Science International 193:118-121.

Sinkins SP, Gould F (2006) Gene drive systems for insect disease vectors. Nature Reviews Genetics 7:427-435.

Sivinski JM (1993) Longevity and fecundity in the Caribbean fruit fly (Diptera: Tephritidae): Effects of mating, strain and body size. Florida Entomologist 76:635-644. 298

Sivinski JM, Dodson G (1992) Sexual dimorphism in *Anastrepha suspensa* (Loew) and other tephritid fruit flies (Diptera: Tephritidae): Possible roles of developmental rate, fecundity, and dispersal. Journal of Insect Behavior 5:491-506.

Skidmore P (1985) The biology of the Muscidae of the world. Springer Science & Business Media, Dordrecht

Smith EP (1982) Niche breadth, resource availability, and inference. Ecology 63:1675-1681.

Souris M (2018) Ecuador. In: Institut de Recherche pour le Développement, IRD. http://www.savgis.org/ecuador.htm

2021

Spalding MG, Mertins JW, Walsh PB, Morin KC, Dunmore DE, Forrester DJ (2002) Burrowing fly larvae (*Philornis porteri*) associated with mortality of Eastern Bluebirds in Florida Journal of Wildlife Diseases 38:776-783.

Spatz DR, Zilliacus KM, Holmes ND, Butchart SHM, Genovesi P, Ceballos G, Tershy BR, Croll DA (2017) Globally threatened vertebrates on islands with invasive species. Science Advances 3:e1603080.

Stanley CR, Liddiard Williams H, Preziosi RF (2018) Female clustering in cockroach aggregations—A case of social niche construction? Ethology 124:706-718.

Steinwascher K (1984) Egg size variation in *Aedes aegypti*: Relationship to body size and other variables. The American Midland Naturalist 112:76-84.

Stiling P, Cornelissen T (2005) What makes a successful biocontrol agent? A metaanalysis of biological control agent performance. Biological Control 34:236-246.

Stillwell RC, Dworkin I, Shingleton AW, Frankino WA (2011) Experimental manipulation of body size to estimate morphological scaling relationships in *Drosophila*. Journal of Visualised Experiments:e3162.

Stone GN (1995) Female foraging responses to sexual harassment in the solitary bee *Anthophora plumipes*. Animal Behaviour 50:405-412.

Tammaru T, Esperk T, Castellanos I (2002) No evidence for costs of being large in females of *Orgyia* spp. (Lepidoptera, Lymantriidae): Larger is always better. Oecologia 133:430-438.

Teder T, Tammaru T (2005) Sexual size dimorphism within species increases with body size in insects. Oikos 108:321-334.

Teixeira D (1999) Myiasis caused by obligatory parasites. Ib. General observations on the biology of species of the genus *Philornis* Meinert, 1890 (Diptera, Muscidae). Myiasis in man and animals in the Neotropical Region:71-96.

Teixeira D, Couri M, Luigi G (1990) Notes on the biology of *Philornis rufoscutellaris* Couri, 1983 (Diptera, Muscidae) and on its association with nestling birds. Revista Brasileira de Entomologia 34:271-275.

Telfer S, Bown K (2012) The effects of invasion on parasite dynamics and communities. Functional Ecology 26:1288-1299.

Tershy BR, Shen K-W, Newton KM, Holmes ND, Croll DA (2015) The Importance of Islands for the Protection of Biological and Linguistic Diversity. BioScience 65:592-597.

Theodosopoulos AN, Hund AK, Taylor SA (2019) Parasites and host species barriers in animal hybrid zones. Trends in Ecology & Evolution 34:19-30.

Thomas JK, Fadul GJ, Keller GP, Chaudhury MF (2018) The use of dried Bovine hemoglobin and plasma for mass rearing new world screwworm. Journal of Insect Science 18 Thompson JN (1994) The Coevolutionary Process. University of Chicago Press

Tigano A, Friesen VL (2016) Genomics of local adaptation with gene flow. Molecular Ecology 25:2144-2164.

Toral-Granda MV, Causton CE, Jäger H, Trueman M, Izurieta JC, Araujo E, Cruz M, Zander KK, Izurieta A, Garnett ST (2017) Alien species pathways to the Galapagos Islands, Ecuador. PLOS ONE 12:e0184379.

Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and animals. Frontiers in Ecology and the Environment 2:183-190.

