# IMPACT AND MOLECULAR ECOLOGY OF *Philornis downsi*:

## AN INTRODUCED PARASITIC FLY OF BIRDS ON

## THE GALÁPAGOS ISLANDS


**RACHAEL YVONNE DUDANIEC**

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**School of Biological Sciences**

**Flinders University, South Australia**

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Cover images: above—nestlings of Darwin's finches; below—adult fly of *Philornis downsi*.

Photos: Sonia Kleindorfer

**DECLARATION**

I certify that this work does not incorporate without acknowledgement any material previously submitted for a degree at this or any other university. To the best of my knowledge the work contained within this thesis has not been previously published or written by any other person with exception to those who have been given due reference. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder of those works.

Rachael Yvonne Dudaniec

22 August 2008

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## THESIS ABSTRACT

Endemic avian populations on islands may experience increased risks associated with introduced pathogens. This study examines the impact and molecular ecology of an invasive fly (*Philornis downsi*), which is a haematophagous ectoparasite of nestling birds on the Galápagos Islands, Ecuador. This parasite causes extreme mortality and fitness costs in Darwin's finches and threatens vulnerable and declining finch species across the archipelago.

This study may be divided into two complementary parts; (1) ecological factors affecting the impact of *P. downsi* on its avian hosts (chapters 2-4); and (2) molecular ecological insights into the genetic structure and reproductive behaviour of *P. downsi* (chapters 5-7). With six years of data across six finch species, *P. downsi* intensity was found to be higher in years with increased rainfall, and finch species with high adult body mass had more parasites in their nests. The percentage of nests with mortality was between 40 % and 100 % for all six host species. Darwin's small tree finches that nested in mixed species aggregations had increased *P. downsi* intensity, and larger nests had more parasites. Evidence is therefore presented for parasite-mediated selection pressures on nesting behaviour and nest characteristics that interact with climate, habitat and host species. Using nine novel microsatellite markers for *P. downsi*, gene flow and dispersal was examined across two climatically contrasting habitats and three islands of the Galápagos. Low genetic differentiation across habitats and islands indicated high dispersal in *P. downsi*, though evidence for population genetic bottlenecks and fine-scale genetic structure within islands was observed. Genetic analyses of *P. downsi* broods within nests revealed a high frequency of multiple mating in female flies, and an almost ubiquitous occurrence of multiple infestations within nests. Patterns of host distribution, parasite intensity, and genetic relatedness of *P. downsi* broods across habitats on Floreana Island provided evidence for host density-dependent oviposition behaviour in female flies.

The scope and approach of this study is unmatched by previous investigations of dipteran ectoparasites of birds, and represents a seminal contribution to the fields of avian parasitology and invasive species biology. The results are particularly applicable to the conservation management of the Galápagos avifauna, and future efforts to control and eventually eradicate the severe threat of *P. downsi* to endemic island populations.

## STATEMENT OF AUTHORSHIP

This thesis contains no material published elsewhere except where reference is made in the text. No other person's work has been used without due acknowledgement in the main text of the thesis. This thesis represents an original independent piece of research. All significant aspects of analysis and interpretation of results were done by myself. The thesis is presented as a series of already published or submitted papers. The nature of the collaborations indicated by the co-authorship of these papers is as follows:

- Sonia Kleindorfer is included in recognition of the contribution she has made to my training as my primary supervisor, for providing funding for the project and access to data collected prior to my Phd, and for comments on manuscript drafts.
- Birgit Fessl is recognised as a co-author due to her role as primary collaborator on the Galápagos Islands, assistance with field work and data collection.
- Michael G. Gardner is included in recognition of his role as my co-supervisor, for providing expertise and practical advice on molecular laboratory techniques and analysis, and commenting on drafts.
- Steven Donnellan is included due to the advice he provided on the molecular genetic analyses, and commenting on drafts.

All research procedures reported in this thesis follow the Guidelines for the Use of Animals in Research (Flinders University, Charles Darwin Research Station, Galápagos National Parks), the legal requirements of Ecuador (the country in which the work was carried out), and were approved by the Animal Welfare Committee of Flinders University.

Rachael Yvonne Dudaniec

# CHAPTER 1: Introduction

Host-parasite interactions can disrupt or alter the ecology and population dynamics of host species while shaping selection pressures on host and parasite life-histories (Richner 1998). In island ecosystems, invasive parasites may be particularly threatening to endemic species that have had limited pathogen exposure, resulting in high susceptibility, mortality and extinction risk (van Riper 1986; Wikelski et al. 2004). Arthropod ectoparasites of birds are frequently associated with high fitness costs, mortality and changes in host behaviour that may alter community interactions and population dynamics. The ecology of invasive avian parasites is largely determined by the distribution, density, and reproductive biology of their host species, and along with host impact, deserves equal attention for a comprehensive understanding of bird-parasite interactions.

Darwin's finches are an iconic group of fourteen bird species that is endemic to the Galápagos Islands (Figure 1.1). Located ~1000 km west of South America, the Galápagos Islands are considered globally to be a natural living laboratory for biological research. Darwin's finches are known as an evolutionary treasure and have inspired extensive advancements in our understanding of adaptive radiation and natural selection. In reflection of his voyage to Galápagos in 1835, Charles Darwin concluded his memoir with the famous lines:

> "Hence, both in space and time, we seem to be brought somewhat near to that great fact —that mystery of mysteries— the first appearance of new beings on this earth."

Current studies of Darwin's finches continue to provide theoretical advancements in the fields of evolutionary biology and behavioural ecology, but not in the absence of anthropogenic threats to their survival. While introduced predators of the endemic avifauna are well known (e.g. rats, cats, predatory birds), the impact of disease and parasitism in Darwin's finches was virtually unstudied until the discovery of a blood-sucking parasitic fly, *Philornis downsi*, within finch nests by B. Fessl and colleagues in 1997 (Fessl et al. 2001). Devastating and obvious high levels of nestling mortality due to *P. downsi* parasitism (Figure 1.2) caused great concern among Galápagos ornithologists and conservationists, and the study of this host-parasite interaction was promptly initiated (Fessl and Tebbich 2002). This thesis represents the first comprehensive study to describe the impacts of *P. downsi* on the fitness and behaviour of Darwin's finches, and in addition, to examine the reproductive and behavioural ecology of *P. downsi* across the Galápagos archipelago using molecular genetic methods.

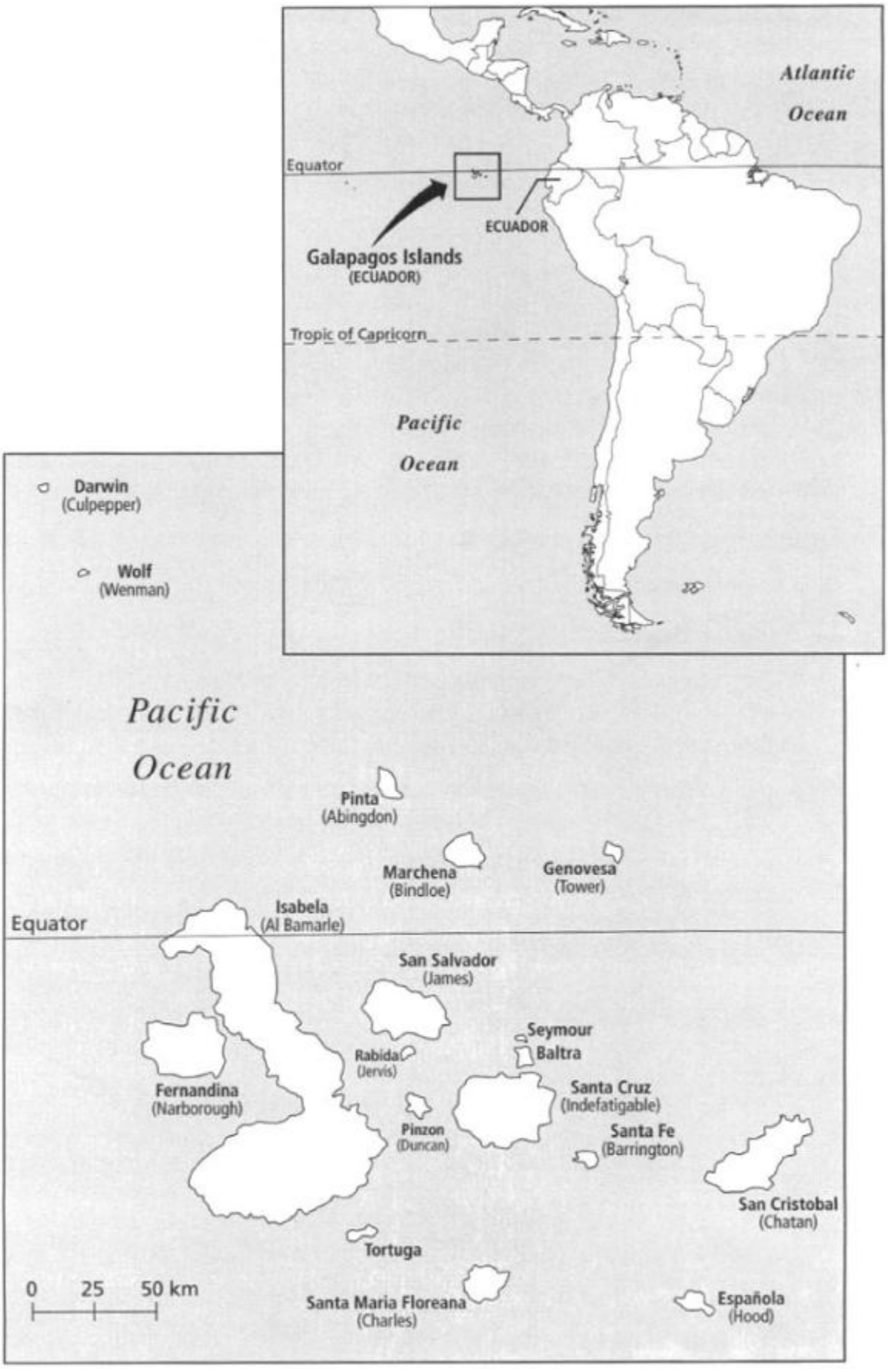


**Figure 1.1** Map of the Galápagos Islands, Ecuador.

**(a)**

**(b)**

**Figure 1.2** (a) Four parasitised nestlings of medium ground finch (*Geospiza fortis*) at ten days of age, and all *P. downsi* larvae and puparia collected from their nest; (b) *P. downsi* third instar larvae in the nesting material of a finch nest. Each larva is approximately 1-1.5 cm in length.

### *Avian host-parasite interactions*

Apart from frequently causing mortality and reduced fledging success, parasites may also directly or indirectly affect avian hosts on morphological, behavioural and physiological levels (Richner 1998). These affects can vary dramatically across host species depending on host characteristics (see Chapter 2). Avian parasites may show variation in impact between hosts according to clutch size (Richner and Heeb 1995), nest structural characteristics (Gwinner 1997), host social behaviour (Whiteman and Parker 2004) or host body mass (Poulin and George-Nascimento 2007) (Chapters 2, 3 and 4). For example, aggregated nesting behaviour has advantages such as reduced nest predation, but also costs such as a higher chance of acquiring and accumulating ectoparasites due to increased proximity and contact among individuals (Côté and Poulin 1995; addressed in Chapter 4). Parasites may also use sound or olfactory cues to locate hosts, which may be intensified where hosts are aggregated (Gibson and Torr 1999), or for nests with larger clutch size (Richner and Heeb 1995; Chapter 3).

Variation in habitat characteristics may affect the impact of parasites upon avian hosts (Chapters 3 and 4), as well as the reproductive behaviour of parasites (Chapters 6 and 7) because of selective pressures brought about by variation in climate and vegetation structure, host species composition or host distribution. For nest-dwelling ectoparasites of birds, nest size and structure may significantly influence the abundance of parasites infesting a nest due to constraints on space, nesting material preference, or interaction effects of other variables such as brood size (Remeš and Krist 2005; Soler et al. 2007; Chapter 3). Parasites may exert strong selection pressure on the immune response of avian hosts (Lindström et al. 2004), which may induce trade-offs with parental care, nestling development, and parasite resistance. In Darwin's finches, bloodsucking *P. downsi* larvae reduce haemoglobin concentration in nestlings (Dudaniec et al. 2006 [Appendix 1]), while upon visual inspection, enlarged nostrils, lesions, and internal bleeding are often evident (Figure 1.3). Such wide-ranging effects of avian parasites point to the vast number of unexplored avenues to consider if we are to accurately assess and mitigate the impacts of harmful avian parasites.

### *Genetics of island parasite invasions*

In the absence of immunological or behavioural defence mechanisms that have coevolved with parasite exposure, island communities may show extreme susceptibility to the impacts of introduced parasites (Parker et al. 2006). Molecular genetic techniques have allowed the

**(a)**

**(b)**

**Figure 1.3** (a) Enlarged nostril of a living nestling caused by burrowing larvae of *P. downsi*; (b) Internal bleeding and lesions in a living nestling caused by larvae of *P. downsi*.

genetic processes of biological invasions to be elucidated, and for invasive parasites this is linked with the distribution and reproductive characteristics of the host species. Thus the genetic structure of parasite populations is closely linked with the population dynamics and associated genetic variation within its host species (Whiteman et al. 2006a; Whiteman et al. 2007). Introduced parasites on islands may show reduced genetic variation while successfully enlarging their ecological niche, especially because natural enemies (such as predators or parasitoids) are often absent (Frankham 2005). Host generalism among invasive parasites is particularly concerning for island populations, which are generally characterised by low host species diversity, resulting in a high prevalence of parasitism within species communities (O'Dowd et al. 2003; Chapter 3).

The genetic structure of introduced organisms is frequently characterised by founder effects – which is a population bottleneck resulting from a small number of individuals becoming reproductively separated from the source population, with a subsequent reduction in genetic variability (Sakai et al. 2001). In invasive species, this loss of genetic variability is found, paradoxically, alongside successful establishment and adaptation to new environments, most likely because of multiple introduction events, high reproductive rates, or migration (Chen et al. 2006; examined in Chapter 6). Dispersal for invasive parasites on islands introduces additional complexity and obstacles for migration and maintaining population genetic variation, particularly in regards to systems where human or weather-mediated dispersal is prevalent or requisite (addressed in Chapter 6). Genetic studies of habitat- and host- generalist parasite species (such as *P. downsi*) can reveal patterns of dispersal across and within habitats, elucidating fine-scale genetic structure and habitat use. On the Galápagos Islands, two contrasting habitats, the arid lowlands and the humid highlands (Figure 1.4) offer a unique opportunity to investigate these patterns. Highly variable molecular genetic markers, such as microsatellites, are particularly suitable for examining such questions, yet have not previously been used for ecological studies of dipteran ectoparasites of birds (Otranto and Stevens 2002; Criscione et al. 2005; Azeredo-Espin and Lessinger 2006).

***Molecular insights into parasite ecology***

The mating system of parasites plays a determinant role in the maintenance and distribution of genetic variation (Chevillon et al. 2007; see Chapters 6 and 7). Reproductive behaviour in parasitic insects may vary with host density, host distribution, and other environmental factors

**(a)**

**(b)**

**Figure 1.4** (a) The arid lowlands of Santa Cruz Island featuring *Opuntia* cacti; (b)The humid highlands of Santa Cruz Island featuring *Scalesia pedunculata*.

determining parasite fecundity (Cronin and Strong 1999; Tripet et al. 2002). Highly resolving genetic markers, such as simple sequence repeats (e.g. microsatellites) have provided the necessary tools for examining parasite mating systems, life-cycles, transmission dynamics, and the evolution of host specificity (Criscione et al. 2005). For example, female multiple mating occurs in a variety of insect species and has implications for effective population size, rates of gene flow and maintaining genetic variation (Chapter 7). Genetic relatedness among parasites within a host has also been associated with the determination of optimal strategies of parasite growth, manipulation of host behaviour, and virulence (Chao et al. 2000; Puustinen et al. 2004; Chapter 7). Thus, molecular markers can address questions about otherwise cryptic or subtle ecological processes within parasite populations.

***The role of genetics in controlling parasitic flies***

The majority of molecular genetic studies of myiasis-causing flies, which encompass blowflies, screw flies and botflies (characterised by their ability to develop in animal flesh) have focused on species of medical, agricultural or veterinary importance, such as the cattle-infesting New World screwworm fly and the botfly *Dermatobia hominis* (Otranto and Stevens 2002; Azeredo-Espin and Lessinger 2006). Such studies are examples of management driven research aimed at minimising the large economic impact of myiasis-causing flies, and provide the theoretical and technical means to apply similar methods to the control of parasitic flies threatening wildlife populations. The primary management strategy for myiasis-causing flies to employ genetic techniques during the last half century is the sterile insect technique (SIT) (Krafsur 1998). Through the large-scale release of laboratory reared sterile male flies of the target species, SIT has successfully suppressed or eradicated myiasis-causing flies of global economic concern (Vreyson et al. 2006; Chapters 6 and 7). The development of SIT programs have benefited enormously from genetic studies, which can identify the existence of multiple, possibly reproductively isolated strains of the target species (Cayol et al. 2002), or reveal levels of polyandry that can be used to infer mating success of released sterile males (Bonizzoni et al. 2002). Through a fine-scale understanding of the genetic processes underlying parasite populations, molecular genetic techniques can help to predict not only behavioural, but also evolutionary responses of dipteran parasites to management practices, such as pheremonal attractants, insecticides, or SIT. Understanding the genetic structure of invasive insects is important for designing management plans that are appropriate at spatial and temporal scales, particularly on islands with discrete parasite populations subject to local ecological variation and fluctuating climatic conditions (Chapters 3, 6, and 7).

***Thesis scope and objectives***

This thesis elucidates contrasting ecological patterns and impacts of parasitism across multiple bird species within an island ecosystem, and provides a foundation from which well-informed conservation management plans can be developed. The following chapters describe the ecology and impact of *P. downsi* on birds and also provide essential knowledge regarding the molecular ecology of the fly that is necessary for an integrative control plan, namely SIT, which is currently being evaluated for its feasibility by Galápagos biologists.

Specifically, the aims of this study are to:

1. Synthesise all literature concerning the genus *Philornis* within a comprehensive review, encompassing species biology and effects on birds in light of the impact of *P. downsi* on the Galápagos Islands.
2. Identify variation in *P. downsi* impact in six species of Darwin's finches across years, and in relation to rainfall and habitat (arid lowlands, humid highlands; Figure 1.3).
3. Examine the roles of host social nesting behaviour, host nest size and host mass in the impact of *P. downsi* parasitism across tree finch species on Santa Cruz Island.
4. Develop microsatellite markers for *P. downsi* for fine-scale genetic analyses.
5. Examine dispersal, gene flow, and population bottlenecks in *P. downsi* across three islands of the Galápagos, and between habitats and sites within islands.
6. Elucidate the reproductive ecology of *P. downsi* by determining female remating and nest re-infestation frequency, enabling patterns of fly oviposition behaviour to be described.

***Organisation of the thesis***

The thesis is presented as a series of papers that have been published or have been submitted for publication in peer-reviewed, scientific journals. Therefore the thesis contains some repetition in content. The thesis is comprised of one published review article and five data-rich papers (the aims of which are stated above), which are assembled into separate chapters. A general discussion of findings and suggestions for future research concludes the thesis. Appendix 2 contains a copy of a published article co-authored by myself that contains molecular methods referred to within Chapter 5. The content of published chapters is presented as published or as for final submission, with the exception of references, which have been removed and incorporated at the end of the thesis. A statement of authorship is provided at the beginning of the thesis, stating the contributions of all co-authors. These contributions do not lessen the originality or my overall contribution to the thesis.

**CHAPTER 2:**

**The effects of the parasitic flies *Philornis* (Diptera: Muscidae) on birds**

Rachael Y. Dudaniec and Sonia Kleindorfer

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**Abstract**

Little is known about the genus *Philornis* (comprising ~50 species), a group of muscid flies that parasitise birds and may be highly detrimental to host nestlings. *Philornis* species affect at least 115 species of bird, particularly in the Neotropics. The main distribution of *Philornis* is in Central and South America, extending to the southern United States. Larvae of the genus *Philornis* reside in bird nests and may feed on either nestling faeces (coprophagous scavengers), the blood of nestlings (semi-haematophagous parasites), or on nestling tissue and fluid (subcutaneous parasites). Depending on the species of *Philornis*, larval development can occur in bird faeces, in nesting material, or inside nestlings. Nestling mortality depends on the species of *Philornis*, the intensity of infection, and nestling susceptibility, which in turn depends on the nestling species, age, brood size, body condition, and the anatomical site of infestation. Consequently, variable effects of *Philornis* parasitism are observed in relation to nestling growth, development, and fledging success. The impetus for this review is the recent discovery of *Philornis downsi* on the Galápagos archipelago combined with high *Philornis*-induced mortality in Darwin's finches. The potential for ectoparasites such as *Philornis* to compromise the viability of small, isolated bird populations is highlighted by this recently documented parasite invasion.

**Introduction**

In 1997, Fessl et al. (2001) observed the presence of the introduced obligate bird ectoparasite, *Philornis downsi* (Diptera: Muscidae), in nests of Darwin's finches on the Galápagos archipelago. *Philornis downsi* is characterised by free-living non-parasitic adults whose larvae develop in bird nests as semi-haematophagous (bloodsucking) external parasites on nestlings (Couri 1985, 1999; Fessl and Tebbich 2002). The date of introduction of the parasite to the Galápagos remains speculative. Until very recently, the earliest known occurrence of *P. downsi* was 1981, but this has been predated by the identification of six archived specimens of *P. downsi* from the island of Santa Cruz from collections made by D. Q. Cavagnaro and R. C. Schuster in 1964 (California Academy of Sciences, San Francisco; Causton et al. 2005).

*Philornis downsi* infestation is reported to cause significant fitness costs in Darwin's finches. For example, high mean number of *P. downsi* parasites per nestling (parasite intensity; Bush et al. 1997) was associated with higher nestling mortality (19 % total brood loss; 8 % partial brood loss) in a comparison of four Darwin finch species (Fessl and Tebbich 2002), and low haemoglobin levels in the small ground finch (*Geospiza fuliginosa*) (Dudaniec et al. 2006 [Appendix 1]). An experimental study subsequently showed impaired nestling development and reduced mass gain over a four day period owing to *Philornis* parasitism in two species of Darwin's finches as well as 62 % parasite-induced mortality (Fessl et al. 2006) (Table 2.1). Although no conclusive evidence is available, *P. downsi* may be implicated in the suspected local extinction of Darwin's warbler finch (*Certhidea fusca*) on Floreana Island by 2004 (Grant et al. 2005). Infestation by *P. downsi* is widespread on Santa Cruz Island: larvae and puparia were found in 97 % of 177 nests of 12 species of birds examined in 1998 and 2000 (Fessl and Tebbich 2002). The parasite was associated with total or partial brood mortality in 27 % of 85 active nests, with significant differences in parasite intensity among species (Fessl and Tebbich 2002). Since 1998, *P. downsi* has been found on all inhabited islands of the Galápagos (Wiedenfeld et al. 2007). The potentially catastrophic effects of *P. downsi* on the Galápagos finches are the impetus for this review. Only by understanding the parasite and its effects can conservation measures be implemented to preserve an iconic group of birds that are widely regarded as an evolutionary treasure (Grant 1999).