Torres-Carvajal O, Pazmiño-Otamendi G, Salazar-Valenzuela D (2019) Reptiles of Ecuador: a resource-rich portal, with a dynamic checklist and photographic guides.

Amphibian & Reptile Conservation 13:209-229.

Travis JM, Dytham C (2002) Dispersal evolution during invasions. Evolutionary Ecology Research 4:1119-1129.

Tye A, Francisco-Ortega J, Bramwell D, Caujapé-Castells J (2011) Origins and evolution of Galapagos endemic vascular plants. The biology of island floras:89-153.

Uhazy LS, Arendt WJ (1986) Pathogenesis associated with Philornis myiasis (Diptera: Muscidae) on Nestling Pearly-eyed Thrashers (Aves: Mimidae) in the Luquillo rain forest, Puerto Rico. Journal of Wildlife Diseases 22:224-237.

UNESCO Galápagos Islands. In. https://whc.unesco.org/en/list/1/

Accessed: 16 Aug 2022

Välimäki P, Kaitala A (2007) Life history tradeoffs in relation to the degree of polyandry and developmental pathway in *Pieris napi* (Lepidoptera, Pieridae). Oikos 116:1569-1580.

Välimäki P, Kaitala A, Madslien K, Härkönen L, Várkonyi G, Heikkilä J, Jaakola M, Ylönen H, Kortet R, Ytrehus B (2011) Geographical variation in host use of a blood-feeding ectoparasitic fly: Implications for population invasiveness. Oecologia 166:985-995.

Van Alphen JJ, Visser ME (1990) Superparasitism as an adaptive strategy for insect parasitoids. Annual Review of Entomology 35:59-79.

van Riper III C, van Riper SG, Goff ML, Laird M (1986) The Epizootiology and Ecological Significance of Malaria in Hawaiian Land Birds. Ecological Monographs 56:327-344.

Van Valen L (1973) A new evolutionary law. Evolutionary Theory 1:1-30.

Vargas RI, Leblanc L, Piñero JC, Hoffman KM (2014) Male Annihilation, Past, Present, and Future. In: Shelly T, Epsky N, Jang EB, Reyes-Flores J and Vargas R (eds) Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies: Lures, Area-Wide Programs, and Trade Implications. Springer Netherlands, Dordrecht, pp. 493-511 Venables B, Ripley B (2002) Modern Applied Statistics With S.

Vilà M, Hulme PE (2017) Impact of biological invasions on ecosystem services. Springer

Visher E, Boots M (2020) The problem of mediocre generalists: Population genetics and eco-evolutionary perspectives on host breadth evolution in pathogens. Proceedings of the Royal Society B: Biological Sciences 287:20201230.

Visser ME, Rosenheim JA (1998) The Influence of Competition between Foragers on Clutch Size Decisions in Insect Parasitoids. Biological Control 11:169-174.

Vitousek PM (1988) Diversity and biological invasions of oceanic islands. Biodiversity 20:181-189.

Wang J (2009) A new method for estimating effective population sizes from a single sample of multilocus genotypes. Molecular Ecology 18:2148-2164.

Wang J (2019) Pedigree reconstruction from poor quality genotype data. Heredity 122:719-728.

Wang J, Santure AW (2009) Parentage and Sibship Inference From Multilocus Genotype Data Under Polygamy. Genetics 181:1579-1594. 301 Warburg MS, Yuval B (1997) Circadian Patterns of Feeding and Reproductive Activities of Mediterranean Fruit Flies (Diptera: Tephritidae) on Various Hosts in Israel. Annals of the Entomological Society of America 90:487-495.

Waters JM, Emerson BC, Arribas P, McCulloch GA (2020) Dispersal Reduction: Causes, Genomic Mechanisms, and Evolutionary Consequences. Trends in Ecology & Evolution 35:512-522.

Watkins G, Cruz F (2007) Galapagos at risk: A socioeconomic analysis.

Weir BS, Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population Structure. Evolution 38:1358-1370.

Welter SC, Pickel C, Millar JG, Cave F, Van Steenwyk R, Dunley J (2005)

Pheromone mating disruption offers selective management options for key pests. California

Agriculture 59:16-22.

Weng Z, Yang Y, Wang X, Wu L, Hua S, Zhang H, Meng Z (2021) Parentage Analysis in Giant Grouper (*Epinephelus lanceolatus*) Using Microsatellite and SNP Markers from Genotyping-by-Sequencing Data. Genes 12

Wessel P, Smith WHF (1996) A global, self-consistent, hierarchical, high-resolution shoreline database. Journal of Geophysical Research: Solid Earth 101:8741-8743.