Little is known about the ecology and biology of *Philornis* flies, as most previous work has concentrated on taxonomy (Aldrich 1923; Dodge 1955; Dodge 1968; Dodge and Aitken 1968; Couri 1984; Skidmore 1985; Carvalho 1989; Couri 1999; Couri and Carvalho 2003). Only a

handful of studies have explored host-*Philornis* biological associations (e.g. Kinsella and Winegarner 1974; Fraga 1984; Couri 1985; Teixeira 1999; Spalding et al. 2002). Given the paucity of information on *P. downsi*, observations from studies of other *Philornis* species (e.g. Oniki 1983; Arendt 1985a; Delannoy and Cruz 1991; Nores 1995) are vital to increase our current understanding of this parasite. It is important to point out, however, that none of the other species of *Philornis* studied had semi-haematophagous larvae like *P. downsi*, which curtails the possibility of direct comparisons.

This review provides a synthesis of the current state of knowledge regarding the general biology and fitness costs of *Philornis* parasitism in birds. We examine the systematics of the genus *Philornis*, its distribution and patterns of host choice, the biology of some *Philornis* species and impacts of *Philornis* species on nestlings in relation to parasite intensity, nestling mortality, growth and development, brood size and fledging success.

**Systematics of the genus *Philornis***

The first species of what is now the muscid fly genus *Philornis* was described as *Aricia pici* from the Dominican Republic (Macquart 1854). Two additional species were described before the new genus *Philornis* was proposed for the fourth species *P. molesta* (Meinert 1890). Initially, *Philornis* was confused and synonymised with *Protocalliphora* (Diptera: Calliphoridae), another genus of bird-infesting parasitic flies, until distinctive diagnostic features were recognized, and it was properly separated and placed in the family Muscidae (Bezzi 1922). Generic arrangement within the family is still in flux, but the most recent placement of the genus is in the subfamily Azeliinae, tribe Reinwardtiini (Skidmore 1985; Couri and Carvalho 2003; Carvalho et al. 2005). *Philornis* (with ~50 species) and the related genus *Passeromyia* (five species) are the only known Muscidae whose larvae are consistently parasites of birds, although this avian association seems to have arisen independently in each group (Couri and Carvalho 2003). The two genera, together with several other parasitic and non-parasitic genera, belong to a monophyletic group characterised by a single synapomorphic character: a puparium enclosed in a cocoon (Couri and Carvalho 2003).

Most *Philornis* species were described in the 1960s (Dodge and Aitken 1968) and the 1980s (Couri 1983, 1984), and keys have only recently become available to identify most of the adults (Couri 1999). The immature stages of *Philornis* and their avian host relationships are known for only about half of the named species (Couri 1999; Teixeira 1999), and many

**Table 2.1.** Characteristics of hosts (adult body length, clutch size, nest type) affected by *Philornis* parasitism where information on parasite intensity, mortality or other fitness impacts is available in the literature. Percentage mortality is expressed in parentheses as the number of nestlings that died out of the number of observed infested nestlings.

| ***Philornis* species** | **Host Species** | **Range of parasite intensity per nestling(n=total infested nestlings)** | **Body length (cm) of host as adult** | **Host clutch-size** | **Host nest-type** | **Percentage mortality of infested nestlings** | **Other fitness impacts** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| **Semi-haematophagous species**<br>*P. downsi* | Darwin's finches (four species) | 4-64<br>(n = 49) | 10-14 | 2-5 | Dome | 27 (total or partial mortality)<br>(23/85) | Nests with small broods had higher parasite intensity and reduced fledging success | Fessl and Tebbich 2002 |
| | small ground finch (*Geospiza fuliginosa*) and medium ground finch (*G. fortis*) | 11-40<br>(n = 13) | 10-14 | 2-5 | Dome | 62 (8/13) | Reduced mass gain in nestlings and reduced fledging success | Fessl et al. 2006a |
| | small ground finch (*G. fuliginosa*) | 1-45 (n = 59) | 10-12 | 2-5 | Dome | 29 (17/59) | Lower haemoglobin level, increased reticulocyte numbers, and reduced fledging success | Dudaniec et al. 2006 |
| | rufous-capped antshrike (*Thamnophilus ruficapillus*) | 1 (n = 1 nest) | 15-20 | 2-3 | Cup | Not recorded | | Mendonça and Couri 1999 |
| **Subcutaneous species**<br>*P. carinatus* | house wren (*Troglodytes aedon*) | 1-22<br>(n >140) | 10-11 | 3-5 | Cavity | 0-19 (42 nests) | Shorter wing chords and tarsi at fledging and reduced mass on day 12 | Young 1993 |
| *P. deceptiva* | pearly-eyed thrasher (*Margarops fuscatus*) | 0-220<br>(n = 448) | 28-30 | 2-4 | Cavity | 46.7<br>(209/448) | Delayed growth and development (body mass and tarsus length) | Arendt 1985b |
| | | 64 and 74<br>(n = 2) | | | | 100<br>(2/2) | Loss of red blood cells, connective tissue and tissue fluids, evidence of cellular immune response | Uhazy and Arendt 1986 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *P. glaucinus* | chestnut-backed antshrike (*Thamnophilus palliates*) | 1-4 (n = 1) | 15-20 | 2-3 | Hanging basket | Not recorded | Loss of upper wing coverts due to presence of larvae | Mendonça and Couri 1999 |
| *P. pici* and *P. seguyi* | brown cacholote (*Pseudoseisura lophotes*) | 3-21 (n = 26) | ~24 | 2-4 | Dome | 30.7 (8/26) | | Nores 1995 |
| | firewood-gatherer (*Anumbius annumbi*) | 3-17 (n = 29) | ~18 | | Dome | 31 (9/29) | | Nores 1995 |
| *Neomusca (Philornis) porteri* | great-crested flycatcher (*Myiarchus crinitus*) | 1-21 (n = 32) | ~23 | 5-6 | Cavity | 3.1 (1/32) | | Kinsella and Winegarner 1974 |
| *Philornis* sp. (unidentified) | Puerto Rican sharp-shinned hawk (*Accipiter striatus venator*) | 1-16 (n = 41) | 28-38 | 2-3 | Dome | 61 (25/41) | | Delannoy and Cruz 1991 |
| | mourning dove (*Zenaidura macroura*) | 17 and 26 (n = 2) | 28-30.5 | 1-3 | Stick platform | 0 (0/2) | | Glasgow and Henson 1957 |
| | great kiskadee (*Pitangus sulphuratus*) | 30-41 (n = 4) | ~27 | 2-5 | Dome | 0 (0/4) | Physical deformation | Oniki 1983 |
| | beechey jay (*Cyanocorax* [*Cissilopha*] *beecheii*) | 7 -12 (n = 3) | 20-30 | 3-5 | Cup | 33 (1/3) | Delayed behavioural development; physical debilitation; reduced lengths of primary 9 and rectrix 1 | Winterstein and Raitt 1983 |
| | aplomado falcon (*Falco femoralis*) | 6-35 (n = 3) | 36-43 | 2-3 | Cup | Not recorded | | Hector 1982 |
| | masked gnatcatcher (*Polioptila dumicola*) | 3 in dead nestling (n = 2) | ~10 | 3-5 | Cup | 50 (1/2) | | Fraga 1984 |
| | firewood-gatherer (*Anumbius annumbi*) | ≥5 in dead nestlings (n = 4) | ~18 | | Dome | 50 (2/4) | | Fraga 1984 |
| | chalk-browed mockingbird (*Mimus saturninus*) | 8 in dead nestling (n = 3) | 25-27 | 3-4 | Cup | 33.3 (1/3) | | Fraga 1984 |
| | screaming cowbird (*Molothrus rufoaxillaris*) | 0-11 (n = 3) | 18-20 | brood parasite | (Host nest) Dome, chamber | 0 (0/3) | The host species removed larvae from nestlings | Fraga 1984 |

previous biological studies faced taxonomic difficulties in recognising the species under study (Hector 1982; Oniki 1983; Winterstein and Raitt 1983). Dodge and Aitken (1968) pointed out that several *Philornis* species may occur in a variety of geographic forms or subspecies (i.e. they are polytypic).

**Distribution and host choice**

The ~50 species of *Philornis* currently known occur throughout the Neotropical Region, and the distribution of up to six species (including *P. porteri* and *P. obscura*) extends to the United States (Dodge 1955; Spalding et al. 2002; Couri and Carvalho 2003). Species of *Philornis* have been collected in Argentina, Brazil, Costa Rica, Cuba, Dominican Republic, Ecuador, Florida, Guatemala, Guyana, Louisiana, Mexico, Panama, Peru, Puerto Rico, Texas, Trinidad, Uruguay, and Venezuela (Dodge 1955, 1968; Dodge and Aitken 1968; Couri 1999; Fessl et al. 2001). *Philornis* species have been reported to infest at least 127 species of birds (Couri 1985; Teixeira 1999; Fessl et al. 2001; Fessl and Tebbich 2002), and they do not show marked host specificity (Couri 1991; Teixeira 1999).

Known hosts for *Philornis* species are mainly Neotropical passerines (listed in Dodge 1955, 1968; Dodge and Aitken 1968; Teixeira 1999), but some infestation has been found in Falconiformes (Hector 1982; Delannoy and Cruz 1991), Galbuliformes, Cuculiformes, Galliformes, Columbiformes, Psittaciformes, Apodiformes, Piciformes, and Strigiformes (Teixeira 1999). The species affecting Darwin's finches, *P. downsi*, is known from collections in Trinidad and Brazil (Dodge and Aitken 1968; Uhazy and Arendt 1986; Mendonça and Couri 1999) and has been reported to infest 26 species of birds in 22 mostly passerine genera, including those on the Galápagos archipelago (Fessl et al. 2001).

**The biology and ecology of *Philornis***

Little is known about the biology of *Philornis* species, as general information is limited to 28 (56 %) of the ~50 described species (Teixeira 1999). Adult *Philornis* flies are non-parasitic and feed on decaying organic matter, fruit or flowers (Teixeira 1999; Fessl et al. 2001). Larval trophic relationships are documented for only 22 species. Apart from the larvae of two species that are coprophagous scavengers, the 20 remaining *Philornis* species for which the larval biology is known have been associated with parasitism in a diverse array of New World bird taxa. *Philornis vespidicola* is an exception as it is only known from a wasp nest (*Paracharitopus frontalis*) (Hymenoptera: Vespidae), but this is considered an unusual and

inadequately explained observation (Dodge 1968; Teixeira 1999).

Where known, the larval habits of *Philornis* species are divided into three groups: coprophagous, semi-haematophagous and subcutaneous. Most (82 %) are parasitic subcutaneous tissue and fluid feeders (e.g. *P. deceptiva*, *P. augustifrons*, and *P. glaucinis*), which burrow into the host's integument and reside beneath the skin between the dermis and body musculature, resulting in the formation of individual cysts (Teixeira 1999; Spalding et al. 2002). These endoparasitic larvae feed on serous fluids, tissue debris and blood of the host, and each larva breathes through a small aperture it cuts in the host's integument (Skidmore 1985; Uhazy and Arendt 1986; Young 1993). Larvae of some *Philornis* species (9 %) are free-living semi-haematophagous parasites (e.g. *P. downsi* and *P. falsifica*), which also live freely and develop in the host's nesting material. However, the larvae periodically visit the integument of nestling hosts to feed, which they do by cutting an opening in the skin and subsequently ingesting blood and fluids of the host (Dodge and Aitken 1968; Teixeira 1999). The free-living larvae of commensal coprophagous species (9 %; e.g. *P. aitkeni* and *P. rufoscutellaris*) feed and develop in accumulated organic debris (primarily faeces) at the bottom of nests of certain Neotropical birds that nest in closed cavities (Dodge 1963; Couri 1999; Teixeira 1999; Couri and Carvalho 2003).

All *Philornis* species choose hosts with altricial or semi-altricial young. Apart from coprophagous species that preferentially infest cavity nests with increased organic matter, the parasites do not select hosts with particular types of nest, having been found in cup-shaped, domed and cavity nests (Table 2.1). Given this lack of specific host selection it is not surprising that some hosts are affected by more than one species of *Philornis* that differ in parasitic strategy (Oniki 1983; Teixeira 1999).

Although most muscids are oviparous, viviparity occurs commonly in the tribe Reinwardtiinae (Skidmore 1985). The Reinwardtiinae includes the species infesting Darwin's finches, *P. downsi*, but there is no definitive evidence regarding the reproductive habit of this species. In Darwin's finches, *P. downsi* larvae have been collected from the nares of young nestlings (Fessl et al. 2006b). This suggests that *P. downsi* larvae develop in nestling nares before moving into the nesting material, where they have been observed later in the nesting cycle (Fessl and Tebbich 2002). Subcutaneous larvae of *P. porteri* have also occasionally been observed in the nares of great-crested flycatchers (*Myiarchus crinitus*) in Florida

(Kinsella and Winegarner 1974), though perhaps incidentally given the endoparasitic habit of this species. Although *Philornis* infestations have been observed at all stages of the nestling feeding phase (Arendt 1985a; Young 1993; Nores 1995), no *Philornis* larvae have been observed in nests before the eggs of the hosts hatch (Arendt 1985b; Young 1993; Fessl and Tebbich 2002). Rather, larval infestation occurs within hours or a few days of nestlings hatching (Kinsella and Winegarner 1974; Spalding et al. 2002).

Larval feeding and growth are completed within four to eight days in endoparasitic species and up to 29 days in coprophagous species (Teixeira 1999). During this time larvae grow to 1 cm or more in length (this differs across *Philornis* species) and pass through three stadia (Fraga 1984; Arendt 1985a; Skidmore 1985; Delannoy and Cruz 1991; Spalding et al. 2002). Larvae then drop from the host to the base of the nest, where each forms a frothy cocoon (or puparium) from salivary gland secretions and in which they pupariate (Dodge 1971; Skidmore 1985), before undergoing pupation (development into adult stage). Larvae exit (in subcutaneous species) or detach from chicks (in semi-haematophagous species) quickly after nestling mortality or before fledging, and proceed to burrow into the nesting material (Teixeira 1999; Spalding et al. 2002). Such larvae may be able to pupariate before they are fully grown, based circumstantially on variations in size among pupae and adult flies (Kinsella and Winegarner 1974; Spalding et al. 2002). The duration of the pupal stage may vary from five to 15 days according to species and environmental conditions, though the majority of species take approximately two weeks to emerge as adults (Glasgow and Henson 1957; Oniki 1983; Delannoy and Cruz 1990; Spalding et al. 2002).

Despite the high degree of host generalism found among parasitic *Philornis*, the life-cycles of *Philornis* species seem to be closely synchronized to the host nestling phase, as the flies require a living host with a breeding period complementary to their own relatively short life spans (Teixeira 1999). Adult *P. downsi* flies were observed emerging from nests of Darwin's finches within days of host fledging (S. Kleindorfer and B. Fessl unpublished). Oniki (1983) observed that larvae of several *Philornis* species had detached from nestlings and started pupation in the nesting material a few days before host fledging. *Philornis* species parasitising multiple bird species might adjust the duration of their larval periods to match the varying nestling periods of their hosts, choose to attack only the youngest nestlings, or be able to pupate outside the nest, as some species do when they occasionally parasitise adult bird hosts (Oniki 1983).

Repeated fly infestations of nests occur throughout the nestling period, and larvae of different instars have been observed simultaneously in individual nests (Oniki 1983; Arendt 1985b; Nores 1995), perhaps indicating the number of fly cohorts a nest might produce (e.g. Young 1993). Observed fluctuations in the number of parasites per nestling over the nestling period also suggest repeated fly infestations (Winterstein and Raitt 1983). Larval numbers per nestling increased with nestling age during the nestling feeding phase in pearly-eyed thrashers (*Margarops fuscatus*) (Arendt 1985b). A brood of nestling house wrens, *Troglodytes aedon*, can sustain up to three or four cohorts of *Philornis* flies, although single cohorts were predominantly observed (Young 1993). There was no indication of larval reinfestation in Darwin's finch nests treated with a 1 % pyrethrin solution when the nestlings were four to six days old, which effectively reduced *P. downsi* intensity to less than two larvae per nest (Fessl et al. 2006a).

*Philornis* parasitism of adult birds has been reported, but it is not comparable with the level or impact of nestling infestations. Of 105 adult pearly-eyed thrashers, 31 % were infested with *P. deceptiva*, but no evidence of parasite-induced mortality was observed (Arendt 1985b). Parasitism of adult birds by *Philornis* species may be opportunistic and is most probably limited by host mobility (Teixeira 1999), feather protection (e.g. in nestlings, Oniki 1983) or sex (Arendt 1985b) or combinations of these factors. In pearly-eyed thrashers, infestation was much higher in females rearing young (46.7 %) than in males (13.3 %) who do not spend long periods in the nest. In the absence of convincing evidence to the contrary, nestling birds seem to be the primary hosts of *Philornis* (Teixeira 1999).

**Impacts of *Philornis* on nestlings**

*Mortality and fledging success*

There have been few studies on the impact of *Philornis* parasitism in nestling birds. Whereas some studies have found significant reductions in nestling fitness and survival caused by *Philornis* larvae (Winterstein and Raitt 1983; Delannoy and Cruz 1991; Young 1993; Nores 1995; Dudaniec et al. 2006; Fessl et al. 2006a,b), others have not (Glasgow and Henson 1957; Kinsella and Winegarner 1974; Oniki 1983). This discrepancy in findings may be partly explained by variation in host species, parasite species, and environmental conditions (Teixeira 1999). Table 2.1 provides an overview of some of the main variables that may affect the fitness costs of *Philornis* parasitism and their impact between avian hosts.

The highest mortality levels (>50 % of broods with total or partial mortality) among birds in relation to *Philornis* parasitism were found by Fraga (1984), Delannoy and Cruz (1991) and Fessl et al. (2006a). *Philornis downsi* on the Galápagos Islands was associated with 62 % brood loss in Darwin's finches (Fessl et al. 2006a), whereas fledging success differed markedly between brood sizes of one (0 %), two (~50 %), and three or four chicks (75-85 %) (Fessl and Tebbich 2002). Notably, two other parasitic Diptera were commonly found in the Darwin finch nests sampled (*Sarcodexia lambdens* and an unidentified endoparasitic Muscidae species), thus the results may not solely reflect the activities of *P. downsi* (Fessl and Tebbich 2002). The brood size dilution effect observed by Fessl and Tebbich (2002) is a widely recognised phenomenon in avian ectoparasitism (Richner and Heeb 1995).

Delannoy and Cruz (1991) found an almost four-fold difference in mortality between *Philornis*-parasitised (61 % brood mortality) and unparasitised Puerto Rican sharp-shinned hawk (*Accipiter striatus venator*) nestlings. Fraga (1984) found 50 % partial brood mortality in *Philornis*-parasitised broods of masked gnatcatchers (*Polioptila dumicoa*) and firewood-gatherers (*Anumbius annumbi*), with 33 % loss in chalk-browed mockingbirds (*Mimus saturninus*). Nearly half of 448 pearly-eyed thrasher nestlings infested with *P. deceptiva* died over a four-year study period, whereas fledging success was approximately two-fold higher in unparasitised (98 %) than in parasitised nests (42-56 %) (Arendt 1985b).

In contrast, Nores (1995) found that fledging success in brown cacholotes (*Pseudoseisura lophotes*) and firewood-gatherers did not differ significantly between nestlings that were simultaneously parasitised by *Philornis pici* and *P. seguyi* (69 % fledged) and those that were unparasitised (75 % fledged). In addition, total parasite-induced mortality was relatively low (5.5 % and 5.6 % in brown cacholotes and firewood-gatherers, respectively) owing to low parasite prevalence (i.e. percentage of hosts infested; 16 %), though a third of the infested nestlings died (Table 2.1). Although behavioural development was delayed in nestling purplish-backed jays (*Cyanocorax* (*Cissilopha*) *beecheii*) infested with *Philornis*, survival to one year of age was not affected by the level of nestling parasitism (Winterstein and Raitt 1983). The lack of evidence linking *Philornis* parasitism with elevated mortality across some studies has been attributed to inconclusive evidence (Oniki 1983), small sample size (Hector 1982), or the absence of any mortality (Glasgow and Henson 1957; Young 1993). Clearly, the variation in fitness costs due to *Philornis* parasitism should be examined in the context of parasite species and intensity as well as host body size, clutch size, and nesting habit of the

hosts (see Table 2.1).

*Parasite intensity*

The correlation between nestling mortality and the number of *Philornis* larvae infesting the brood does not show a consistent relationship, either within or between host species. The single most important factor likely to explain this discrepancy is the species of both host and parasite (see also discussion above) (Table 2.1). But even with constancy in these two basic elements (host and parasite species), variation in mortality under different parasite intensities may derive from other factors, such as nestling age, size, nutritional condition, or anatomical site of larval infestation (Delannoy and Cruz 1991). Environmental stochasticity may generate further variation, such as in rainfall, which was found to positively influence infestation prevalence (Delannoy and Cruz 1991; Arendt 2000). Notably, all information on this subject is derived from studies of endoparasitic *Philornis* species, creating an imperative for further research into species with ectoparasitic and coprophagous habits.

Mortality among bird hosts resulting from *Philornis* infestation differs according to mean parasite intensity. Four nestlings of great kiskadee (*Pitangus sulphuratus*) observed with individual loads of 30-41 unidentified *Philornis* larvae were deformed but not dying (Oniki 1983), but other studies found that larval loads of five (Arendt 1985b), six (Delannoy and Cruz 1991), or 13 (Nores 1995) per nestling were sufficient to cause mortality in different host species, though the mass of nestling hosts might be a determining factor here. Arendt (1985a) found that when larvae infested sensitive areas around the nestling's head, less than five larvae were sufficient to cause debilitation in pearly-eyed thrashers.

Brood parasitism may play an interesting role in reducing the negative impact of *Philornis* for both the avian host and brood parasite. For example, Fraga (1984) reported adult bay-winged cowbirds (*Molothrus badius*) removing *Philornis* larvae from their nestlings and those of their coexisting brood parasite, the screaming cowbird (*Molothrus rufoaxillaris*). The high fitness benefits associated with this parental care may explain the host specialisation of this brood parasite (Fraga 1984).

Parasite prevalence may increase towards the end of the breeding season (Arendt 1985a, b; Young 1993), perhaps owing to building seasonal fly populations, although not all studies have found this trend (Nores 1995; Fessl and Tebbich 2002). Such variation in prevalence as

well as intensity have been proximately related to temporal variation in resource abundance (e.g. related to rainfall) (Arendt 1985b; Arendt 2000), resulting in changes in fly densities (Delannoy and Cruz 1991).

*Nestling growth and development*

Nestling pearly-eyed thrashers parasitised by *Philornis* experienced shorter and delayed growth increments in the tarsus and ninth primary pin feather during the first week of life compared to unparasitised nestlings (Arendt 1985a). Growth of flight components, including the ulna and exposed shaft of the ninth primary were not adversely affected by parasitism, which suggests that birds maintain sufficient energy transfer to flight components under physiological stress (Arendt 1985a). However, in another study, lengths of the ninth primary and first rectrix were found to be significantly greater in uninfested than infested nestling purplish-backed jays (Winterstein and Raitt 1983). Young (1993) found that parasitised nestling house wrens fledged with significantly shorter wings and showed a trend toward shorter tarsi than parasite-free nestlings. Body mass may be negatively affected by *Philornis* parasitism, after controlling for increases in weight due to larval biomass (Winterstein and Raitt 1983; Arendt 1985a). In some cases, however, infested and uninfested nestlings had similar mass at fledging (Young 1993).

A recent study of Darwin's finches found that nestlings in experimentally manipulated parasite-free nests (treated with 1 % pyrethrin solution) had an almost two-fold positive difference in mass across four days compared with untreated nests during the feeding phase (Fessl et al. 2006a). Such strong effects may be explained by the recent introduction of *P. downsi* to the Galápagos archipelago as hosts may not have developed strong behavioural or immunological defence mechanisms. Furthermore, island populations may be particularly susceptible to parasitism because the ability of hosts to disperse is restricted, allowing high prevalence and impact of parasitism (Price 1980; Delannoy and Cruz 1991).

*Nestling vulnerability to parasitism*

Vulnerability and survival of nestlings parasitised by *Philornis* has been related to host age, with stronger negative impacts in younger nestlings (Nores 1995). Older nestlings are thought to be less vulnerable because they have greater mobility, might be partly protected by feathers, or may actively deter flies, whereas younger nestlings are naked, less active, and perhaps have softer tissue sites for larval entry (Hector 1982; Oniki 1983; Nores 1995;

Teixeira 1999). Hatchlings and one-week-old nestlings of the brown cacholote and firewood-gatherer bore greater numbers of larval *P. pici* and *P. seguyi* than did older nestlings (Nores 1995), and increased intensity was correlated with increased mortality in other host-*Philornis* studies involving the pearly-eyed thrasher (Arendt 1985b) and Puerto Rican sharp-shinned hawk (Delannoy and Cruz 1991). It should be noted that the identification of *P. sequyi* in Nores (1995) may be in doubt as there is a lack of information for this species (e.g. see Teixeira 1999).

Site specificity (i.e. the preferred anatomical location) of *Philornis* larvae on nestlings also varies with nestling age, and may influence mortality because body areas vary in sensitivity to injury (Delannoy and Cruz 1991). Again, the species of *Philornis* and host involved is likely a major underlying component of this variability. For example, anatomical site specificity will depend on whether the species of adult *Philornis* deposits its eggs or larvae in the nesting material or directly onto nestlings, as the former is likely to be associated with parasitism of ventral surfaces (Arendt 1985a). Unfortunately, the only information found on the subject of site specificity is limited to endoparasitic *Philornis* species, though larvae of semi-haematophagous *P. downsi* have been positively identified inside the nares of Darwin's finch nestlings (Fessl et al. 2006b), and this has not been reported in endoparasitic species. Arendt (1985b) and Uhazy and Arendt (1986) found that young pearly-eyed thrasher nestlings (one to nine days old) had more larvae on the head, mouth, and dorsal areas of the trunk, but larvae were concentrated on the legs and ventral surfaces of older nestlings (10 to 19 days old). This suggests that adult flies may exploit readily accessible sites for oviposition (Uhazy and Arendt 1986). Site-specificity may disappear later in the nestling period, when populations of both flies and hosts are most dense (Arendt 1985b). However, Nores (1995) found no significant variation in anatomical distribution of *Philornis* larvae during nestling development in brown cacholotes and firewood-gatherers, and Oniki (1983) also found that larvae infested all ages of several hosts examined, without notable preference for sites.

**Conclusion**

A review of the available literature on *Philornis* flies confirms the paucity of ecological and biological information available for this genus. For example, very little is known about the adult stage in *Philornis*, and little is known of its reproductive biology (Arendt 1985b; Skidmore 1985; Teixeira et al. 1990; Teixeira 1999). Although a handful of studies have examined avian host-*Philornis* parasite interactions, much work remains to be done.

The fitness costs of *Philornis* parasitism in birds may be severe, with high incidences of nestling mortality, although this is not always the case (Table 2.1). Host populations that are newly colonised by *Philornis* parasites, such as Darwin's finches on the Galápagos archipelago, may experience significant initial mortality costs. These observations raise some important questions concerning the sustainability of small, isolated bird populations under threat from parasite invasion, and the potential for avian hosts to develop an adaptive response to introduced pathogens (Altizer et al. 2003). The study of *P. downsi* on the Galápagos archipelago provides a useful opportunity to increase our understanding of avian-parasite coevolution in the wild, particularly on species-poor islands where resistance to alien insect introductions is often low (Causton et al. 2005). In birds, ectoparasites have been linked with mediating selection on life-history trade-offs at morphological, behavioural, and physiological levels (Richner 1998). The available information on host-*Philornis* interactions reviewed here suggests that *Philornis* parasites contribute substantially to these associations.

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**CHAPTER 3:**

**Interannual and interspecific variation in intensity of the parasitic fly, *Philornis downsi*, in Darwin's finches.**

Rachael Y. Dudaniec, Birgit Fessl, Sonia Kleindorfer

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## Abstract

An integrative approach to managing host-parasite interactions that threaten species communities will benefit from identifying variation in parasite impact across host species, and host-parasite responses to annual climatic variation. We examine interannual, inter- and intraspecific variation in *Philornis downsi* intensity – an introduced blood sucking fly that causes high fitness costs in Darwin's finches on the Galápagos Islands. We sampled 131 nests of six finch species (with nestling survival ≥ 6 days post hatching) between 1998 and 2005 on Santa Cruz Island. *P. downsi* total (per nest) and mean (per nestling) intensity differed across species and years. The woodpecker finch (*Cactospiza pallida*), and the large tree finch (*Camarhynchus psittacula)* had the highest total parasite intensity. Both species had comparatively large adult body mass, and we found a positive association between adult body mass and total parasite intensity among nestlings. *P. downsi* total and mean intensity was highest during the El Niño year of 1998. Surprisingly, despite a three-fold difference in rainfall across lowland and highland habitats in other than the El Niño year, there was no difference in parasite intensity per nest between habitats. However, species composition of hosts and intraspecific brood size vary across habitats. Highland nests with larger broods and lower mean (per nestling) parasite intensity had higher fledging success. There was no significant effect of total parasite intensity on fledging success for intraspecific analyses. The percentage of nests with nestling mortality in each habitat ranged between 40-100 % for all six host species.

**Introduction**

Parasites are increasingly being recognised as significant threats to the preservation of avian populations (Brown et al. 1995; Tompkins and Begon 1999) due to their measurable effects on mortality and population dynamics (Arendt 1985a, b; Friend et al. 2001). Variation in parasite impact across host species, coupled with environmental factors determining parasite fecundity are both issues to consider when allocating conservation resources to parasite-affected ecosystems. Low parasite species diversity is typically found within insular endemic communities, which can lead to increased risks associated with introduced parasites for immunologically naïve hosts (Wikelski et al. 2004; Parker et al. 2006). High impacts of parasites are documented for a variety of island bird species (e.g. blood parasites, van Riper et al. 1986; *Philornis* ectoparasites, Arendt 2000; Fessl and Tebbich 2002) as well as for other island vertebrates (Pickering and Norris 1996; Crooks et al. 2001). Founding parasite populations on islands may lose genetic variability while enlarging their ecological niche: This loss of genetic variability and wider niche use is often associated with lower host specificity, especially in the absence of natural enemies or biotic regulators (Roque-Albelo and Causton 1999; Goüy de Bellocq et al. 2002; Nieberding et al. 2006).

The parasitic Dipteran *Philornis downsi* (Muscidae) was first formally identified from Darwin finch nests in 1997 and has since been found on 11 of 13 Galápagos Islands in nests of 14 endemic species (Fessl et al. 2001; Wiedenfeld et al. 2007). *P. downsi* is considered a serious threat to Galápagos finch populations (Fessl and Tebbich 2002; Dudaniec and Kleindorfer 2006 [Chapter 2]; Fessl et al. 2006a) and is the only parasite known to cause measurable fitness costs in Darwin's finches. This raises concern for several endemic bird species of the Galápagos Islands that are threatened or in decline (Wikelski et al. 2004), such as the mangrove finch (*Camarhynchus heliobates*) (Dvorak et al. 2004), the medium tree finch (*Camarhynchus pauper*), and the (presumably) locally extinct warbler finch (*Certhidea fusca*) on Floreana Island (Grant et al. 2005). The only other known ectoparasites of Darwin's finches are feather mite species (Lindström et al. 2004), while blood parasites have not been detected (Dudaniec et al. 2005).

Climatic fluctuations (e.g. in temperature, precipitation or humidity) often correlate with parasite prevalence and intensity (De Lope et al. 1993; Heeb et al. 2000; Dawson et al. 2005). For example, arthropod parasites may increase in number under conditions of higher humidity (in rice-rat botflies: Bergallo et al. 2000; bird fleas: Heeb et al. 2000; and chewing lice: Moyer

et al. 2002) or precipitation (in *Philornis* flies: Arendt 1985a; and fire ants: Roque-Albelo and Causton 1999). Such interrelations between abiotic conditions and parasite dynamics underscore the necessity to explore these relationships within host-parasite interactions. The Galápagos Islands are strongly impacted by El Niño events (e.g. 1982/83 and 1997/98) that bring sharp increases in rainfall, air and sea temperatures to the archipelago, resulting in rapid changes to population abundance and dynamics in many taxa (Glantz 2001). Knowledge of the interrelationships between parasite, host, and climate will augment management strategies aiming to control invasive pathogens within insular communities, especially under conditions of limited resources.

We currently lack information on interspecific variation in the abundance and fitness costs of *P. downsi* across habitats and years on the Galápagos Islands, even though annual fluctuations in *P. downsi* prevalence and intensity are expected due to significant annual variation in climatic conditions (Grant 1999a). We examine *P. downsi* intensity and fitness costs in six species of Darwin's finches on Santa Cruz Island using data that were collected over six study years (1998 to 2005, excluding 1999 and 2003). Previous studies on the fitness costs of *P. downsi* intensity have pooled all study species for the analyses (Fessl and Tebbich 2002). Here, we provide species-specific data on fledging success and parasite intensity across habitats in relation to (1) annual rainfall, (2) host species and body mass; and (3) brood size. Our aim is to present descriptive data on patterns and impacts of fly prevalence in different finch species to identify host species that may be considered at elevated risk of *P. downsi* parasitism.

**Methods**

*Study site and study species*

*Philornis downsi* parasites and data on nesting success were collected from Santa Cruz Island in the Galápagos archipelago over six field trips between January and March in 1998, 2000, 2001, 2002, 2004, and 2005. Nests from six species of Darwin's finches were sampled: small ground finch, *Geospiza fuliginosa*; medium ground finch, *Geospiza fortis*; small tree finch, *Camarhynchus parvulus*; large tree finch, *Camarhynchus psittacula*; woodpecker finch, *Cactospiza pallida*; and warbler finch, *Certhidea olivacea* (Table 3.1). The latter four species are members of the 'tree finch' group and are found to nest almost exclusively in the highlands (300-600 m elevation), which are an evergreen humid forest dominated by *Scalesia pedunculata* (Asteraceae) (5-15 m height). Nests were randomly sampled from one site with

replicate study plots near Los Gemelos in the humid highlands (0˚ 37'S, 90˚ 23'W), and among three sites from the arid lowlands (0˚ 40'S, 90˚ 13'W): 1) Garrapatero 2) Mina Negra, and 3) west of Puerto Ayora township. All sample sites constituted approximately 2000 $m^2$. Nests in the lowlands (0-100 m elevation) are mainly found in cacti of *Opuntia echios gigantean* (1.5-4 m height), and less commonly in the trees *Croton scouleri, Bursera graveolens, Pisonia floribunda,* and *Piscidia carthagenensis* (Fessl and Tebbich 2002). All of Darwin's finches typically build a dome shaped nest (~15-20 cm diameter) with a circular side entrance with a diameter of approximately 4-8 cm (Grant 1999a; Kleindorfer 2007a, b). Nest characteristics vary subtly among Darwin's finches, though larger species typically build larger nests with wider entrances (Kleindorfer and Dudaniec in review [Chapter 4]).

*Annual rainfall*

Annual precipitation data were provided by the Charles Darwin Research Station and taken at Bahía Academia, Puerto Ayora, Santa Cruz (latitude: 0º 44'S, longitude: 90º 18'W, elevation: 2 m ASL), and at Bella Vista, Santa Cruz (latitude: 0º 42'S, longitude: 90º 22'W, elevation: 194 m ASL) for each study year (Table 3.2). Although highland study sites were located at higher elevations than the Bella Vista measurement site, precipitation levels are not expected to be significantly different over this small geographical scale, with a difference in elevation of approximately 100 m. Average air temperatures between the January to March breeding period in all study years were as follows: 1998 = 27.6˚C; 2000 = 25.4˚C; 2001 = 25.8˚C; 2002 = 26.0˚C; 2004 = 26.3˚C; 2005 = 26.2˚C (provided by the Charles Darwin Research Station). Because parasite intensity tends to increase under conditions of high rainfall and high temperature, we predict higher *P. downsi* intensity in years with high rainfall.

*Host brood size*

Darwin's finches typically have a smaller clutch size in the highlands (two or three eggs) compared with the lowlands (three to five eggs), even within species (Kleindorfer 2007a). Brood size was defined as the number of nestlings that hatched, or the number that were present in the nest at the onset of nest monitoring. We measured brood size at 116 nests and collected data on fledging success from 110 nests for which brood size information was available (Table 3.1). Although no nests had a clutch size of one, brood sizes of one were observed due to hatching failure or nestling mortality. The number of nestlings ranged from one to four per nest and was determined by direct nest inspection or by the presence of dead nestlings. Clutches of five eggs were observed in the lowlands in 2000 and 2002, but all brood

sizes were less than five across all study years due to partial brood loss in nestlings less than six days old, possibly combined with prevailing drought conditions in the lowlands (Table 3.2). In addition, all nests sampled in years with the highest annual rainfall (1998, 2001, and 2002) were from the highlands, where brood size was typically two or three nestlings, which is the modal clutch size of Darwin's tree finches.

*Host body mass*

Adult birds of the six study species were caught using mist-nets in both the highlands and lowlands. Adult body mass (g) was measured using a digital scale (Tanita 1479) and was analysed only in relation to parasite intensity in 1998 due to unequal species sampling across years. Based on evidence from previous studies documenting a positive relationship between host size and parasite intensity, we predict a positive association between *P. downsi* intensity and body mass.

*Parasite life history*

For a detailed description of the life-cycle and larval biology of *P. downsi* see Fessl et al. (2006b). In brief, *P. downsi* is an obligate avian parasite in its three larval stages, whereas adult flies feed on organic matter (Dudaniec and Kleindorfer 2006 [Chapter 2]). First instar larvae infest the nares of newly hatched nestlings (usually at one to three days old), and have not been encountered in the nesting material (Dudaniec, personal observation). Second and third instar larvae attach externally and continue to feed on nestling blood and tissues over four to six days, frequently resulting in infected wounds and internal bleeding (Dudaniec and Kleindorfer 2006 [Chapter 2]). Most larvae of *P. downsi* appear to reach their third instar phase at the time of host fledging. Third instar larvae are between 0.5-1.5 cm long and are easily detected and collected. Second larval instars are rarely present in the nesting material, but are quite easily detected, with a length of approximately 0.4-0.6 cm. The larvae pupariate at the base of the nesting material for approximately two weeks before emerging as adult flies (Teixeira 1999; Dudaniec and Kleindorfer 2006 [Chapter 2]).

*Parasite collection from nests*

To minimize the effects of nestling age on variation in mean parasite intensity (number of parasites per nestling, defined by Bush et al. 1997) as found by Fessl and Tebbich (2002), only nests with nestlings that survived to at least six days are included in all analyses. This criterion significantly reduced our sample size of the six study species from 249 nests found

and monitored since 1998 to 131 nests at which nestlings survived to at least six days of age (Table 3.1). Nesting status was determined from repeated 20-min observations (every two days) of parental activity at each nest, as well as by nest inspection. Active nests were classified into three breeding stages; (1) incubation, (2) early feeding stage (nestlings < 6 days), and (3) middle to late feeding stage (nestlings ≥ 6 days). Nestling age was determined by tarsus length (mm) and body mass (g) using callipers and a digital scale respectively, or by the date of hatching.

Darwin's finches fledge at 12-14 days (Fessl and Tebbich, 2002) and fledging success was calculated from the percentage of nestlings in a nest that survived to leave the nest. Causes of nesting failure were identified as predation (in cases where the nest or nest entrance was destroyed) (Table 3.1), abandonment (no sign of parents and no fat deposit in dead nestlings), or parasitism (parents observed attending nestlings during the previous 24 hrs and dead nestlings with fat deposit) (Fessl and Tebbich, 2002). Inactive nests were collected and stored in individual sealed plastic bags and later dismantled for counting *P. downsi* larvae, puparia, or puparia cases from the thick nest bottom layers (Fessl and Tebbich, 2002). The number of puparia cases was included in the total number of parasites. Although often fragmented, the puparia cases could be counted based on the presence of the distinctive puparium cap for each individual (Wiedenfeld et al. 2007).

*Statistics*

*P. downsi* total intensity in nests was log transformed and *P. downsi* mean intensity was square root transformed for normality. Ordinal regression (Logit link) was used to assess the effects of *P. downsi* total intensity, brood size, and year on fledging success across all species in each habitat (Table 3.3). Fledging outcome for all species was divided into three categories: complete nesting failure (0 %), partial fledging success (< 100 %), and total fledging success (100 %).We examined each habitat separately because of known differences in species composition and intraspecific clutch size across habitats (Grant 1999a). For intraspecific analyses, we used ANOVA to examine *P. downsi* total intensity (dependent variable) against the independent variables fledging success and brood size. Fledging success was examined for two categories due to small sample size: 100 % = complete fledging success, and < 100 % = partial or total brood loss; brood size ranged from one to four. Because brood size is lower in the highlands compared with the lowlands, we predict lower fitness costs (i.e. higher fledging

**Table 3.1** Data on host reproduction and parasitism for six Darwin finch species collected in 1998, 2000, 2001, 2002, 2004, and 2005 are summarised (SGF = small ground finch, MGF = medium ground finch, STF = small tree finch, LTF = large tree finch, WF = warbler finch, WP = woodpecker finch) for the lowlands (L) and the highlands (H). Total and partial brood loss refers to all recorded causes of mortality. The variable '% of nests depredated' includes both partial and total loss of nestlings due to predation. Shown are number (#), percent (%) or mean ± s.e. (sample size).

| | SGF | | MGF | | STF | | LTF | | WF | | WP | | Total # of nests |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | L | H | L | H | L | H | L | H | L | H | L | H | |
| **Total # nests** | 26 | 43 | 19 | 0 | 1 | 69 | 0 | 17 | 0 | 47 | 0 | 27 | **249** |
| **# nests with nestlings ≥ 6 days** | 18 | 16 | 15 | - | 1 | 33 | - | 5 | - | 21 | - | 22 | **131** |
| **Prevalence (%) of *P. downsi* **** | 100 (18) | 100 (16) | 100 (15) | - | 100 (1) | 100 (33) | - | 100 (5) | - | 100 (21) | - | 100 (22) | **131** |
| **Total intensity **** | 40.4±6.0 (18) | 29.3±3.4 (16) | 39.3±6.0 (15) | - | 37±0(1) | 36.2±2.4 (33) | - | 50.8±4.5 (5) | - | 42.2±4.6 (21) | - | 66.8±7.6 (22) | **131** |
| **Mean intensity**** | 15.8±3.0 (18) | 12.9±1.4 (15) | 18.8±3.2 (12) | - | 37±0(1) | 20.5±2.3 (29) | - | 18.5±2.0 (5) | - | 21.2±2.5 (15) | - | 30.4±3.0 (21) | **116** |
| **Mean brood size**** | 3.0±0.2 (18) | 2.33±0.2 (15) | 2.25±0.2 (12) | - | 1±0 (1) | 2.0 ±0.1 (29) | - | 2.8 ±0.2 (5) | - | 2.33±0.1 (15) | - | 2.3 ±0.1 (21) | **116** |
| **% Total brood loss**** | 33(6) | 38(6) | 40(6) | - | 100(1) | 51(17) | - | 20(1) | - | 38(8) | - | 32(7) | **125** |
| **% Partial brood loss**** | 33(6) | 6.3(1) | 10(1) | - | - | 21(7) | - | 40(2) | - | 14(3) | - | 23 (5) | **125** |
| **% of nests depredated*** | 27.3 (22) | 66.6 (39) | 21.0 (19) | - | 100 (1) | 50 (62) | - | 54.5 (11) | - | 37.1 (35) | - | 23.1 (26) | **215** |

*calculated for total # nests**calculated for nests with nestlings ≥ 6 days old, where data available

success) of parasitism in the lowlands if there is evidence of a brood size dilution effect (Richner and Heeb 1995).