Whitlock MC (1996) The Red Queen beats the Jack-Of-All-Trades: The limitations on the evolution of phenotypic plasticity and niche breadth. The American Naturalist 148:S65-S77.

Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, 'invasive traits' and recipient communities: Challenges for predicting invasive potential. Diversity and Distributions 14:569-580.

Wickham H (2009) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

Wickman P-O, Karlsson B (1989) Abdomen size, body size and the reproductive effort of insects. Oikos 56:209-214.

Wiedenfeld DA, Jiménez GU, Fessl B, Kleindorfer S, Carlos Valarezo J (2007) Distribution of the introduced parasitic fly *Philornis downsi* (Diptera, Muscidae) in the Galápagos Islands. Pacific Conservation Biology 13:14-19.

Wikelski M, Foufopoulos J, Vargas H, Snell H (2004) Galápagos Birds and Diseases: Invasive Pathogens as Threats for Island Species. Ecology and Society 9 Wilkinson GS, Johns PM (2005) Sexual selection and the evolution of mating systems in flies. In: Yeates DK and Weigmann BM (eds) The Biology of the Diptera. Columbia University Press, New York, pp. 312-329

Williams H, Richardson AMM (1983) Life history responses to larval food shortages in four species of necrophagous flies (Diptera: Calliphoridae). Australian Journal of Ecology 8:257-263.

Winkler JD, Stölting KN, Wilson AB (2012) Sex-specific responses to fecundity selection in the broad-nosed pipefish. Evolutionary Ecology 26:701-714.

Wolinska J, Lively CM, Spaak P (2008) Parasites in hybridizing communities: the Red Queen again? Trends in Parasitology 24:121-126.

Wong GKL, Jim CY (2018) Abundance of urban male mosquitoes by green infrastructure types: implications for landscape design and vector management. Landscape Ecology 33:475-489.

Woodworth BL, Atkinson CT, LaPointe DA, Hart PJ, Spiegel CS, Tweed EJ, Henneman C, LeBrun J, Denette T, DeMots R, Kozar KL, Triglia D, Lease D, Gregor A, Smith T, Duffy D (2005) Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. Proceedings of the National Academy of Sciences of the United States of America 102:1531.

Woolhouse MEJ, Webster JP, Domingo E, Charlesworth B, Levin BR (2002) Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nature Genetics 32:569-577.

Wright NA, Steadman DW, Witt CC (2016) Predictable evolution toward flightlessness in volant island birds. Proceedings of the National Academy of Sciences 113:4765-4770.

Xue R-D, Ali A (1994) Oviposition, fecundity, and body size of a pestiferous midge, *Chironomus crassicaudatus* (Diptera: Chironomidae). Environmental Entomology 23:1480-1484.

Yamagishi M, Kakinohana H, Kuba H, Kohama T, Nakamoto Y, Sokei Y, Kinjo K (1993) Eradication of the melon fly from Okinawa, Japan, by means of the sterile insect technique. Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques.

Yeates D, Dodson G (1990) The mating system of a bee fly (Diptera: Bombyliidae). II. Factors affecting male territorial and mating success. Journal of Insect Behavior 3:619-636.

Yi X, Latch EK (2022) Nonrandom missing data can bias Principal Component Analysis inference of population genetic structure. Molecular Ecology Resources 22:602-611.

Young BE (1993) Effects of the parasitic botfly *Philornis carinatus* on nestling house wrens, *Troglodytes aedon*, in Costa Rica. Oecologia 93:256-262.

Young HS, Dirzo R, McCauley DJ, Agwanda B, Cattaneo L, Dittmar K, Eckerlin RP, Fleischer RC, Helgen LE, Hintz A, Montinieri J, Zhao S, Helgen KM (2015) Drivers of Intensity and Prevalence of Flea Parasitism on Small Mammals in East African Savanna Ecosystems. Journal of Parasitology 101:327-335.

Yuval B (2005) Mating Systems of Blood-Feeding Flies. Annual Review of Entomology 51:413-440.

Zerbino DR, Birney E (2008) Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. Genome Research 18:821-829.

Zhang L, Rohr J, Cui R, Xin Y, Han L, Yang X, Gu S, Du Y, Liang J, Wang X, Wu Z, Hao Q, Liu X (2022) Biological invasions facilitate zoonotic disease emergences. Nature Communications 13:1762.