**Results**

P. downsi *intensity across species, habitats, and years*

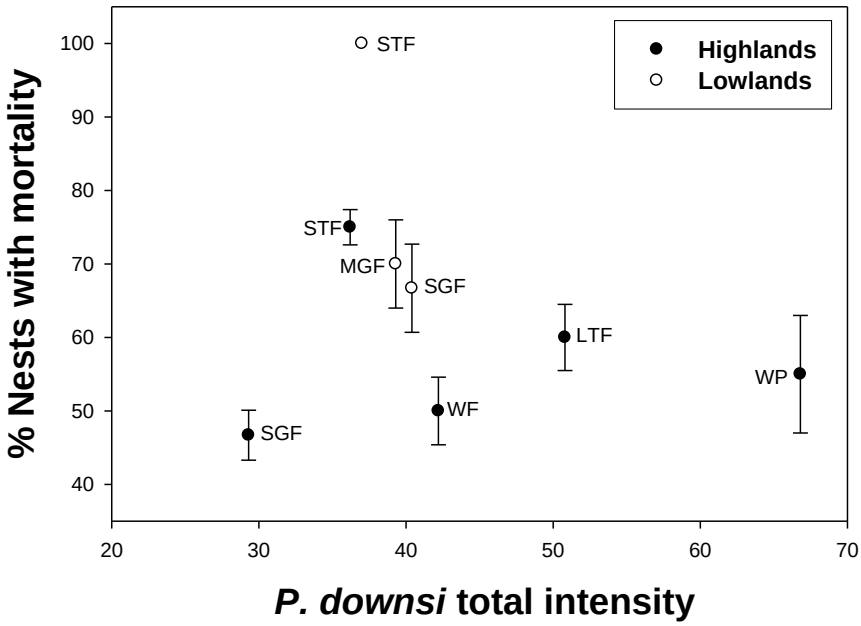
*P. downsi* total intensity differed significantly across species (ANOVA: $F_{131} = 3.15$, df = 5, P < 0.02) (Table 3.1, Figure 3.1), with a trend for a difference across years ($F_{131} = 2.22$, df = 5, P = 0.057) but not habitats ($F_{131} = 0.18$, df = 1, P > 0.6). The interaction terms were not significant (all P > 0.08). Tukey Post Hoc tests showed that total parasite intensity in the woodpecker finch was significantly higher than in small ground finch (P < 0.01), small tree finch (P < 0.01), warbler finch (P < 0.03), and medium ground finch (P < 0.05), but did not differ significantly from the large tree finch (P > 0.9) (Figure 3.1). *P. downsi* mean intensity differed significantly across years ($F_{116} = 2.57$, df = 5, P < 0.04) (Table 3.2), with a trend for a difference across species ($F_{116} = 2.28$, df = 5, P = 0.053) (Figure 3.1), but not habitats ($F_{116} = 0.94$, df = 1, P > 0.3). *P. downsi* total intensity in nests did not differ according to brood size using the pooled data for all species ($F_{116} = 1.2$, df = 3, P > 0.3). However, not surprisingly, a dilution effect was evident, with lower mean intensity in nests with larger brood size ($F_{116} = 13.3$, P < 0.01).

*Host body mass and* P. downsi *intensity*

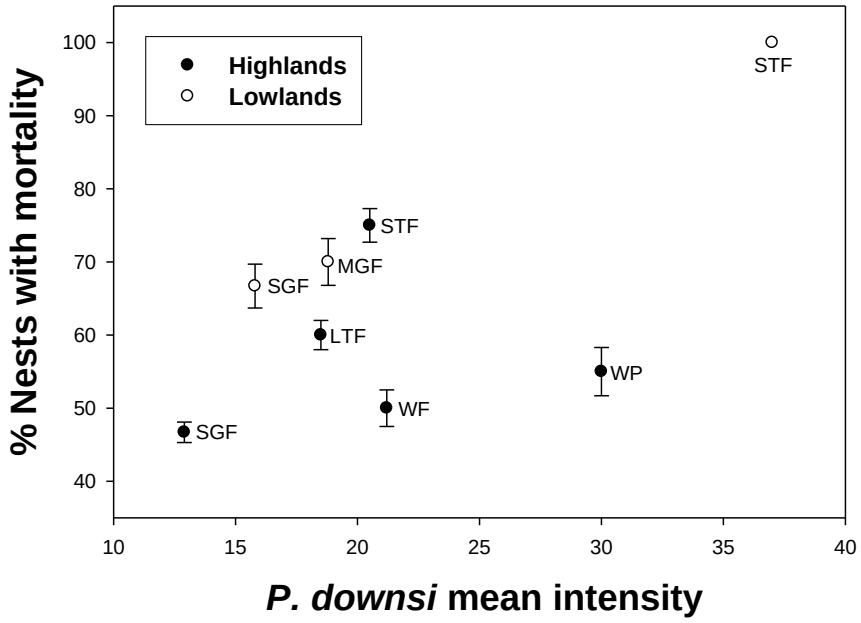
There was a positive association with host adult body mass and total parasite intensity across species (Pearson Correlation = 0.920, P < 0.03): large tree and woodpecker finches had the highest parasite intensity. Total intensity (mean ± s.e.) for each species and corresponding average adult body mass for birds caught in 1998 were as follows: warbler finch (9.4 g) = 37.2 ± 6.7; small tree finch (12.2 g) = 42.4 ± 4.3; small ground finch (13.3 g) = 57 ± 0; large tree finch (17.4 g) = 54 ± 0; woodpecker finch (22 g) = 71.8 ± 11.5. The medium ground finch was not sampled in 1998.

*Inter- and intraspecific analyses: fledging success and* P. downsi *intensity*

Ordinal regression analyses of highland nests showed that *P. downsi* total intensity significantly affected fledging success across the study species (Table 3.3). There was increased fledging success in highland nests with larger brood sizes, with a trend for higher fledging success during years with low rainfall (Table 3.3). There was also a negative effect of *P. downsi* mean intensity on fledging success and a difference in fledging success across




**(a)**




**(b)**

**Figure 3.1** *P. downsi* total (a) and mean (b) intensity (± s.e.) in relation to % nests with mortality for six species: SGF (small ground finch, n = 33), STF (small tree finch, n = 33), MGF (medium ground finch, n = 10), WF (warbler finch, n = 22), LTF (large tree finch, n = 5), WP (woodpecker finch, n = 22) in both the highlands (dark circles) and lowlands (open circles). Nestling mortality includes nests in which total or partial brood mortality occurred (sample size in Table 3.1).

**Table 3.2.** *P. downsi* mean intensity (± s.e., # nests) for all species in relation to annual rainfall (mm) and habitat (lowlands, highlands) per study year.

| Year | Annual rainfall lowlands (mm) | Annual rainfall highlands (mm) | *P. downsi* mean intensity | |
|---|---|---|---|---|
| | | | Lowlands | Highlands |
| 1998 | 1752 | 2119 | - | 29.2 ± 2.8 (28) |
| 2000 | 188 | 796 | 10.7 ± 2.4 (12) | 15.0 ± 2.0 (18) |
| 2001 | 293 | 825 | - | 18.0 ± 1.7 (12) |
| 2002 | 578 | 1591 | - | 18.8 ± 2.3 (19) |
| 2004 | 161 | 806 | 21.7 ± 2.9 (16) | 22.0 ± 4.2 (5) |
| 2005 | 186 | 813 | 23.6 ± 2.0 (3) | 21.8 ± 4.7 (3) |

**Table 3.3.** Statistics for ordinal regression analyses (Logit link) of three fledging success categories (1 = 0 %, 2 = < 100 %, 3 = 100 %) in relation to *P. downsi* mean and total intensity. Data are analysed separately for species in the highlands (n = 98 nests) and lowlands (n = 34 nests). Model 2 excludes brood size, as mean intensity is a direct computation from brood size.

| **Model** | | **Highlands** | **Lowlands** |
|---|---|---|---|
| **1** | total intensity | **P = 0.046** | P = 0.219 |
| | Year | P = 0.051 | P = 0.381 |
| | brood size | **P = 0.027** | P = 0.264 |
| | model fit $\chi^2$ | 10.05* | 7. 63 |
| | Nagelkerke $r^2$ | 0. 128 | 0.279 |
| **2** | mean intensity | **P = 0.048** | P = 0.287 |
| | total intensity | P = 0.110 | P = 0.778 |
| | Year | **P = 0.032** | P = 0.229 |
| | model fit $\chi^2$ | 9.17* | 7.58 |
| | Nagelkerke $r^2$ | 0.118 | 0.277 |

* significant at P < 0.05

years (Table 3.3). In the lowlands, ordinal regression analyses showed there was no significant effect of *P. downsi* total intensity, brood size, and year on fledging success and fledging success was not significantly predicted by *P. downsi* mean intensity, total intensity, or year (Table 3.3). Within individual host species, there was no difference in parasite intensity across brood sizes or categories of fledging success using ANOVA (brood size, all $P > 0.6$; fledging success, all $P > 0.1$), nor were the interaction terms significant.

**Discussion**

*Species differences in parasite intensity*

*P. downsi* total intensity was significantly different across six species of Darwin's finches on Santa Cruz Island, with a trend across years. Two tree finch species with large body mass, the large tree finch and the woodpecker finch, had the highest total parasite intensity (Figure 3.1). This may be of conservation significance as these species are considered to be declining in the study area (Kleindorfer et al. unpublished), and on Floreana Island, large tree finches are possibly endangered (Grant et al. 2005). We acknowledge that the sample size per species was small, and that the study was not specifically designed as an interspecific comparison from the outset. However, given the iconic status of Darwin's finches and the well documented fitness costs of this invasive parasite, we feel that it is warranted to discuss some of the implications of the patterns we found.

*Host body mass*

In 1998, highland species with high adult body mass showed increased *P. downsi* intensities (mean number of infesting larvae per nest). Host body mass may indirectly influence parasite intensity if nest size varies with host size and nest space is a limiting factor for parasite numbers (e.g. in *Protocalliphora* species: Whitworth 1976). Nestlings of finch species with higher adult body mass have larger nestlings (Grant 1981) with presumably increased blood volume and a greater surface area for larvae to feed and attach, allowing a greater number of parasites to be sustained (Poulin 1991). The nares of Darwin's finch nestlings have been identified as the oviposition site of adult *P. downsi* flies and might also vary with host size, providing an additional limitation on the number of larvae that can be deposited (Fessl et al. 2006b), but this is yet to be examined. Large host size has been associated with increased clutch size of an ectoparasitoid of wasps (*Eulophus pennicornis*) (Bell et al. 2005), and with increased ectoparasitoid prevalence in bumblebees (*Bombus terrestris*) (Müller et al. 1996) However, parasitoid survival and fecundity may be negatively affected by host size (Bell et al.

2005), which points to potential trade-offs that influence the optimal parasite clutch size for a given host size.

In contrast to the pattern found for 1998, the heavy-bodied medium ground finch (~19 g) (not sampled in 1998) had parasite intensities that were more comparable with species of light body mass (Figure 3.1). Also, nests of the light-bodied warbler finch (9 g) had the third highest parasite intensity out of the six study species, though this was not significantly different from that of the lighter-bodied species (Table 3.1, Figure 3.1). This may be due to variation in nest characteristics (e.g. concealment, vegetation structure, size) that increase a host's parasite susceptibility (Gold and Dahlsten 1989; Gwinner 1997). Although abundant in the highlands of Santa Cruz (Kleindorfer, personal observation), *P. downsi* parasitism is suspected to have contributed towards the presumed local extinction of the warbler finch (*Certhidea fusca*) on Floreana Island (Grant et al. 2005).

Preliminary analyses of nestling mass-specific parasite intensity across species (using estimated nestling mass at day 6) show that lighter-bodied species have a greater number of parasites per gram of host tissue, accounting for differences in host mass and clutch size between nests (e.g. large tree finch (8 g) = 2.3 ± 0.3; small tree finch (6 g) = 3.6 ± 0.4; warbler finch (4 g) = 5.3 ± 0.6). Nestling mass data were not available to examine this in the current study, yet this indicates that host mass is not a predictor of *P. downsi* fitness costs across host species per se, but is rather a predictor of the total parasite intensity within nests. Our results show that *P. downsi* intensity varies with adult body mass of host species in the highlands of Santa Cruz Island, though more data are required from the lowlands to examine these patterns more thoroughly.

*Effects of rainfall and year*

The difference in interspecific *P. downsi* mean and total intensity found across years can be explained by high mean intensity found in the El Niño year of 1998 (~ 29 parasites per nestling) followed by low mean parasite intensity in 2000 (~10-14 parasites per nestling). The years 1998 and 2000 corresponded with high and low annual rainfall in the highlands respectively, with 1998 exceeding the recorded rainfall for all other study years by at least 500 mm (Table 3.2). Notably 1998 experienced the highest average air temperature over the breeding period, which is consistent with findings that small increases in temperature can lead to large increases in fly population density (Goulson et al. 2005). Darwin's finches increase

their clutch size and the number of breeding attempts in years of high rainfall (Grant 1999a), resulting in a significant increase in finch numbers during El Niño events (Grant 1999b; Glantz 2001). Despite presumably larger clutch sizes, the number of parasites per nestling was highest during the El Niño year of 1998 (Table 3.2). Our data indicate that brood size was actually smaller during 1998, which was likely due to high partial nestling mortality prior to nestlings reaching six days of age, thus creating an apparent, but not factual reduction in brood size. Therefore, it remains possible that annual differences in clutch size can explain the annual difference in mean parasite intensity we observed.

The positive response of host numbers to increased rainfall, and not rainfall or temperature per se, is likely to be more dominant in determining *P. downsi* intensity. The relationship between rainfall and the impact of *P. downsi* is presumably affected by several variables, as indicated by comparable parasite intensities between years with moderate (e.g. 2002) and low (e.g. 2004) annual rainfall (Table 3.2). Fly populations may fluctuate rapidly in response to changes in precipitation and temperature according to conditions prevailing on daily or weekly timescales (Mahon et al. 2004). Such responses are not reflected when analysing annual rainfall in relation to intensity data obtained in one season, as in the current study. Other studies of *Philornis* show a positive relationship between total intensity and prevalence with rainfall (Nores 1995; Arendt 2000). However, dry conditions may reinforce the impact of *P. downsi* due to difficult breeding conditions (Fessl et al. 2006a). There is a need for predictive models stemming from short-term meteorological data that could inform an integrated control program for the fly on the Galápagos Islands (Goulson et al. 2005).

*Habitat*

Assessment of inter-habitat differences in parasite population dynamics is advantageous for prioritising locations for in situ parasite control efforts. As found in previous studies on Santa Cruz Island (Fessl and Tebbich 2002; Dudaniec et al. 2006), we found no difference in *P. downsi* intensity between the highlands and lowlands (Table 3.2). However, given the three-fold difference in rainfall between habitats, it is likely that this finding would be altered if intensity and rainfall data were available over a broader temporal span (e.g. days or weeks) (Arendt 1985a, b). However, conspecific flies may develop physiological adaptations to habitats with contrasting climates, resulting in populations with increased desiccation or cold tolerance (e.g. in *Drosophila* species: Gibbs et al. 2003). In contrast to Santa Cruz Island, *P. downsi* intensity on Floreana Island was significantly higher in the highlands than lowlands

(O'Connor and Kleindorfer, unpublished data), thus different patterns are likely to occur across the archipelago.

*Fledging success and brood size*

Experimental and observational studies since 1998 have documented high fitness costs of *P. downsi*, including decreased gain in nestling mass (Fessl et al. 2006a), reduced fledging success (Fessl and Tebbich 2002), high blood loss in nestlings (between 18-55 %) (Fessl et al. 2006b), and reduced haemoglobin (Dudaniec et al. 2006; Fessl et al. 2006a). Nestling mortality in Darwin finch species due to *P. downsi* parasitism was high, with 19-62 % total brood loss across years, pooling all species (Fessl and Tebbich 2002; Fessl et al. 2006a). In the current study, fledging success across all species was highest in the El Niño year of 1998 (although there were many cases of partial brood loss), which is consistent with trends documenting increased finch survival in years of extreme rainfall (Grant 1999a). High *P. downsi* mean intensity and small brood size were associated with reduced fledging success across all species in the highlands, but this was not evident intraspecifically (Figure 3.1). However, this finding was upheld within species across habitats (small ground and small tree finch only). The variation in *P. downsi* intensity and brood size within individual species in each habitat is most likely not sufficient to reveal any significant effects on fledging success.

The difference in fledging success across habitats in relation to brood size and *P. downsi* intensity may be explained by the different causes of nesting failure (i.e. due to different levels of food abundance and predation risk) between habitats (Kleindorfer 2007a). Lowland nestling mortality is more commonly due to nestling starvation (Fessl et al. 2006b), whereas in the highlands food is abundant across the year and mortality within infested nests is most likely attributable to *P. downsi* parasitism in the absence of evidence for alternative causes of mortality. We only monitored the first clutches of the breeding season, thus high levels of host reproduction may have preceded a subsequent increase in parasite population density (e.g. Arendt 1985a) that could impact host reproductive attempts later in the breeding season.

Avian ectoparasites can have fitness costs that vary in magnitude with brood size (Richner and Heeb 1995). A parasite dilution effect was evident across all species, with larger broods having lower *P. downsi* mean intensity. The opposite pattern has also been found in parasitic flies infesting bird nests (e.g. in *Protocalliphora* species: Dawson et al. 2005), and could be influenced by parasite reinfestation during the nesting period. The brood size dilution effect

we found implies that finch species in the highlands may be at elevated risk from *P. downsi*, where the brood size of all finches was smaller compared with that of lowland finches (Kleindorfer 2007a). Notably, both species with the highest parasite intensity (large tree and woodpecker finches) nest almost exclusively in the highlands.

## Conclusion

We have shown that parasite intensity varies across host species and in relation to annual rainfall, which are pertinent factors to consider within a conservation plan for managing *Philornis* parasitism. Current efforts to understand the fly's reproductive and dispersal behaviour will fill a further prerequisite for the development of appropriate control methods. The severe impact of *P. downsi* makes this parasite likely to be the largest threat to endemic bird species on the Galápagos Islands and presents an immediate task of conservation priority.

## Acknowledgements

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**CHAPTER 4:**

**Love thy neighbour? Social nesting pattern, host mass, and nest size affect ectoparasite intensity in Darwin's tree finches.**

Sonia Kleindorfer and Rachael Y. Dudaniec

*Behavioural Ecology and Sociobiology* (accepted pending revision)

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**Abstract**

Social nesting behaviour is commonly associated with high prevalence and intensity of parasites in intraspecific comparisons. Little is known about the effects of interspecific host breeding density for parasite intensity in generalist host-parasite systems. Darwin's small tree finch (*Camarhynchus parvulus*) on Santa Cruz Island, Galápagos Islands, nests in both heterospecific aggregations and at solitary sites. All Darwin finch species on Santa Cruz Island are infested with larvae of the invasive blood-sucking fly *Philornis downsi*. Here, we test the prediction that total *P. downsi* intensity (the number of parasites per nest) is higher for nests in heterospecific aggregations than at solitary nests. We also examine variation in *P. downsi* intensity in relation to three predictor variables: (1) nest size, (2) nest bottom thickness, and (3) host adult body mass, both within and across finch species. The results show: (1) total *P. downsi* intensity was significantly higher for small tree finch nests with many close neighbours, (2) finches with increased adult body mass built larger nests (inter- and intraspecific comparison), (3) parasite intensity increased significantly with nest size across species and in the small tree finch alone (4) nest bottom thickness did not vary with nest size or parasite intensity. These results provide evidence for an interaction between social nesting behaviour, nest characteristics and host mass that influences the distribution and potential impact of mobile ectoparasites in birds.

**Introduction**

The study of social nesting behaviour has generated many insights into the costs and benefits of aggregations, but less is understood about the mechanisms underlying their formation (Tinbergen 1964; Clotfelter and Yusukawa 1999). Past studies have focused on identifying the costs and benefits of group living, which were used as post-hoc evidence for the cause of the aggregation (e.g. Silva et al. 1994). Despite the logical fallacy of such an argument (Williams 1966), understanding current costs and benefits of aggregations will provide information on the strength of natural and/or sexual selection to maintain them (Aviles 1999). Social nesting behaviour may have advantages such as reduced nest predation (Hamilton 1971), but also costs such as higher parasite prevalence and intensity, or sometimes increased predator attraction (Valera et al. 2007). Parasite intensity is influenced by many factors including the mode of parasite transmission, which will vary in efficacy in relation to host density (reviewed in Fenton et al. 2002; Whiteman and Parker 2004). Evidence suggests that parasites limit host group size for specific parasite transmission modes (because of different transmission efficacy in relation to group size and parasite dispersal), but at present there is no evidence that parasite avoidance is the cause of particular patterns of group living (Poulin 1999).

Group-living in conspecific birds is associated with a higher probability of acquiring and accumulating contact-transmitted ectoparasites (e.g., mites, feather lice) due to increased proximity and physical contact among group members (Poulin 1991; Brown and Brown 1996). Transmission of mobile ectoparasites, such as blood-sucking flies is predicted to not differ between group-living and solitary individuals because mobile parasites are not dependent on host contact or proximity for transmission (Poulin 1991), though this prediction is not always supported (Duncan and Vigne 1979). Parasite intensity within groups can show high variation and some individuals typically have very few, or a lot of parasites compared to the mean for the group (reviewed in Wilson et al. 2002). The ‘encounter-dilution effect’ in relation to host density is known across taxa (in feral horses: Duncan and Vigne 1979; wasps: Wieslo 1984; and sticklebacks: Poulin and Fitzgerald 1989) and is analogous to the predicted decrease in predation probability for individuals within larger groups (e.g., the “selfish herd” model developed by Hamilton 1971). However, mobile parasites may show the opposite pattern when the probability of parasites detecting hosts increases with host group size and when an individual parasite can affect multiple hosts within host aggregations (Mooring and Hart 1992).

In this study, we examine nesting aggregation in Darwin's tree finches in relation to parasitism by the fly *Philornis downsi* (Muscidae). Darwin's small tree finch (*Camarhynchus parvulus*) nests either with 0-1 neighbouring nests within 20 m of the focal nest (referred to as solitary nesting; SN) or with 2-4 heterospecific nests (referred to as mixed species nesting associations) (Kleindorfer et al. in review). Mixed species nesting associations are known for birds, but have rarely been studied in relation to parasitism (Burger 1981; Mönkkönen and Forsman 2002). Birds in mixed nesting associations often have lower nest predation (Kleindorfer et al. in review), but studies also suggest that such associations (and large groups in general) may be easier to locate for both predators and parasites (Nicolas and Sillans 1989; Danchin and Wagner 1997). Increased propensity for nesting in mixed nesting associations may therefore represent an 'ecological trap' if fitness consequences of introduced parasites or predators are increased in comparison to solitary nesting (reviewed in Robertson and Hutto 2006).

Larvae of the introduced parasitic fly *P. downsi* cause high fitness costs in Galápagos finches (Fessl and Tebbich 2002; Dudaniec et al. 2006; Fessl et al. 2006a). The parasite was first formally identified from Darwin finch nests in 1997, has 100% prevalence for all Darwin finch species on Santa Cruz Island, and occurs on all but two of the 13 major islands (Wiedenfeld et al. 2007). The parasite causes low nestling mass, low haemoglobin concentration (Dudaniec et al. 2006; Fessl et al. 2006a), high blood loss (between 18-55 %), and low fledging success (19-100 % brood loss) (Fessl and Tebbich 2002; Fessl et al. 2006b). In 2006, *P. downsi* was given the highest risk rating for introduced species to the Galápagos Islands (Causton et al. 2006), and is a major conservation concern for small and endemic bird populations (Dvorak et al. 2004; Wikelski et al. 2004; Grant et al. 2005).

It is not clear why we see high levels of intraspecific variation in *P. downsi* intensity, though finch species with large adult body mass have more *P. downsi* in their nests (Dudaniec et al. 2006, 2007 [Chapter 3]). While parasite fitness may depend on features such as host mass and immune defence, parasite fecundity does not always covary with host characteristics (e.g., parasitic flies of barn owls: Roulin 1999). Host nest size may help to explain intraspecific variation in parasite intensity, especially if the parasite depends on host nest characteristics (e.g., burrowing depth) for survival (in *Protocalliphora* species: Whitworth 1976; Gold and Dahlsten 1989; Remeš and Krist 2005). Nest size may also affect host quality in terms of

immunity, both intra- and interspecifically (Soler et al. 2007). *Philornis downsi* larvae use the bottom layers of finch nests for refuge and as a substrate for pupariation whereby densely packed puparia are enclosed by tightly woven cocoons in the nesting material. Therefore, the thickness of the nest bottom layer may be a specific spatial limiting factor for parasite intensity in Darwin's finches.

At an intraspecific level, parasite intensity in birds generally increases with the level of sociality (e.g., colonial nesting species) (Côté and Poulin 1995). To date, few studies have examined interspecific variation in social nesting behaviour in relation to parasite intensity for generalist avian parasites (Tella 2002). Here, we examine Darwin's tree finches on Santa Cruz Island, Galápagos archipelago, to test the following predictions: (1) total *P. downsi* intensity (number of parasites per nest, defined by Bush et al. 1997) is higher for nests in mixed species nesting associations than solitary nests; (2) Hosts with high adult body mass build larger nests with increased nest bottom thickness; and (3) *P. downsi* total intensity increases with nest size (within and across finch species) and nest bottom thickness (across finch species).

**Methods**

*Location and study species*

This study was conducted on Santa Cruz Island in the highland area surrounding Los Gemelos (0º37´S, 90º 21´W) during the breeding season (January - March) from 2000-2004, excluding 2003. Although Darwin's tree finches occur in both the lowlands and highlands of Santa Cruz Island, they attain their highest density in the highland *Scalesia* zone (300-750 m), an area that is dominated by the endemic composite tree *Scalesia pedunculata* (Asteraceae) (Eliasson 1984). We sampled nests from five species of Darwin's finches: small ground finch, *Geospiza fuliginosa*; warbler finch, *Certhidea olivacea*; small tree finch, *Camarhynchus parvulus* (Figure 4.1); large tree finch, *Camarhynchus psittacula*; woodpecker finch, *Cactospiza pallida.* The latter three species are members of the 'tree finch' group and occur either exclusively or at higher density in the highlands, while the small ground finch breeds at higher density in the lowlands (0-100 m) but is common in the highlands (Kleindorfer 2007a). The warbler finch nests exclusively in the highlands.

*Nest distance of neighbours*

We recorded the location (GPS Garmin 12 XC) and the number of active nests within a 20 m radius per focal small tree finch nest, but did not include unused display nests in the analysis

(see also Kleindorfer 2007a). Twenty meters was selected as a distance because it is the maximum distance at which males of the small tree finch respond to playback of song by other males (Christensen et al. in review; Kleindorfer unpublished data). Active nests were identified by males singing at nests, incubation, or parents feeding nestlings. Recording the distance of all nests surrounding the focal nest within 20 m was aided by placement of two intersecting 10 m ropes below the focal nest. Decimal longitude and latitude co-ordinates were transformed into UTM Coordinates (Universal Transverse Mercator) in the form of Eastings and Northings. These values were then used to calculate the distance between all nests from a common zero point. Distances of all nests from focal small tree finch nests were sorted, and could be examined for the cut-off value of 20 m. Because the distribution is bimodal, nests with zero or one neighbour within a 20 m radius were termed solitary nests, and nests with ≥ two neighbours (range was two to four) were termed mixed species nesting associations (defined in more detail in Kleindorfer et al. in review).

*Nest monitoring*

Darwin's finches are usually socially monogamous (Grant and Grant 2008) and build domed shaped nests. When breeding commences, males build a display nest and sing to attract a mate (Lack 1947; Kleindorfer 2007a). Females then visit the singing male and often enter and inspect the nest. A female either rejects the male and his display nest, accepts the male and builds a new nest together with the male, or accepts both the male and the display nest for nesting (Kleindorfer 2007a). During nest building, males primarily build the outer nest, while females primarily collect nesting material to reinforce the inner bottom layers (S Kleindorfer personal observation). We monitored active nests from the first stage of nest building by the male until the nesting outcome was known (Kleindorfer 2007a, b). Nesting phase was determined from repeated 20-min observations (every two days) of parental activity at each nest, as well as by nest inspection. Active nests were classified into three breeding stages: incubation, early feeding (< 6 days post-hatching), and mid to late feeding (6 - 12 days post-hatching). We monitored a total of 199 small tree finch nests. For this study, we restrict the sample size to 43 nests that met two criteria: (1) nestlings survived until ≥ 6 days post-hatching (to minimize the effects of nestling age on variation in total parasite intensity) (see Fessl and Tebbich 2002), and (2) accurate information was obtained for the number and proximity of active nests within 20 m of the focal nest (see below; Kleindorfer et al. in review) (Table 4.1). The focal nest was always the small tree finch, *C. parvulus* (Figure 4.1) and for analyses of nesting pattern (mixed species versus solitary nesting) and parasite

intensity, only intensity of the focal nest was used. Data on *P. downsi* intensity were also collected from nests of four other finch species in which nestlings survived ≥ 6 days post-hatching that were also members of mixed associations (Table 4.1). We do not have data for solitary nests for these species because our comparison of *P. downsi* intensity in mixed nesting associations versus solitary nests is for the small tree finch only.

*Nest size and nest site characteristics*

After the nesting attempt was completed, we collected each nest and measured the following variables: (1) nest height (cm), (2) nest width (cm), (3) diameter of the entrance (cm), and (4) thickness of the nest bottom (cm) (measured by placing a ruler from the top of the nest to the base of inside of the entrance hole, and subtracting this length from the nest height). We also measured the following nest site characteristics: height of the nest (m) in the nesting tree, and percentage of leaf cover above, below, and to the sides of the nest, which was visually estimated as a percentage of each nest surface area covered 1 m to each side of the nest. For the analysis, we used the mean percentage nest cover per nest.

*Parasite life-history and collection*

*Philornis downsi* is an obligate avian parasite in its three larval stages, whereas adult flies feed on organic matter (Fessl and Tebbich 2002; Dudaniec and Kleindorfer 2006 [Chapter 2]). The fly is oviparous, and larval development is triggered by carbon dioxide (Muth 2007). First instar fly larvae are found in nestling nares after 8-24 hours post-hatching (Fessl et al. 2006b). Mature second and third instar larvae attach externally and feed on nestling blood and tissues (Dudaniec and Kleindorfer 2006 [Chapter 2]; Fessl et al. 2006b). The larvae feed for about four to six days before pupating in tight clusters at the base of the nesting material for approximately two weeks (Dudaniec and Kleindorfer 2006 [Chapter 2]). Most larvae of *P. downsi* reach their third instar phase at the time of host fledging. All nests (100%) with nestlings ≥ 6 days contained *P. downsi* larvae, puparia or puparia cases. Inactive nests were stored in individual sealed plastic bags and subsequently dismantled to count the number of parasites from the thick nest bottom layers (Fessl and Tebbich 2002). *P. downsi* puparia, puparia cases, second and third instar larvae are easily detected and collected from nests, whereas first instars have not been found in the nesting material (Wiedenfeld et al. 2007; R Dudaniec personal observation).

**Table 4.1.** Total *P. downsi* intensity (number of parasites per nest) across five Darwin finch species in relation to host nest characteristics. We show sample sizes for nests at which nestlings survived to ≥ 6 days post hatching and for which we have data on the distance of all neighbouring nests, and species summaries for nest size variables (shown as means ± s.e.).

| | **warbler finch** | **small tree finch** | **small ground finch** | **large tree finch** | **woodpecker finch** |
|---|---|---|---|---|---|
| **Sample size** | 9 | 17 | 9 | 4 | 4 |
| **Adult body mass (g)*** | 9.4 | 12.2 | 13.3 | 16.7 | 22.0 |
| **Parasite intensity** | 41 ± 6 | 23 ± 3 | 33 ± 3 | 39 ± 9 | 57 ± 4 |
| **Nest height (cm)** | 11.2 ± 0.3 | 13 ± 0.3 | 14.4 ± 0.3 | 16.4 ± 0.4 | 16.5 ± 0.4 |
| **Nest width (cm)** | 8.9 ± 0.4 | 9.8 ± 0.3 | 10.2 ± 0.3 | 12.8 ± 0.3 | 13.3 ± 0.4 |
| **Nest entrance (cm)** | 4.2 ± 0.1 | 4.6 ± 0.1 | 5.2 ± 0.3 | 7.6 ± 0.2 | 8 ± 0.5 |
| **Nest bottom (cm)** | 3.44 ± 0.2 | 4.12 ± 0.2 | 4.22 ± 0.4 | 4.88 ± 0.3 | 5.13 ± 0.4 |
| **% Vegetation cover** | 44.2 ± 3.4 | 53.2 ± 2.9 | 45.6 ± 3.5 | 42.5 ± 4.8 | 41.3 ± 7.5 |
| **Nesting height (m)** | 4.2 ± 0.4 | 6.2 ± 0.3 | 4.1 ± 0.4 | 6.9 ± 0.2 | 6.5 ± 0.2 |

*Adult body mass (mean) was not calculated from the birds sampled in this study and are taken from Dudaniec et al. (2007) [Chapter 3].

**Figure 4.1.** Darwin's small tree finch (male) (*Camarhynchus parvulus*) (photo: Sonia Kleindorfer).

*Statistical analysis*

Summary statistics are presented as means ± s.e. All statistical tests are two-tailed. Data for total *P. downsi* intensity met the underlying assumption for normality and were not transformed. Nest site concealment data were calculated as percentages and were arcsine square root transformed. We used principal components analysis (PCA), varimax with Kaiser normalisation rotation method, to reduce the nest size variables (width, height) to one derived variable. We examined nest bottom thickness as a separate variable. We used ANOVA to examine *P. downsi* intensity across species and in relation to nesting pattern. The residuals of the ANOVA were normally distributed. We used multiple regression analysis to examine the role of the derived nest size variable (PCA scores), nest bottom thickness, nest vegetation cover, nesting height, and the number of neighbouring nests for *P. downsi* total intensity. Sample size was inadequate to analyse STF adult size and body mass with nest parasite intensity. All analyses were performed using SPSS 14.0 for Windows.

**Results**

*Heterospecific nesting density*

No nests of the focal species (small tree finch, *C. parvulus*) were found within 20 m of another small tree finch nest, therefore all nearest neighbours within 20 m were heterospecific. The majority of nests at which nestlings survived until ≥ 6 days post-hatching (28/43; 65 %) were in mixed species nesting associations, while the rest were classified as solitary nests (15/43; 35 %). Using ANOVA, we examined parasite intensity in relation to host species and nesting pattern (mixed versus solitary nests). Intensity of infestation by *P. downsi* differed significantly across host species ($F_{4, 42} = 5.06$, $P < 0.004$) and was highest in woodpecker finch > warbler finch > large tree finch > small tree finch > small ground finch (Table 4.1). Nesting pattern was significantly related to parasite intensity ($F_{1, 42} = 7.3$, $P < 0.02$), but not the interaction term host species x nesting pattern ($F_{4, 42} = 0.7$, $P > 0.5$). Thus, nests with many neighbours had more parasites per nest than solitary nests (Figure 4.2), and host species composition within the nesting aggregations did not covary with parasite intensity.

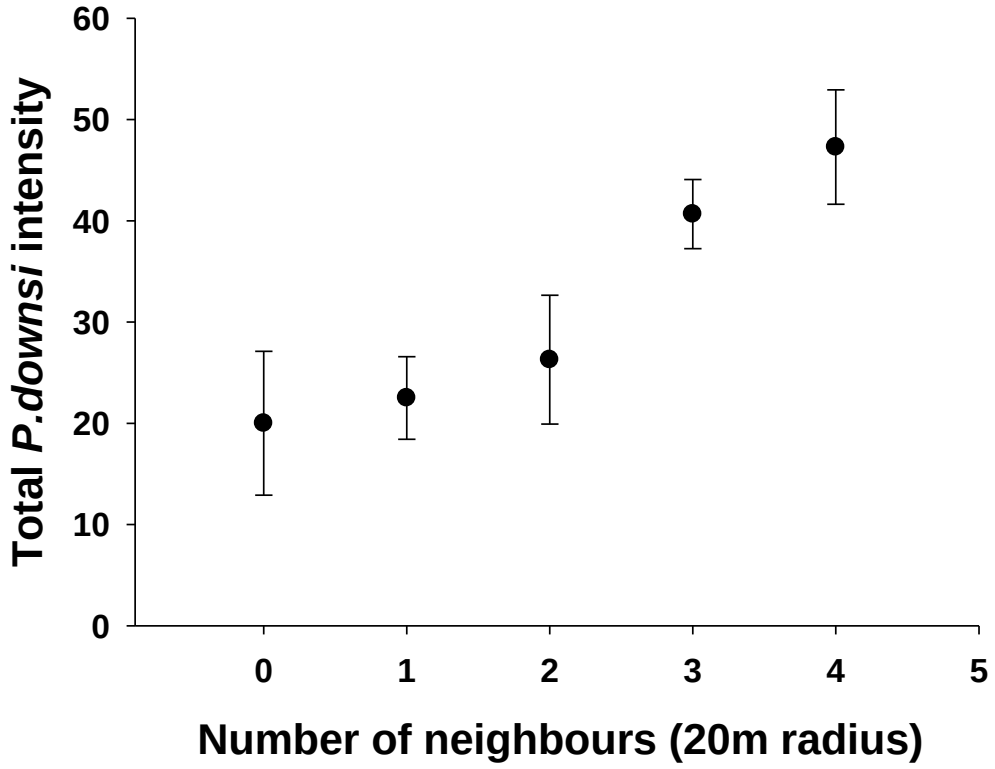


**Figure 4.2.** Total *P. downsi* intensity (mean ± s.e.) of the focal small tree finch nest increases with the number of neighbours within a 20 m radius (n = 43 nests).

*Nest size, nest bottom thickness, and parasite intensity*

Nest size differed significantly across species in proportion to adult body mass. That is, small species had small nest size and large species had large nest size (Figure 4.3) ($r = 0.88$, $t = 11.5$, $P < 0.0001$). The eigenvalue was 1.9 for the principal components analysis of nest size, and the derived factor explained 94% of the variance, with high factor loadings (0.97, 0.97 for nest height and width respectively). Using the derived nest size variable (PCA1), we found a significant correlation between nest size and *P. downsi* intensity ($b = 0.4$, $t_{1, 41} = 2.9$, $P < 0.01$) (Figure 4.4). Within the small tree finch only, there was a significant positive correlation between nest size and *P. downsi* intensity ($b = 0.6$, $t_{1, 15} = 3.0$, $r = 0.6$, $P < 0.01$) (Figure 4.5). However, there was no significant relation between nest bottom thickness and *P. downsi* intensity across species ($b = 0.1$, $t_{1, 41} = 0.5$, $r = 0.1$, $P > 0.6$) or with nest size (PCA1) within species (linear regression, all species: $P > 0.3$).

*Multivariate approach: nesting density and nest size*

Table 4.2 shows the results of a multiple regression analysis to examine total *P. downsi* intensity in relation to the derived nest size variable (PCA1), percentage vegetation cover at the nest, nesting height, and the number of neighbours within a 20 m radius. Both nest size and the number of neighbours were significant predictors of total *P. downsi* intensity.

**Discussion**

*P. downsi* intensity was higher for nests in mixed species nesting associations (65% of nests) than solitary nests (35% of nests), and increased with the number of neighbouring nests surrounding the focal small tree finch nest (Figure 4.2). This association was unaffected by the species composition of neighbouring nests. Because we only included nests that survived to day six post-hatching for our measure of *P. downsi* intensity, and mixed associations had lower nest predation (Kleindorfer in review), we had more nests in mixed associations than solitary ones. However the percentage of mixed associations and solitary nests was comparable to a previous study (Kleindorfer et al. in review). Previous experimental and observational studies showed that *P. downsi* causes high nestling mortality (reviewed in Dudaniec and Kleindorfer 2006 [Chapter 2]). Our current finding therefore raises the question: Why do Darwin's tree finches frequently nest in mixed associations given predicted high fitness costs due to parasitism that increase with the size of the nesting aggregation?

**Table 4.2.** Multiple regression results for total *P. downsi* intensity (the number of parasites per nest) (dependent variable) against the following independent variables: PCA nest size, nest cover, nesting height, and the number of neighbours (within 20 m) (n = 43).

| | **B** | **SE** | **Beta** | **t** | **P** |
|---|---|---|---|---|---|
| **(Constant)** | 37.97 | 11.75 | | 3.23 | 0.003 |
| **PCA nest size** | 5.64 | 2.39 | 0.33 | 2.36 | 0.024 |
| **Vegetation cover** | -0.27 | 0.18 | -0.18 | -1.49 | 0.142 |
| **Nesting height** | -1.33 | 1.47 | -0.12 | -0.90 | 0.372 |
| **# Neighbours** | 6.81 | 1.64 | 0.50 | 4.15 | 0.001 |

a Dependent variable: total *P. downsi* intensity.

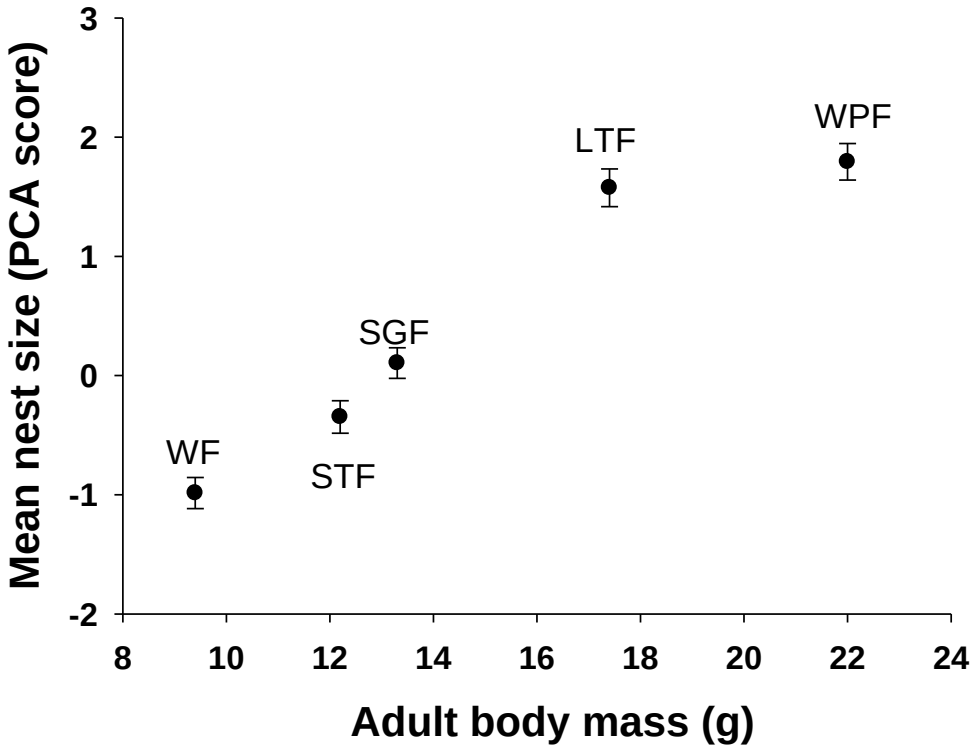


**Figure 4.3.** The positive relation between host adult body mass (g) (mean per species) and mean nest size (± s.e). (derived PCA variable). The species abbreviations are: warbler finch (WF), small ground finch (SGF), small tree finch (STF), large tree finch (LTF), and woodpecker finch (WPF).

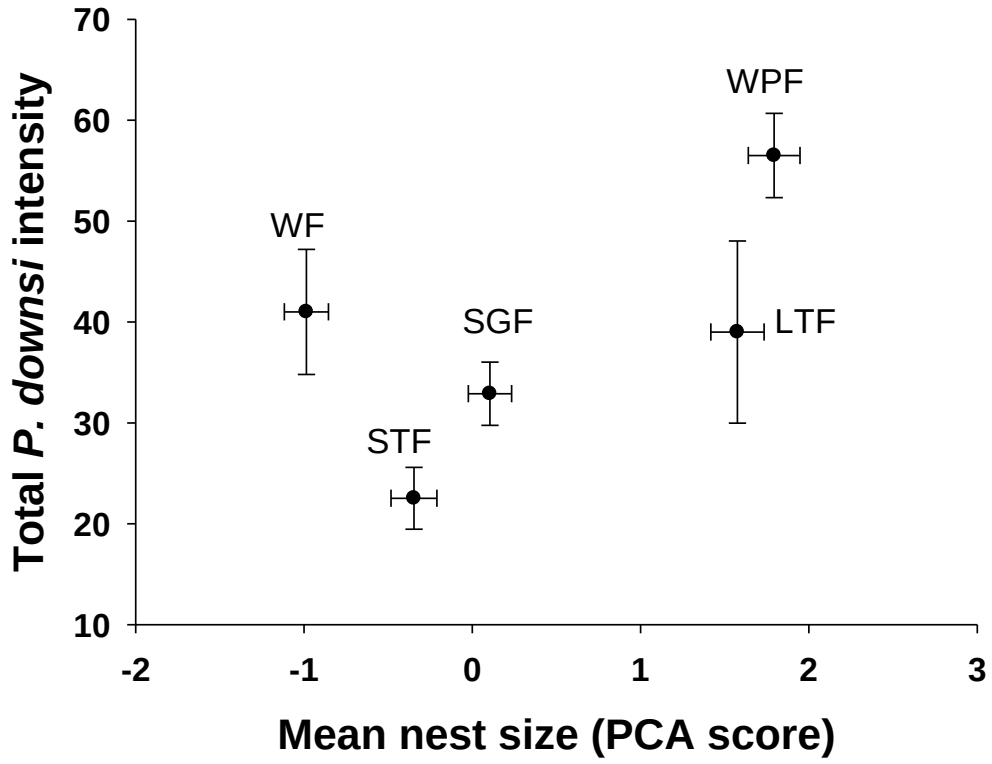


**Figure 4.4.** Total *P. downsi* intensity (mean ± s.e) increases with nest size (± s.e) (derived PCA variable). The species abbreviations are: warbler finch (WF), small ground finch (SGF), small tree finch (STF), large tree finch (LTF), and woodpecker finch (WPF).

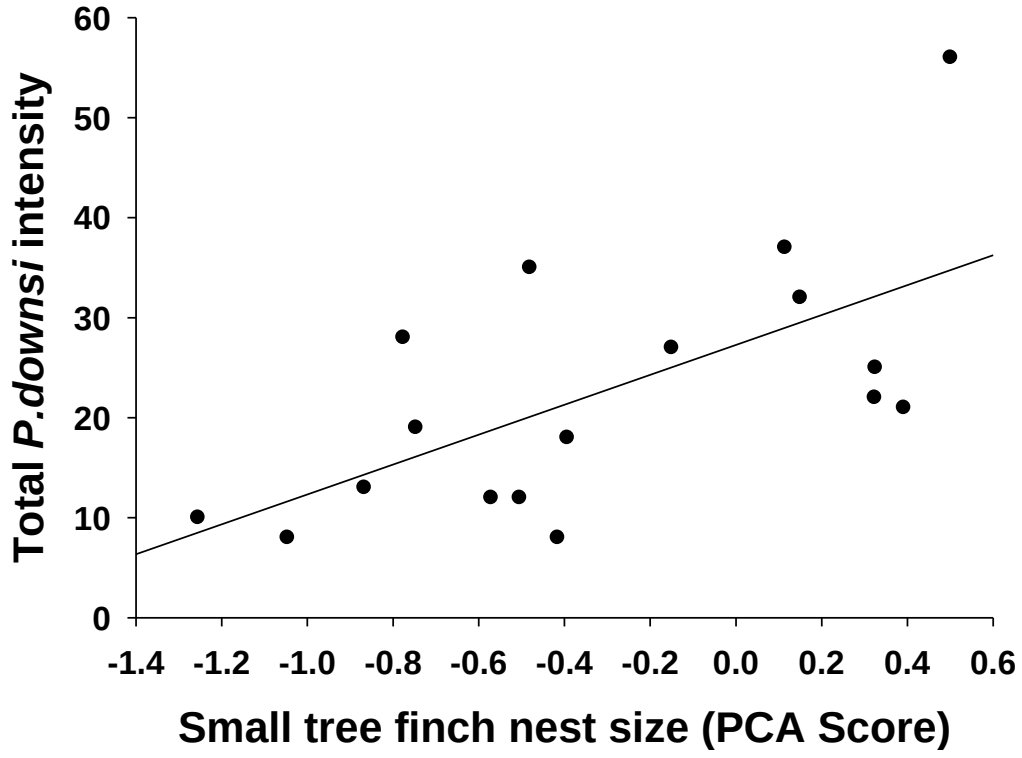


**Figure 4.5.** Data for the small tree finch (*Camarhynchus parvulus*) only: a positive correlation between nest size and total *P. downsi* intensity (number of parasites per nest) ($r$ = 0.61, $P < 0.02$; n = 17 nests).

Our findings may indicate the existence of an ecological trap (Robertson and Hutto 2006), in which the fitness benefits of nesting in aggregations (e.g., increased predator vigilance) have been overcome by increased impacts of a recently introduced parasite. Ecological traps triggered by sudden changes that result in high fitness costs may initiate rapid population decline (Robertson and Hutto 2006).

Mixed nesting associations are composed of between two and four species, and all contain a nest of one large finch species (Kleindorfer in review) – either a large tree finch or woodpecker finch nest. Notably, parasite intensity was highest for large finch species, and host body mass and nest size were both covariates of *P. downsi* intensity (large birds built big nests) (Figure 4.3). Within the small tree finch, larger males built larger nests, and larger nests had higher *P. downsi* intensity (Figures 4.4 and 4.5). We could not directly examine small tree finch adult body size and mass with *P. downsi* intensity due to small sample size (only four nests). The interspecific differences in parasite intensity within mixed associations may be indicative of 'apparent competition' (Holt and Kotler 1987), whereby indirect interactions between one host species and another are formed in response to survival costs from a mutual parasite. However this is largely determined by parasite behaviour and requires further examination (Holt and Kotler 1987). Nest bottom thickness is an important substrate for the puparia of *P. downsi*. However, nest bottom thickness was not significantly correlated with nest size, and did not significantly predict *P. downsi* intensity. Combined, these findings suggest that host body mass and social nesting pattern are important causal agents in *P. downsi* intensity, but not nest size or bottom thickness.

*Host nesting behaviour and parasitism*

Social nesting aggregations have many benefits (e.g. increased predator vigilance, opportunity for extra-pair copulations), but are also frequently associated with increased resource competition, increased conspicuousness to predators, and a higher risk of disease transmission (Danchin and Wagner 1997; Wagner 1997; Richardson and Bolen 1999). Nesting aggregations in territorial species can reflect local variation in resource densities. For example, the presence of established males or of dominant conspecifics in an area may act as an indicator of habitat quality for individuals acquiring their first territory (Stamps 1988; Robertson and Hutto 2006). In Darwin's tree finches, nest site vegetation characteristics were not associated with nesting pattern (mixed species versus solitary nesting), while tree finch nests in mixed associations had a higher nest defence response and reduced predation

compared with solitary nests (Kleindorfer et al. in review). Aggregations may be formed according to cues obtained through 'public information' (which may be observed as habitat copying) and result in a by-product of negative fitness consequences, despite the benefits of aggregations.

Previous research has focused on the mechanisms for how parasites may disrupt or modify host social systems to favour the expression of social or anti-social behaviour (Mooring and Hart 1992; O'Donnell 1997; Sorci et al. 1997). Larger colony size and high ectoparasite intensity were associated with increased levels of corticosterone in cliff swallows (Raouf et al. 2006), which can impair reproductive or cognitive function. The cost of ectoparasitism for host reproductive success increased with colony size in the family Hirundinidae, yet this was countered by a greater investment in immune function among highly colonial species, particularly in nestlings (Møller et al. 2001). Selective pressures on host social behaviour may be exacerbated, or altered by parasitism, but they are likely to vary considerably with the hosts' parasite defence mechanisms, parasite life history and the influence of other ecological pressures (e.g., resource availability and predation).

*Parasite transmission and host grouping behaviour*

Because *P. downsi* is not a contact-transmitted parasite, theory predicts that the risk of transmission in mixed nesting associations is not likely to exceed the risk to the population as a whole. Therefore, neither solitary nor grouping behaviour should be disfavoured (O'Donnell 1997). In support of this, an inter-specific comparison of 45 passerines by Poulin (1991) showed that group-living species had a greater prevalence of contact-transmitted mites than solitary species, but the abundance of mobile parasitic flies (Hippoboscidae) did not differ with social behaviour. However, mobile parasites may have a higher impact within larger host groups when the probability of a parasite detecting its host increases with host group size, and where an individual parasite can use multiple hosts (Mooring and Hart 1992). This latter argument seems to apply to *Philornis* parasites, which are transmitted via mobile adult flies that have the capacity to parasitise multiple nests.

Parasitic flies may be attracted to a group of hosts via visual or olfactory cues (Gibson and Torr 1999) and/or carbon dioxide emissions (Nicolas and Sillans 1989), which are more concentrated in larger host aggregations. From the parasites' perspective, an encounter with a high density group of hosts represents an efficient opportunity to deposit a maximal number

of offspring within a short time period (Mooring and Hart 1992). Though smaller inter-nest distances among colonially nesting birds have been associated with higher intensity of contact-transmitted chewing lice (*Meropoecus* and *Brueelia* spp.) (Hoi et al. 1998), analogous investigations involving mobile parasites are lacking, particularly in the absence of knowledge regarding parasite dispersal behaviour.

The spatial distribution of hosts is an important factor for the evolution of dispersal behaviour and host specialisation of parasites (Hoi et al. 1998; Tripet et al. 2002). Our data suggest that *P. downsi* females disperse short distances between nests and deposit larvae in multiple nests within close proximity, which may possibly act as a safeguard against stochastic effects between nests that may jeopardize parasite survival (e.g., nest predation). Alternatively, female flies may invest more offspring within individual nests that occur in areas of high host density to maximize offspring reproductive success upon dispersal. Such parasite dispersal behaviour may partly explain the increased total *P. downsi* intensity for finch nests within mixed species associations. Current investigation into the genetic relatedness between and among *P. downsi* offspring in nests may reveal patterns of fly oviposition behaviour and dispersal (Dudaniec et al. 2008a [Chapters 6 and 7]; Dudaniec et al. 2008b [Chapter 5]).

*Nest characteristics, host mass, and parasite intensity*

Host adult body mass varied with host nest size across species (Figure 4.3), and larger nests had increased parasite intensity. Is host body mass or nest size a stronger predictor for *P. downsi* intensity? Higher parasite intensity with increasing nest size in the small tree finch (Figure 4.5) suggests that nest size may limit the number of parasites that can be sustained, and may influence the successful growth and survival of *P. downsi* through within-host, density-dependent competition (Hughes et al. 2004). However, nest bottom thickness did not covary with *P. downsi* intensity. Host adult mass is a better predictor of ectoparasite intensity than nest size characteristics, possibly because larger finch species have larger nestlings that provide more resources for parasites (Poulin 1991; Poulin and George-Nascimento 2007).

The relationship between nest size, host mass, and parasite intensity is likely to be multi-faceted, as suggested by the high intensity found in nests of the light-bodied, small-nested warbler finch (Figure 4.4). The warbler finch had the second highest *P. downsi* intensity across six finch species studied on Santa Cruz Island, despite the observation that large finch species generally had more parasites (Dudaniec et al. 2007 [Chapter 3]). This may reflect

interspecific variation in nest-site characteristics and host behaviour that influence parasite susceptibility (Gold and Dahlsten 1989). The nesting biology of warbler finches is largely unstudied. Warbler finch nests occurred within all mixed nesting associations (Kleindorfer et al. in review), which we found is a significant predictor of increased *P. downsi* intensity. Anecdotal reports indicate that warbler finches may take over newly built nests of heterospecific finches (Lack 1947; S Kleindorfer personal observation), introducing additional variables. A focal nesting study of the warbler finch and fly egg laying behaviour is required to further examine the mechanisms driving the contrary patterns found within this species.

**Conclusion**

The findings of this study do not support the prediction that mobile, non-contact-transmitted ectoparasite intensity is comparable between group living or solitary hosts (Poulin 1991; Mooring and Hart 1992). We found higher *P. downsi* intensity in aggregated nests, and in nests of large hosts. Based on the known benefits of mixed species associations in reducing predation from owls and rats, the increased costs of nesting in aggregations as a result of *Philornis* parasitism appear to be roughly comparable (Kleindorfer et al. in review; Dudaniec et al. 2006). Nevertheless, limitations of statistical power in previous studies may be obscuring significant differences in the net costs and benefits of mixed species nesting as exemplified by alternative sources of predation. If the added costs of mixed species nesting, owing to increased depredation by *P. downsi*, prove to be greater than the benefits from reduced predation by owls and rats, then mixed species nesting would constitute an ecological trap (Kleindorfer et al. in review; Robertson and Hutto 2006). It is possible that such costs of parasitism will be substantially greater on smaller-bodied finches, as these smaller finches have smaller nestlings, which generally exhibit a greater number of parasites per gram of tissue (Dudaniec et al. 2007 [Chapter 3]). Relative to body mass, the warbler finch appears to be particularly vulnerable to *P. downsi*, exhibiting significantly higher levels of parasitism in this study compared with the small tree finch, which is 27% heavier (Table 4.1). Notably, the warbler finch has been pushed to the brink of extinction on one island of Galápagos, owing at least partially to depredation by *P. downsi* (Grant et al. 2005). Thus an informed response to the question of whether mixed species nesting associations present an ecological trap may have to be answered separately for each species of Darwin's finches. One should keep in mind, moreover, that even small differences in opposing predation trends may have major ecological and evolutionary consequences, as the cumulative effects of natural selection will

tend to magnify the consequences of such differences over multiple generations. Further research on Darwin's finches will be needed to provide a more precise answer to the question of whether mixed species nesting associations constitute an ecological trap – one that, if its existence is confirmed, would represent a serious challenge to the continued survival of this iconic group of birds.

**Acknowledgements**

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**CHAPTER 5:**

**Isolation, characterisation and multiplex polymerase chain reaction of novel microsatellite loci for the avian parasite *Philornis downsi* (Diptera: Muscidae).**

Rachael Y. Dudaniec, Michael G. Gardner, Sonia Kleindorfer

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**Abstract**

An enrichment technique was used to isolate 11 di, tri, and tetra microsatellites for the parasitic fly *Philornis downsi* (Diptera: Muscidae). These loci were polymerase chain reaction amplified in single-plexes or two-plexes for *P. downsi*. The loci showed low to moderate polymorphism, exhibited between three and four alleles, and observed heterozygosity ranged from 0.05 to 0.86. These new markers will be useful for population-level and paternity analyses and will provide valuable information about the ecology of this high-impact parasite of vulnerable bird species.

**Main Text**

The genus *Philornis* (comprising ~50 species) is a group of muscid flies whose larvae parasitise birds. *Philornis* fly larvae are known to infest at least 127 species of birds across a range of taxa (Dudaniec and Kleindorfer 2006 [Chapter 2]). The genus has a wide geographical distribution and occurs from South and Central America into the southern United States (Dudaniec and Kleindorfer 2006 [Chapter 2]). *Philornis downsi* is an introduced ectoparasite of Darwin's finches on the Galapágos Islands, Ecuador. Severe fitness costs to infested nestlings have been documented, including reduced nestling growth, lowered haemoglobin, and high mortality (Dudaniec et al. 2006 [Appendix 1]; Fessl et al. 2006). *Philornis downsi* is also known from collections made in Trinidad and Brazil, and has been reported to affect at least 26 species of birds (Dudaniec and Kleindorfer 2006 [Chapter 2]). Here, we report the first microsatellite loci to be developed for any species of *Philornis*. These loci will be useful for investigations in to the reproductive behaviour and population genetic diversity of this parasite.

Genomic DNA was extracted from 12 whole male *P. downsi* flies and 12 whole female flies (excluding the abdomen) preserved in 100 % ethanol using a LiCl technique (Gemmell and Akiyama 1996). The enrichment procedure used is detailed in Gardner et al. (2008) [Appendix 2]. To maximise microsatellite recovery, pooled DNA was twice enriched for AC, AAT, or AAAG microsatellite containing fragments. We screened (Gardner et al. 2008) 440, 100, or 208 separate insert positive clones for AC, AAT, and AAAG repeats respectively. Of these 44 (10 %), 7 (7 %), 25 (12 %) were putatively identified as microsatellite repeat positive clones and PCR amplified with vector primers (T7 promoter and M13 reverse) and sequenced. The presence of microsatellites with > 8 uninterrupted repeats were confirmed in 35 (80 %), 4 (57 %) and 10 (40 %) of these cases. Forward and reverse primers were designed for 47 unique microsatellite containing sequences. These primers were trialled in DNA from 10 unrelated *P. downsi* samples collected from two habitat zones (highlands/ lowlands) across three islands of the Galapágos (Santa Cruz, Floreana and Isabela). Amplification reaction mixtures and conditions followed Gardner et al. (2008) [Appendix 2]. A product of expected size was amplified in 28 of the putative loci for *P. downsi*. Primers were redesigned for 6 sequences where primers had failed to yield a product. Four of these (67 %) amplified successfully, resulting in a total of 32 putative loci. Eleven polymorphic loci were chosen based on repeatability of amplification and size of amplicon for multiplex PCR reactions and simultaneous capillary runs (Table 5.1).

**Table 5.1.** Characteristics of *Philornis downsi* microsatellite loci isolated in this study.

| Locus no. | Clone name | Gen-bank no. | Primer sequences (5'-3') | Clone repeat sequence | Size Range (base pairs) | K | N | H(O) | H(E) | PIC | Null |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pd1*[d] | P2AC24 | EF608562 | F: CATTGTCTACTTTCAGGCTG<br>R: CTCATTGTGATTCCATTGC | $(AC)_{11}$ | 315-327 | 4 | 38 | 0.684 | 0.631 | 0.564 | -0.0426 |
| *Pd2*[d] | P4ACX10b | EF608556 | F: GGGAGATGCCAAGCACTTAC<br>R: CGTTCTGGGTAACTCAGCC | $(AC)_{12}$ | 230-240 | 3 | 37 | 0.568 | 0.621 | 0.540 | 0.0443 |
| *Pd4*[c] | P4ACX14 | EF608557 | F: GCACCCATTTCATCAATG<br>R: CTTAGACGCCTGAAGAAGC | $(GT)_9$ | 252-258 | 4 | 38 | 0.579 | 0.685 | 0.612 | 0.0804 |
| *Pd5*[c]* | P1G3 | EF608560 | F: CCATTCACCAAAGTCTGTTCA<br>R: GGGTAAAAGCAGAAAGTCTCC | $(TTC)_7$ | 358-363 | 4 | 37 | 0.162 | 0.594 | 0.541 | 0.5604 |
| *Pd6*[a] | P2AC28 | EF608564 | F:GCTATATTTGGTCACTAACGAAG<br>R:CAGGATATGTTGTTGTAGAGTTG | $(AC)_{11}$ AA$(AC)_3$ | 241-263 | 4 | 38 | 0.868 | 0.744 | 0.685 | -0.0849 |
| *Pd7*[b] | P1AAT36 | EF608558 | F: GGCACATTTTAGCAATGC<br>R: CCACCCACACAAATTGAG | $(AAT)_2$ AAA$(AAT)_8$ AG $(AAT)_1$ | 201-210 | 3 | 38 | 0.474 | 0.568 | 0.469 | 0.0747 |
| *Pd8*[b] | P2G4 | EF608555 | F: GCCTGCTCTTCTGTTGAG<br>R: CCTTATTCACACATTACAATCTG | $(TTCA)_9$ | 349-365 | 4 | 38 | 0.658 | 0.619 | 0.563 | -0.0430 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pd9*[c] | P2G41b | EF608561 | F: GGCGTATAATAGATAAGGCTG<br>R: GAACCCATTTAGTAGGAATC | $(AAG)_{12}$ | 194-217 | 3 | 38 | 0.711 | 0.625 | 0.543 | -0.0837 |
| *Pd10*[a] | P2AC46b | EF608563 | F: GTCATTCACACAGTGACACG<br>R: CAACACCAAGCATAGGAAGG | $(AC)_{10}$ | 189-193 | 3 | 38 | 0.553 | 0.579 | 0.498 | 0.0252 |
| *Pd11*[a] | P1AAT47 | EF608559 | F: TGTTGTTATTATTATTATTA<br>R: CTTGATGATACGCTCCCAG | $(AAT)_9$<br>$ACA(AAT)_1$ | 70-111 | 3 | 36 | 0.056 | 0.055 | 0.054 | -0.0060 |
| *Pd18*[d] | P4ACX10 | EF608565 | F: CCTGGATGTGTGTGTGTG<br>R: CTCAGCCAAACTCAAAGTGC | $(AC)_9$ | 176-180 | 3 | 38 | 0.684 | 0.627 | 0.521 | -0.0548 |

Superscripts a, b, c and d indicates the forward primer of this locus was 5' labelled with 6-FAM, VIC, PET or NED respectively. ***** Locus not in Hardy Weinberg equilibrium. K = number of alleles; N = number of individuals typed; $H_O$= observed $H_E$ = expected heterozygosity; PIC = polymorphic information content; Null = estimated frequency of null alleles.

Multiplex Ready Technology (MRT) primers were used to obtain a common sequence on the amplicons. These primers allowed the simultaneous amplification of multiple loci with the same fluorescent dye incorporated into the amplicons (Hayden et al. in press). Five loci were amplified in single-plex reactions and six loci in three separate 2-plex reactions (Table 5.1). Amplifications were performed in 1X Multiplex-Ready Buffer, 75nM dye-labelled tagF primer, 75nM tagR primer, 40nM of primer, 0.15 U Immolase polymerase, and 10-50ng genomic DNA. A single reaction, 2-stage PCR profile was used to amplify all multiplex-ready markers. Initial denaturation was at 95 $^{\circ}$C for 10 min. The first PCR phase consisted of 5 cycles of 92 $^{\circ}$C for 1 min, 50 $^{\circ}$C for 90s, 72 $^{\circ}$C for 1 min, followed by 20 cycles of 92 $^{\circ}$C for 30s, 63 $^{\circ}$C for 90s, and 72 $^{\circ}$C for 1 min. The second PCR phase consisted of 40 cycles of 92 $^{\circ}$C for 15s, 54 $^{\circ}$C for 60s, 72 $^{\circ}$C for 1 min, with a final extension of 72 $^{\circ}$C for 10 min.

We established a reproducible microsatellite typing method with amplification of up to two loci in one PCR reaction followed by a simultaneous detection using capillary electrophoresis. The amplicons ranged from 60 to 380 base pairs in length (Table 5.1). Loci Pd7 and Pd8; loci Pd6 and Pd10; and loci Pd9 and Pd4 were coamplified using VIC, FAM or PET respectively. Amplicons from all the 8 PCR reactions (11 loci in total) were mixed in the ratio 3 VIC: 4 FAM: 2 PET: 4 NED and then desalted with AMPure magnetic bead cleanup and resuspended in 40ul of sterile water (Agencourt Bioscience Corporation). Electrophoresis was performed on an ABI3730 instrument (Applied Biosystems) by the Australian Genome Research Facility, Adelaide. Genotypes of 39 *P. downsi* individuals, collected from 20 independent bird nests from five sites on the island of Santa Cruz (0°37'S, 90°, 21'W) during January to March 2004, were analysed using Genemapper v.3.7 (Applied Biosystems).Allele frequencies, heterozygosity, polymorphic information content (PIC) and null allele estimates were calculated using CERVUS (Kalinowski et al. 2007). Analysis using GENEPOP (Raymond and Rousset 1995) revealed that none of the locus combinations exhibited significant linkage disequilibrium, whereas one locus (Pd5) deviated from Hardy-Weinberg expectations after sequential Bonferonni procedures were implemented (Hochberg 1988). These novel loci will provide a foundation for further research on this poorly studied Dipteran genus by allowing investigations in to reproductive ecology and population genetic structure. Such research will provide invaluable information for a conservation strategy to protect bird species threatened by *Philornis* parasites.

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**CHAPTER 6:**

**Genetic variation in the invasive avian parasite, *Philornis downsi* (Diptera, Muscidae) on the Galápagos archipelago.**

Rachael Y. Dudaniec, Michael G. Gardner, Steve Donnellan, Sonia Kleindorfer

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## Abstract

Understanding the dispersal and genetic structure of invasive insects across islands is important for designing management plans that are appropriate at spatial and temporal scales. For invasive parasites, population dynamics are largely determined by the distribution and density of their host species. The introduced parasitic fly, *Philornis downsi*, parasitises nestlings of endemic birds on all major islands of the Galápagos archipelago. The fly's high mortality and fitness impacts are of conservation concern for vulnerable and declining species of Darwin's finches. Using microsatellite data in Bayesian clustering and landscape genetic analyses, we examine gene flow and dispersal in *P. downsi* between three islands and across habitats (highlands, lowlands) and examine for the presence of population bottlenecks. We also examine variation at the mitochondrial gene CO1 across islands to establish if cryptic species were present. Both the mitochondrial and microsatellite data were consistent with there being a single species across islands. We found low genetic differentiation between islands and strong evidence for inter-island gene flow, or shared recent ancestry among individuals. Landscape genetic analysis identified two genetic clusters: one encompassing Santa Cruz and Isabela, and one on Floreana Island. There was no evidence of genetic differentiation between habitats and molecular variance was mainly attributable to within individuals. The combined P. downsi population was found to have undergone a population bottleneck. *Philornis downsi* populations have high connectivity within and between islands, with low levels of genetic differentiation between Floreana and the other two islands examined. The genetic bottleneck found across islands suggests there was a small founding population or few introduction events of *P. downsi*. The high dispersal capacity and wide habitat use of P. downsi highlights the significant threat that this parasite poses to the Galápagos avifauna. Our findings are relevant for assessing the viability of methods to control *P. downsi* on Galápagos, such as the sterile insect technique.

## Introduction

Biological invasions threaten biodiversity and ecosystem function, with pronounced negative effects on islands in particular (Benning et al. 2002; O'Dowd et al. 2003; Causton et al. 2005). Genetic studies of invasive species can identify the adaptive potential of invaders to deal with new environmental conditions (Azzurro et al. 2006) or help to predict evolutionary responses to management practices (e.g. pesticides, biological control agents) (Sakai et al. 2001). Population bottlenecks affect many invasive species because they frequently experience founding effects that reduce genetic variability, but paradoxically, invasive species still manage to successfully establish and adapt to new environments (Chen et al. 2006). However, the effects of bottlenecks may be countered by the occurrence of multiple introductions, high reproductive rates, and subsequent migration between locally bottlenecked populations that are genetically differentiated (Frankham 2005).

For invasive arthropod parasites, these factors are inextricably linked with the distribution, genetics, and behaviour of host species (Roderick 1996; McCoy et al. 2003; Criscione et al. 2005). The recent integration of molecular ecology with parasitology has provided a path for answering a number of questions concerning the genetic structure of parasite populations, which can uncover a wealth of information regarding ecological and evolutionary processes for invasive parasites (Criscione et al. 2005). Highly variable multilocus genotypes are particularly suited to analyses of non-equilibrium or bottlenecked populations because they provide adequate variation for assessing recent gene flow and identifying migrants (Davies et al. 1999).

The introduced fly, *Philornis downsi*, is an avian ectoparasite that is considered to be a serious threat to the persistence of endemic finch populations on the Galápagos Islands (Fessl and Tebbich 2002; Dudaniec and Kleindorfer 2006 [Chapter 2]; Fessl et al. 2006a). Recently, *P. downsi* was given the highest risk ranking affecting endemic fauna in the Galápagos archipelago (Causton et al. 2005). The impacts of other pathogens affecting Galápagos birds such as avian pox virus (Kleindorfer and Dudaniec 2006) and intestinal protozoans (Dudaniec et al. 2005) are of less concern, but may also cause high fitness impacts under certain conditions. The fly was first formally identified from Darwin finch nests in 1997 and has since been found on 11 of 13 major islands in nests of 14 endemic species (Fessl and Tebbich 2002; Dudaniec and Kleindorfer 2006 [Chapter 2]). However, *P. downsi* colonised the islands at least 40 years ago, as the fly was identified recently from collections made in 1964

(Dudaniec and Kleindorfer 2006 [Chapter 2]). The blood-feeding larvae of *P. downsi* are associated with 62-100 % nestling mortality in Darwin's finches (Fessl and Tebbich 2002; Fessl et al. 2006a,b), as well as physiological costs (Dudaniec et al. 2006) and reduced growth rates in nestlings (Fessl et al. 2006). Little is known about the ecology and biology of *Philornis* flies and the dispersal behaviour and population genetics of the genus *Philornis* or of any other myiasis-causing parasite of birds (reviewed in Dudaniec and Kleindorfer 2006 [Chapter 2]).

One potential control method to eradicate *P. downsi* is the sterile insect technique (SIT), which is renowned for its effectiveness at eradicating or suppressing fruit fly and screw-worm fly populations across the globe (Hendrichs et al. 2002; Vreyson et al. 2006). SIT involves the large-scale release of laboratory-reared sterile male (and/or female) flies that eventually suppress fly populations by reducing population fecundity [reviewed in 20]. SIT requires a thorough understanding of the reproductive ecology and population dynamics of the target species. The effectiveness of SIT is affected by the occurrence of genetically divergent 'strains' of the target species across the geographic area under control because this is detrimental to the mating success of sterile flies (Cayol et al. 2002; Hendrichs et al. 2002; Peireira et al. 2007). Specifically, high genetic divergence may reflect differences in behaviour and/or morphological characteristics that result in mating incompatibility among populations of the target species (McInnes et al. 1996; Cayol et al. 2002). Thus, target populations that show low genetic divergence are not likely to show reproductive isolation and influence the success of a particular sterile strain.

The Galápagos archipelago offers a unique system to examine the population genetics of an introduced avian parasite that causes severe fitness costs and that is still within a relatively early phase of invasion. We collected parasites in 2004, 2005 and 2006 from three islands of the Galápagos. Using mitochondrial data, we firstly determine whether the three island populations from which we sampled are of the one fly species. We then use microsatellite data to examine gene flow within and among islands to: (1) determine whether dispersal and genetic divergence are occurring among islands and between habitats within islands (wet highlands, arid lowlands), (2) determine the presence of population bottlenecks resulting from the invasion process, and (3) determine whether inter-island genetic differentiation may be of concern to the potential success of an archipelago-wide SIT program for controlling *P. downsi*.

## Methods

*Study species*

*Philornis downsi* (family Muscidae; subfamily Azeliinae; tribe Reinwardtiini) is a semi-haematophagous obligate avian parasite in its three larval stages, whereas adult flies are non-parasitic and feed on organic matter (Dudaniec and Kleindorfer 2006 [Chapter 2]). Adults lay eggs inside the nares of newly hatched nestlings (usually at one to three days old), which hatch into first instar larvae (Fessl et al. 2006b; Muth 2007). Second and third instar larvae attach externally and feed on nestling blood and tissues over four to six days (Dudaniec and Kleindorfer 2006 [Chapter 2]). Most larvae of *P. downsi* appear to reach their third instar phase at the time of host fledging. The larvae pupariate at the base of the nesting material and remain for approximately two weeks before emerging as adult flies (Teixeira 1999; Dudaniec and Kleindorfer 2006 [Chapter 2]).

*Study area and sample collection*

*Philornis downsi* were collected from three islands of the Galápagos: Santa Cruz (986 $km^2$; 0º37´S, 90º 21´W), Floreana (173 $km^2$, 1º28´S, 90º 48´W), and Isabela (4588 $km^2$, 0º58´S, 90º 58´W). Fly samples were collected from nests during the January to March finch breeding season in 2004, 2005 and 2006 from two contrasting habitats, the arid lowlands (0-100 m asl) and the humid highlands (300-600 m asl) (Table 6.1) (see also Kleindorfer et al. 2006; Kleindorfer 2007). The lowlands are characterised by low rainfall, and are dominated by the trees *Acacia macracantha*, *Bursera graveolens*, *Croton scouleri*, *Opuntia* spp., *Pisonia floribunda*, and *Zanthoxylum fagara* (Fessl and Tebbich 2002). In contrast, the highlands have much higher rainfall (Dudaniec et al. 2007 [Chapter 3]; Kleindorfer 2007), abundant moss and lichen, and are dominated by the endemic tree *Scalesia pedunculata*, or *S. cordata* (Asteraceae) on Isabela Island.

We sampled from one site in each habitat on both Floreana (lowlands, adjacent to the town of Puerto Velasco Ibarra: 1° 16´S, 90° 29´W; highlands, base of Cerro Pajas: 1° 17´S, 090° 27´W) (Figure 1) and Isabela (lowlands: adjacent to town of Puerto Villamil: 0º 57´S, 91º 00´W; highlands: 0º 50´S, 91º 01´W), while on Santa Cruz we sampled from three sites in the lowlands: (1) Garrapatero: 0º 39´S, 90º 28´W; (2) Itabaca: 0º 29´S, 90º 17´W; (3) Punta Estrada, near Puerto Ayora: 0 º 45´S, 90º 18´W, and one site in the highlands (Los Gemelos:

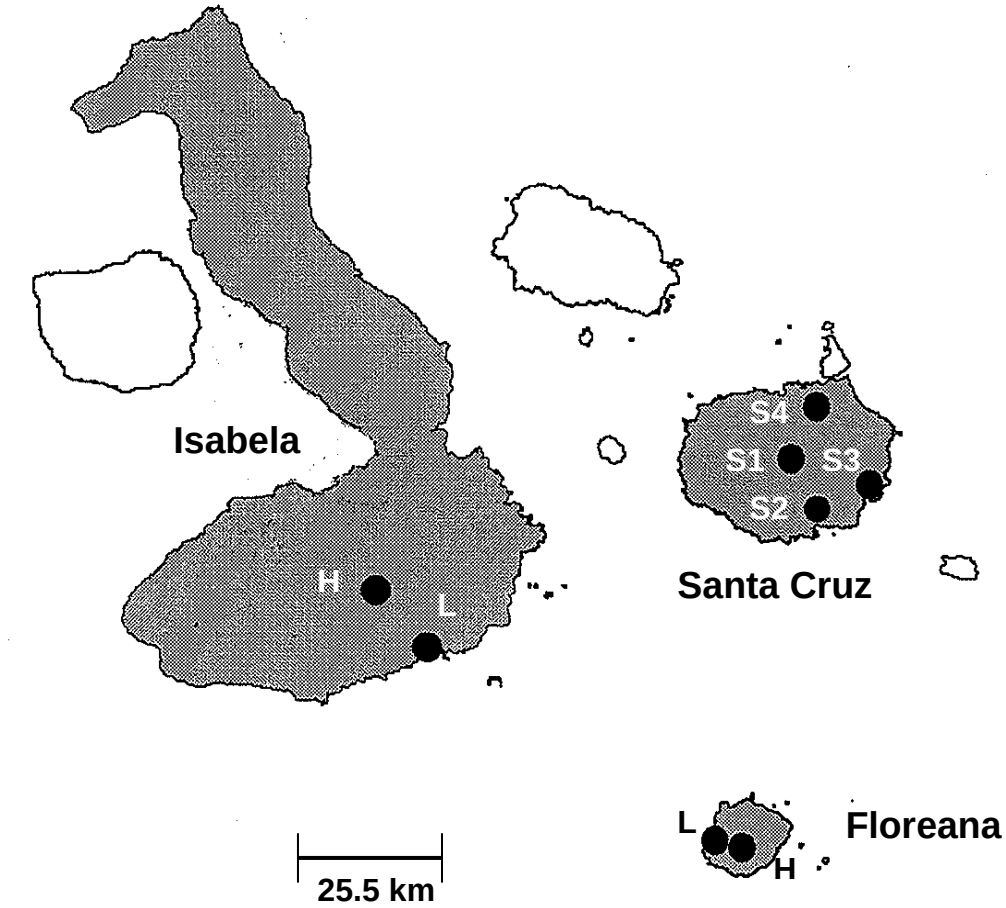



**Figure 6.1.** Map of Santa Cruz, Floreana, and Isabela Islands with sampling locations. Sampling sites on Santa Cruz: S1 = highlands; S2 = lowlands, Punta Estrada; S3 = lowlands, Garrapatero; S4 = lowlands, Itabaca. On Floreana and Isabela, one site each in the lowlands (L) and highlands (H) are indicated.

0º 37´S, 90º 22´W) (Figure 6.1). All sample sites were approximately 2000-4000 $m^2$, except for the highland site on Isabela, where our sample site was only 100 $m^2$ because habitat fragmentation has reduced the *Scalesia* forest to small remnant patches. The distance between highland and lowland sites was much shorter on Floreana (3-5 km) than on Santa Cruz and Isabela (both 15-25 km), while on Santa Cruz, the distance between all four sites (1 highland, 3 lowland) varied between 15 and 27 km. Data were obtained from all three islands in 2004, from just Floreana in 2005, and from Santa Cruz and Floreana in 2006 (Table 6.1).
For the purpose of our study, larvae, puparia and puparia cases were sampled from 64 bird nests of five Darwin finch species (*Geospiza fuliginosa*, n = 25, *Geospiza fortis*, n = 15, *Camarhynchus parvulus*, n = 3, *Camarhynchus pauper*, n = 4; *Cactospiza pallida*, n = 1), while one nest was opportunistically sampled from each of the Galápagos mockingbird (*Nesomimus parvulus*) and the yellow warbler (*Dendroica petechia aureola*). Fourteen recently fledged nests were sampled for *P. downsi* where the finch species was unknown. GPS coordinates were recorded at each nest location. Inactive nests were collected and sealed in individual plastic bags and later dismantled for counting of *P. downsi* individuals. All flies were immediately preserved in 95 % ethanol.

*DNA extraction and microsatellite typing*

DNA extraction was carried out using the salting out procedure described in Miller et al. (1988) with the exception that all samples (3 $mm^2$ tissue from each individual) were homogenised and washed three times in 10 mm TRIS prior to digestion with Proteinase K to remove traces of ethanol, excess lipids, and other potential contaminants. Across all three islands, 1012 *P. downsi* individuals (larvae and pupae) were genotyped (Table 1) using eight microsatellite markers (Dudaniec et al. 2008a [Chapter 5]): Pd1 [GenBank: EF608562] Pd2 [EF608556], Pd4 [EF608557], Pd6 [EF608564], Pd7 [EF608558], Pd8 [EF608555], Pd9 [EF608561], Pd10 [EF608563]. Multiplex PCR conditions were followed as described in Dudaniec et al. (2008) [Chapter 5]. Samples were genotyped on an ABI 3730 capillary electrophoresis DNA analyser (Applied Biosystems). A fluorescently labeled size standard (GS500 (-250) LIZ) was run with the samples and alleles were scored using GENEMAPPER version 3.7 (Applied Biosystems). To minimise and estimate genotyping error, each run of the DNA analyser contained eight repeated samples and a control sample run each time. In total, this resulted in 70 individual samples (14.5 % of all samples genotyped) being re-amplified and genotyped at least once.

**Table 6.1.** Sample sizes of bird nests and *P. downsi* individuals. The number of nests and the number of individuals analysed (following construction of dataset comprising unrelated individuals) for each island and habitat (highland/ lowland) across three islands, Santa Cruz, Floreana, and Isabela in 2004, 2005 and 2006.

| | | **# Bird nests sampled for *P. downsi*** | | **# Individuals analysed** | |
|---|---|---|---|---|---|
| **Year** | **Island** | **Highland** | **Lowland** | **Highland** | **Lowland** |
| **2004** | Santa Cruz | 3 | 18 | 11 | 51 |
| | Floreana | 1 | 4 | 1 | 9 |
| | Isabela | 2 | 1 | 7 | 2 |
| **2005** | Floreana | 11 | - | 28 | - |
| **2006** | Santa Cruz | - | 2 | - | 5 |
| | Floreana | 11 | 10 | 30 | 14 |
| | Total | 28 | 35 | 77 | 81 |

*Mitochondrial DNA sequencing*

An 822-bp region of the 3´ end of the *CO1* gene was amplified in five *P. downsi* individuals collected from Santa Cruz (1 highlands), Floreana (1 highlands, 1 lowlands), and Isabela (1 highlands, 1 lowlands). Samples were amplified using primers M202 (forwards, C1-J-1751; Simon et al. 1994) and M70 (reverse, UEA10; Lunt et al. 1996). Amplifications were performed in 10x *Taq*Gold buffer, 25 mM $MgCl_2$, 10 mM total dNTP's, 200 nM each primer, 0.2 U *Taq*Gold polymerase, and 10–50 ng DNA. Amplification conditions were an initial denaturation at 94°C for 9 min, followed by 34 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 1 min, with a final extension of 72°C for 6 min. Sequencing was performed using the ABI Prism™ Big Dye Terminator Cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. Products were sequenced on ABI 3700 (version 3.7) automated DNA sequencers. SeqEd (version 1.0.3) (Applied Biosystems) was used to edit chromatogram files to determine bi-directional consensus sequences and to manually align sequences across samples.

*Allele frequencies and data set construction*

We calculated allele frequencies using RELATEDNESS 5.0.8 (Queller and Goodnight 1989) by randomly selecting one individual per sample (n = 64) to eliminate the possibility of including related individuals (a sample is defined as all *P. downsi* individuals collected from a single bird's nest). Exact tests were performed for each microsatellite locus to test deviation from Hardy-Weinberg equilibrium using GENEPOP (Raymond and Rousset 1995). All loci were in Hardy Weinberg equilibrium after sequential Bonferroni correction (Hochberg 1988) and these allele frequencies were used for all further analyses. Genetic relatedness among *P. downsi* offspring within nests of Darwin's finches is low, and the individuals found within each nest are produced by up to approximately five ovipositing females that have each mated with between one and five males (as found by sib-ship reconstruction analysis by Dudaniec et al. in review [Chapter 7]). To eliminate the effect of sibs in the data, we selected unrelated individuals that were identified using the sib-ship reconstruction method implemented in the program COLONY 1.2 (Wang 2004). Each sample of *P. downsi* individuals taken from an independent bird nest was run in COLONY 1.2, which uses a maximum likelihood method that partitions individuals into pure full-sib families (i.e. monogamous female parent), or full-sib families nested within half-sib families (i.e. polyandrous female parent) using progeny genotypes without known parental genotypes (Chapman et al. 2003; Wang 2004). Three runs were performed per sample with different random seed numbers (12, 80, and 243) to ensure

data convergence, and a conservative error rate of 5 % was implemented based on evidence from the re-genotyping of 70 individuals, in which genotyping error ranged from 0-5 % across loci.

We selected one individual per reconstructed maternal family (i.e. one family = the offspring assigned to one putative female parent). In nested-half sib families (i.e. one mother, multiple fathers), individuals were only selected from full sib families with the largest number of members that had the highest posterior probability. Only individuals genotyped at all eight loci were included in the analysis and individuals were not sampled from families that contained Class I or Class II typing errors (identified by COLONY 1.2) (Wang et al. 2004). These criteria resulted in a sample size of 158 individuals sampled from 63 bird nests (with between one and six unrelated individuals per sample) (Table 6.1). To examine the probability that two randomly selected individuals from the same population will have the same multi-locus genotype, a Probability of Identity (PI) analysis was performed using GIMLET (Valière 2002). The output is a cumulative multi-locus PI value, estimated both with and without sample size correction. PI values were calculated for the dataset of 158 individuals using equations of unbiased PI, which assumes that individuals are unrelated, and PI for sibs, which assumes that all individuals are siblings (Valière 2002).

*Inter-island genetic differentiation*

Heterozygosity, and pairwise *F*st (Weir and Cockerham 1984) was calculated to examine genetic differentiation between islands (Santa Cruz, Floreana, Isabela) using MICROSATELLITE ANALYSER (MSA) 4.05 (Dieringer and Schlötterer 2003). Genotypic differentiation was tested between islands using option 3 with 10 0000 Markov chain iterations in GENEPOP (Raymond and Rousset 1995). P-values for multiple tests were adjusted using sequential Bonferroni correction (Hochberg 1988). The AMOVA method (Excoffier et al. 1992) was conducted in GENALEX version 6 (Peakall and Smouse 2006) to partition the total genetic variation into three levels: among islands, among individuals, and within individuals using the Codom-genotypic distance calculation and 9999 permutations.

*Population bottleneck analysis*

Recently colonised species may experience a population bottleneck, resulting in a reduction in the number of alleles and expected heterozygosity at polymorphic loci. However, alleles may be lost at a faster rate than the loss of heterozygosity, so observed heterozygosity is higher

than the expected heterozygosity at equilibrium (Luikart and Cornuet 1998). The program BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996) was used to test for the presence of a recent population bottleneck for *P. downsi* by analysing within-population heterozygosity and allele frequency using the constructed dataset of individuals sampled from all islands. Both the stepwise mutation model (SMM) and two-phase model of mutation (TPM) were used, with the latter model being considered the most appropriate for microsatellites. The variance for the TPM was set at 5 % and the proportion of SMM in TPM was set at 95 % (Piry et al. 1999). To determine differences in gene diversity across loci, the Wilcoxin sign-rank test was used as recommended for data sets with less than 20 loci (with 10 000 permutations) (Cornuet and Luikart 1996). We also examined the allele frequency distribution in order to see whether it is approximately L-shaped (as expected under mutation-drift equilibrium) or not (indicating that a recent bottleneck has provoked a mode shift) as described in (Luikart et al. 1998).

*Genetic structure among islands*

For inferring genetic structure among the three sampled islands, we conducted two complementary individual-based Bayesian clustering analyses using STRUCTURE 2.1 (Pritchard et al. 2000) and the landscape genetics program GENELAND (Guillot et al. 2005) without *a priori* knowledge of population units and limits. Both software packages implement a Bayesian clustering method that uses a MCMC technique to define the number of populations in a sample that are at Hardy Weinberg Equilibrium. The methods implemented in these two programs differ in that GENELAND determines the optimal number of populations or 'clusters' and then allocates individuals (probabilistically) to these clusters using geographic coordinates, whereas STRUCTURE carries out the allocation sequentially for different numbers of clusters, and then flags the number of clusters with the highest likelihood (Excoffier and Heckel 2006). In STRUCTURE, the following run parameters were used: admixture without population information used, correlated allele frequency model, a burn-in period of 100 000 simulations followed by a run length of 1 million Markov Chain Monte Carlo (MCMC) simulations and three iterations for each number of potential clusters (defined as $k$ = 1-5) to check for consistency of results. Estimation of $k$ was taken to be the values of $k$ with the highest Pr (X|$k$).

In contrast to STRUCTURE, the algorithm implemented in GENELAND (Guillot et al. 2005) is considered to be a powerful clustering method under conditions of low genetic differentiation among populations (Berry et al. 2004; Coulon et al. 2006). The model infers genetic

discontinuities between populations in space from multilocus genotypes obtained from geo-referenced individuals (Guillot et al. 2005; Fontaine et al. 2007). All individuals from the same sample (i.e. same bird nest) were allocated the same GPS coordinates. GPS coordinates were available for 57/63 nests (Santa Cruz: n = 18; Floreana: n = 36; Isabela: n = 3) (138 individuals in total). Samples for which GPS coordinates were missing were excluded from the analysis. To firstly infer the number of genetic clusters (*k*) in our data set, we used the Dirichlet model, which assumes independent allele frequencies with the following parameters: 1000 000 MCMC iterations, uncertainty attached to spatial coordinates = 0, variable number of populations = TRUE, minimum $k$ = 1, maximum $k$ = 5, and spatial information included in the model = TRUE. This procedure was performed three times to establish consistency of $k$ across runs. The established $k$ was then run five times to check the consistency of individual assignment to the inferred populations across runs. The same parameters were used but $k$ was fixed at the modal number found in the first analyses. These five runs were post-processed (with a burn-in of 1000 x 100 iterations) to obtain posterior probabilities of population membership for each individual. Consistency of results across the five runs was checked visually. Inferred populations were further examined for heterozygosity, allelic richness (corrected for sample size), observed ($H_o$) and expected ($H_e$) heterozygosity, inbreeding coefficients (*F*is), and genetic differentiation (estimated using *F*st) using FSTAT v. 2.9.3 (Goudet 2001).

## Results

*Mitochondrial sequencing*

Sequences of the *CO1* mitochondrial gene fragment in five individuals across islands showed almost no variation, with two individuals (one Santa Cruz highland and one Floreana highland) having an identical single nucleotide substitution (T-G). This supports the existence of one sampled species across the three islands.

*Genetic diversity and differentiation*

Probability of identity (PI) analyses showed that the microsatellite loci had sufficient power and resolution for the analyses. The unbiased PI value was $1.333^{-06}$, and the PI for sibs was $2.610^{-3}$. This equates to one individual in approximately 751 880 having a non-unique genotype where individuals are unrelated (unbiased), and one individual in approximately 383 individuals having a non-unique genotype if all individuals are siblings.

The total number of alleles observed at each locus was as follows; Pd1 = 4; Pd2 = 3; Pd4 = 4; Pd6 = 5; Pd7 = 3; Pd8 = 4; Pd9 = 3; Pd10 = 3 (Table 6.2). There was significant genotypic differentiation across the three islands (Fisher's Exact method: $X^2$ = 72.75; df = 16; P < 0.001). Mean allelic richness across loci was almost identical on each island (Santa Cruz: 3.50; Floreana 3.63; Isabela: 3.5) and the range of observed heterozygosity across loci was also similar (Santa Cruz: 0.45-0.70; Floreana: 0.45-0.73; Isabela: 0.44-0.89). The number and size of alleles from each island population were the same at each locus with two exceptions: there was a unique allele at locus Pd6 on Isabela (allele frequency = 0.055), and at locus Pd7 on Floreana (allele frequency = 0.012), which were each detected only in a single individual. Pairwise *F*st analysis showed low, but significant levels of genetic differentiation between Santa Cruz and Floreana (*F*st = 0.02, P < 0.02) Isabela and Floreana (*F*st = 0.04, P < 0.02), but not between Santa Cruz and Isabela (*F*st = 0.01, P > 0.1). The low genetic differentiation found between islands was reflected in an AMOVA, which showed that just 2 % of the molecular variance was attributable to variation among islands (sum of squares (SS) = 14.23; df = 2; variance components (V) = 0.052), 4 % among individuals (SS = 413.06; df = 155; V = 0.133), and 94 % within individuals (SS = 385.5; df = 158; V = 2.44).

*Bottleneck analysis*

Combining individual from all islands (n = 158), a clear excess of heterozygosity (He) relative to the equilibrium heterozygosity ($H_{eq}$) was observed, indicative of a population bottleneck under the TPM model (Wilcoxon sign-rank test; P < 0.01 ) and under the SMM model (P < 0.01). A mode-shift distortion in the distribution of allele frequencies was evident (Figure 6.2).

*Bayesian clustering analysis*

Individual-based cluster analysis using STRUCTURE (Pritchard et al. 2000) did not detect any genetic structuring in *P. downsi* collected across the three islands (Figure 6.3a), with individual assignment being evenly proportioned across variable numbers of *k*. This implies high levels of inter-island ancestry brought about by frequent dispersal and subsequent gene flow across the three islands sampled. However, when incorporating geographic coordinates of sampling locations into Bayesian analyses using GENELAND (Guillot et al. 2005), two distinct genetic clusters were consistently found across runs (Figures 6.3b and 6.4). The first cluster includes all individuals sampled from Santa Cruz and Isabela Islands (n = 62), while the second cluster includes all individuals sampled from Floreana Island (n = 76).

**Table 6.2.** Allele frequencies for eight microsatellite loci in *P. downsi* within two genetic clusters. Clusters were inferred using landscape genetic analysis: Cluster 1 = Santa Cruz and Isabela Island (n = 62); Cluster 2 = Floreana Island (n = 76); na = not applicable because allele was absent.

| | | **Santa Cruz and Isabela** | **Floreana** |
|---|---|---|---|
| *Pd1* | 315 | 0.319588 | 0.219512 |
| | 323 | 0.134021 | 0.256098 |
| | 325 | 0.154639 | 0.109756 |
| | 327 | 0.391753 | 0.414634 |
| *Pd2* | 230 | 0.273196 | 0.268293 |
| | 236 | 0.582474 | 0.439024 |
| | 240 | 0.14433 | 0.292683 |
| *Pd4* | 252 | 0.35567 | 0.304878 |
| | 254 | 0.298969 | 0.353659 |
| | 256 | 0.036082 | 0.02439 |
| | 258 | 0.309278 | 0.317073 |
| *Pd6* | 241 | 0.391753 | 0.390244 |
| | 251 | 0.221649 | 0.280488 |
| | 259 | na | 0.012195 |
| | 261 | 0.139175 | 0.109756 |
| | 263 | 0.247423 | 0.207317 |
| *Pd7* | 201 | 0.371134 | 0.439024 |
| | 207 | 0.27835 | 0.268293 |
| | 210 | 0.340206 | 0.292683 |
| | 213 | 0.010309 | na |
| *Pd8* | 349 | 0.139175 | 0.195122 |
| | 353 | 0.087629 | 0.04878 |
| | 357 | 0.134021 | 0.097561 |
| | 365 | 0.639175 | 0.658537 |
| *Pd9* | 194 | 0.324742 | 0.414634 |
| | 200 | 0.469072 | 0.45122 |
| | 217 | 0.206186 | 0.134146 |
| *Pd10* | 189 | 0.216495 | 0.329268 |
| | 191 | 0.231959 | 0.134146 |
| | 193 | 0.551546 | 0.536585 |

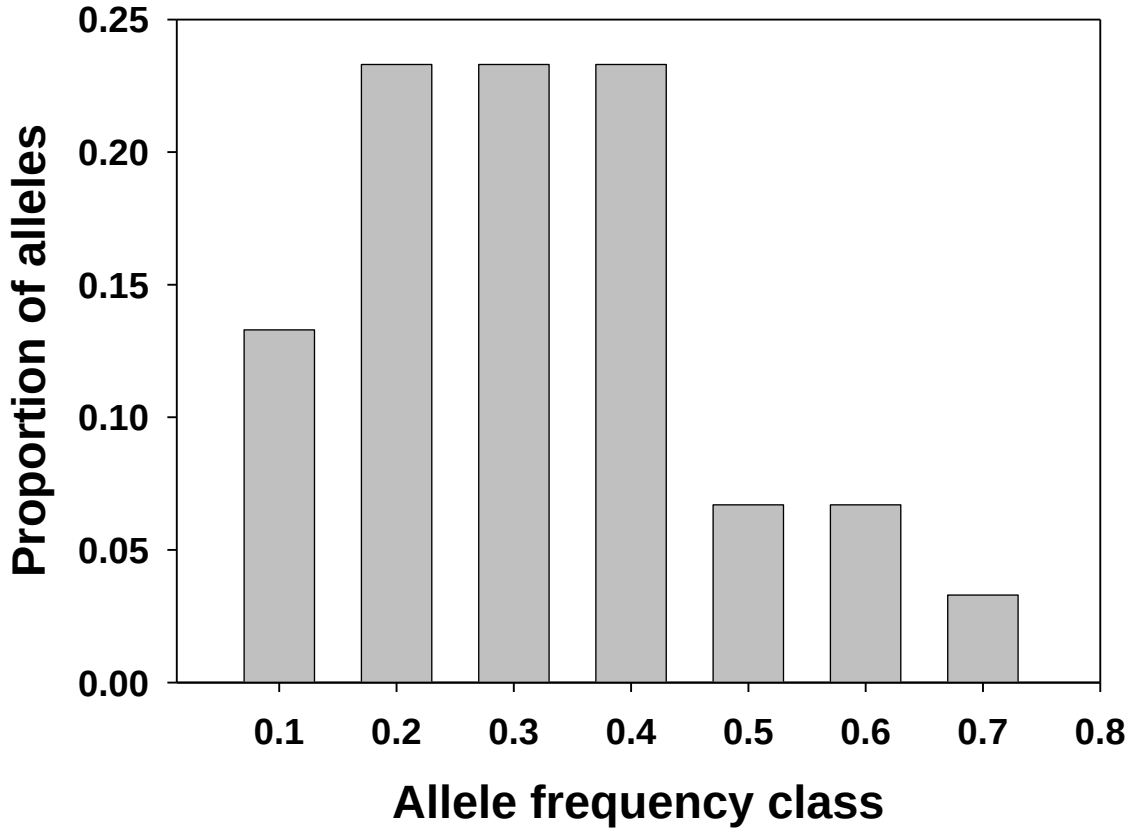
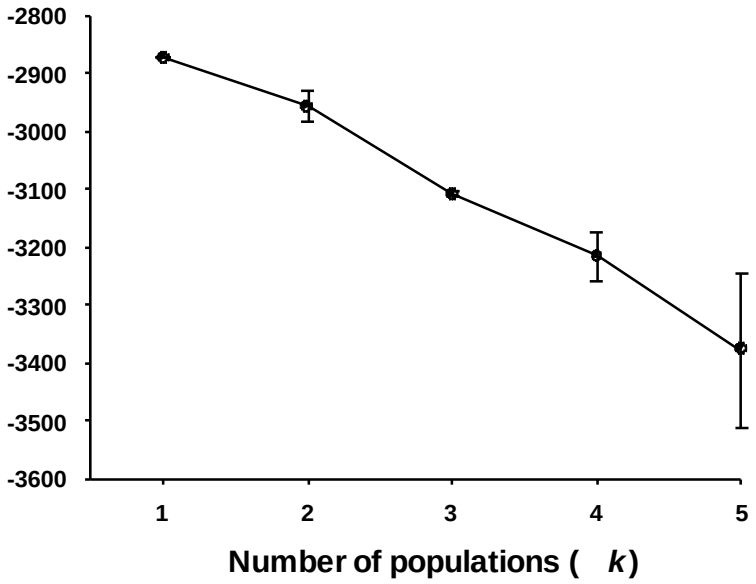
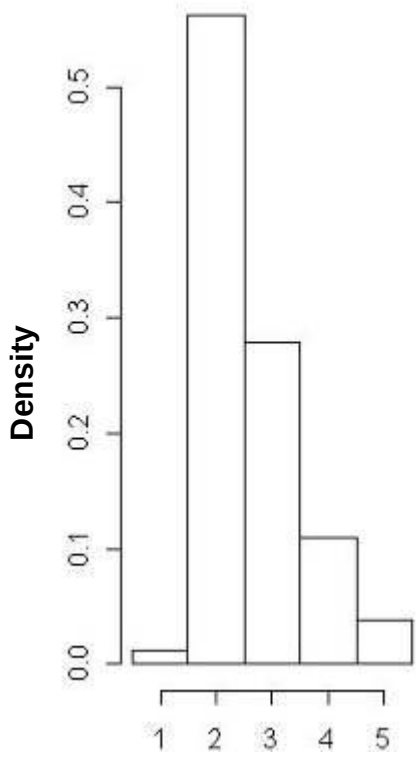



**Figure 6.2.** Distribution of *P. downsi* allele frequencies across islands, indicating a mode-shift. Bars represent the proportion of alleles found in each allele frequency class. Deviation from an L-shaped distribution is indicative of a mode-shift in allele frequency due to a recent genetic bottleneck.

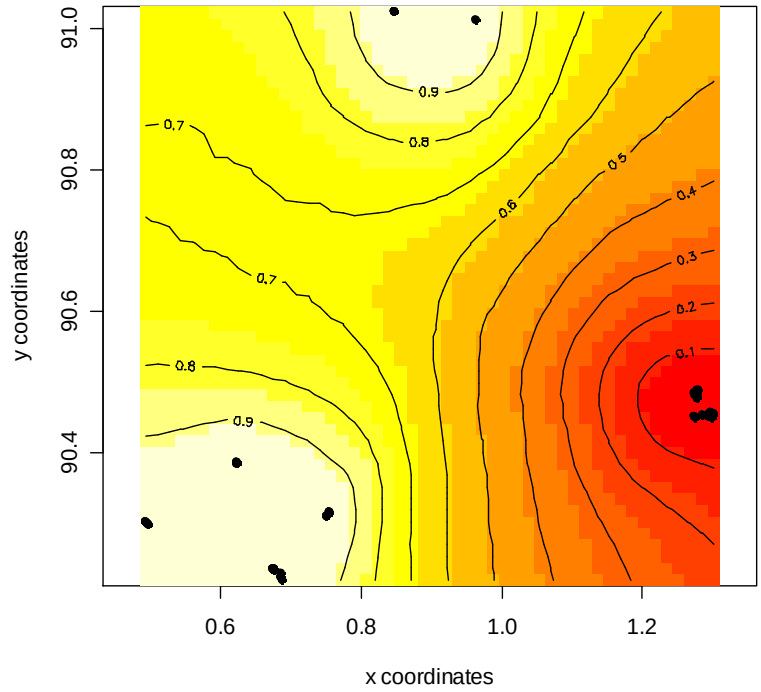



a)

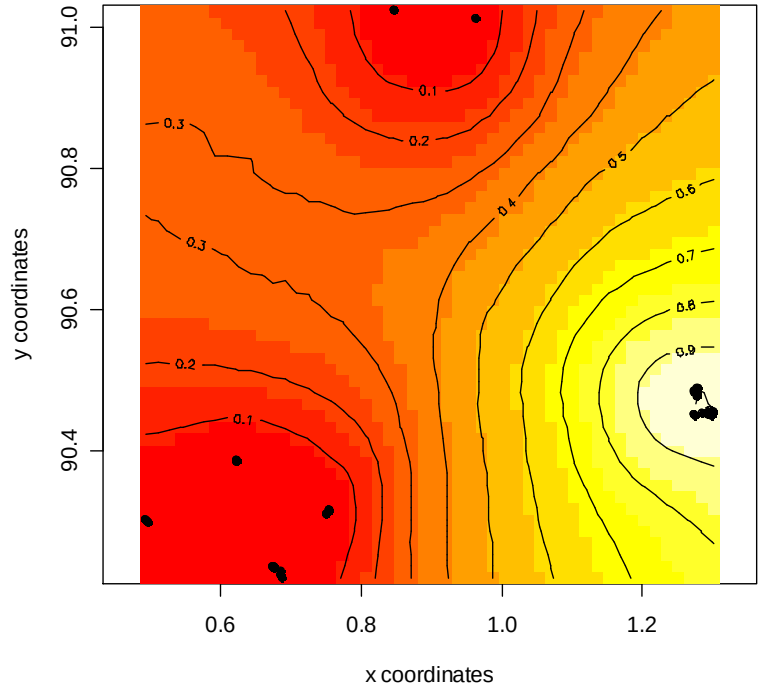



b)

**Figure 6.3.** Estimated number of populations from STRUCTURE (a) and GENELAND (b) analyses. STRUCTURE analyses: (a) mean (±SD) probabilities of the data (LnPr[X| *k*]) over three replicate STRUCTURE runs plotted as a function of the putative number of clusters (*k*); (b) Posterior density distribution of the number of clusters estimated from GENELAND analysis in three replicates.




a)




b)

**Figure 6.4.** Genetic assignment of *P. downsi* individuals across three islands using Bayesian clustering analysis. Two genetic clusters are identified: (a) including all individuals from Santa Cruz (n = 62) (bottom left) and Isabela (n = 9) (centre top), and (b) all individuals from Floreana Island (n = 76) (bottom right). Black dots represent independent geographic sampling points (i.e. location of bird nests). Note that two geographic sampling points on Isabela Island were within 5m of each other and are not distinguishable. Assignment probabilities were between 0.98 and 1.0 across all individuals.

*Genetic diversity and differentiation among inferred clusters*

The two clusters identified by GENELAND displayed comparable genetic diversity with regard to allelic richness and differed slightly in heterozygosity across loci (Tables 6.2 and 6.3). Although two clusters were detected, measures of genetic differentiation (*F*st) between them demonstrated the low divergence between individuals on Floreana Island and those on Santa Cruz and Isabela (*F*st = 0.024; 95% Confidence Interval (CI) = 0.014 - 0.034; P < 0.05). Tests of departure from HW equilibrium showed no significant deviation in either of the two clusters across all loci.

## Discussion

In combination with the microsatellite data, our mitochondrial findings are consistent with there being one species of *Philornis* on the islands from which we sampled. A population bottleneck was detected in the entire sample of individuals from the three islands, which is consistent with the pattern expected from an invasive, recently colonised species (Sakai et al. 2001; Azzurro et al. 2006; Chen et al. 2006). We report low genetic differentiation between island populations of the invasive avian parasite *P. downsi* on the Galápagos archipelago. Fly populations on Santa Cruz, Floreana, and Isabela showed strong evidence for high inter-island gene flow. However, low levels of divergence were detected between individuals from Floreana Island and those from Santa Cruz and Isabela when incorporating geographic sampling information. The molecular variance was mainly explained at the level of individuals, and not by island, which further demonstrates the low genetic differentiation between islands. Bayesian clustering analysis with geographic data assigned individuals to two genetic clusters; one comprises individuals from Santa Cruz and Isabela, and the second comprises all individuals from Floreana Island (Table 6.3, Figure 6.4). This might indicate that gene flow in *P. downsi* between Floreana and the other islands is restricted to some extent, or that this island underwent a distinct founding process. Pairwise *F*st between the three islands further indicated that flies on Floreana may be slightly genetically divergent from flies on the other two islands.

**Table 6.3.** Genetic variation at eight microsatellite loci for two genetically distinct *P. downsi* clusters identified in GENELAND. Sample size is 62 for the Santa Cruz and Isabela cluster and 76 for the Floreana cluster for all loci. A: allelic richness (estimated for a sample size of 75 individuals); $H_o$:observed heterozygosity; $H_e$: expected heterozygosity; *F*is (inbreeding coefficients) were calculated after Weir and Cockerham (1984). None of the loci had a significant heterozygote deficiency or excess after sequential Bonferroni correction.

| | **Santa Cruz and Isabela** | | | **Floreana** | | |
|---|---|---|---|---|---|---|
| **Locus** | **A** | **$H_o$/$H_e$** | ***F*is** | **A** | **Ho/He** | ***F*is** |
| **Pd1** | 4.0 | 0.63/0.71 | 0.038 | 4.0 | 0.71/0.71 | 0.091 |
| **Pd2** | 3.0 | 0.46/0.57 | 0.114 | 3.0 | 0.68/0.66 | 0.091 |
| **Pd4** | 4.0 | 0.67/0.69 | 0.105 | 3.0 | 0.64/0.67 | 0.037 |
| **Pd6** | 5.0 | 0.68/0.72 | 0.090 | 4.0 | 0.73/0.72 | -0.012 |
| **Pd7** | 3.0 | 0.56/0.67 | 0.131 | 3.0 | 0.73/0.66 | 0.037 |
| **Pd8** | 4.0 | 0.48/0.55 | 0.023 | 4.0 | 0.61/0.52 | 0.034 |
| **Pd9** | 3.0 | 0.67/0.64 | -0.083 | 3.0 | 0.68/0.61 | -0.094 |
| **Pd10** | 3.0 | 0.57/0.61 | 0.186 | 3.0 | 0.68/0.59 | -0.154 |
| **Mulitlocus** | 3.6 | 0.59/0.65 | 0.074 | 3.6 | 0.67/0.65 | 0.005 |

The Bayesian clustering method implemented in STRUCTURE is considered to be best able to infer correct individual assignments when genetic differentiation between populations is well defined (Pritchard et al. 2000). Furthermore, the ability to distinguish the source of an individual decreases under conditions of high dispersal and associated low genetic differentiation (Cornuet et al. 1999; Berry 2004). The level of genetic differentiation (*F*st) between populations is found to be a useful predictor of the performance of assignment methods (Cornuet et al. 1999). In the current study, the inability of STRUCTURE to confidently assign individuals to any cluster with certainty may reflect the lack of power to do so due to the low genetic differentiation (i.e. *F*st) between sampling locations. Thus, we conclude there was an insufficient signal in the data to confidently assign individuals under the model of Pritchard et al. (2000), despite reasonably high PI values across loci. Our results are therefore testament that taking the spatial context of individuals into account improved the efficiency of our analysis, as found by Fontaine et al. (2007). Verifying the usefulness of STRUCTURE to assign individuals correctly where genetic differentiation is low and dispersal is common requires further study using empirical field data (Cornuet et al. 1999; Berry 2004).

The current study lacks genetic data from mainland *P. downsi* populations and data from all islands of the Galápagos where *P. downsi* occurs, which will be necessary for a detailed examination of founder effects, bottlenecks, introduction events and colonisation pathways. Thus, without knowing where *P. downsi* populations originally came from, or where they most recently arrived on the Galápagos archipelago, a comprehensive invasion history can not be constructed on a demographic or evolutionary scale (Davies et al. 1999; Whiteman et al. 2007). However, our findings lay the foundation for a more thorough understanding of the process of *P. downsi* invasion on the Galápagos archipelago. It is possible that *P. downsi* arrived on Ecuadorian cargo ships that were transporting fruit to the islands for human consumption (Silberglied 1978; Roque-Albelo and Causton 1999) , while it is also suggested that the fly came with imported pigeons (discussed in Wiedenfeld et al. 2007). Strong winds and air currents present during El Niño events on the Galápagos are believed to contribute to insect dispersal between islands (Peck 1994), while transport of humans and materials is also suspected to aid inter-island insect dispersal. In four other invasive insect species, the dates of colonisation on each island suggest a wind-mediated southeast to northwest direction of colonisation across the islands (Roque-Albelo and Causton 1999). Such patterns remain unexplored for *P. downsi*.

*Invasion processes*

Recently colonised invaders are often subject to a reduction in genetic variation and population bottlenecks because populations are not in genetic equilibrium (Sakai et al. 2001; Azzurro et al. 2006; Chen et al. 2006). We provide evidence for a population bottleneck in *P. downsi* across the three islands examined, which could be due to a small founding population, low immigration rates, or few introduction events (Lambrinos 2004; Chen et al. 2006). The low allelic diversity across loci and population bottleneck in *P. downsi* is further evidence for a small effective population size upon initial colonisation. However, the occurrence of multiple introductions can not be excluded, particularly in the absence of comparisons with potential source populations (e.g. from Ecuador, Trinidad, or Brazil) (Dudaniec and Kleindorfer 2006 [Chapter 2]). Despite the presence of a population bottleneck and the (most likely related) low genetic diversity in *P. downsi*, the fly has clearly succeeded at establishing and spreading itself across the archipelago in high numbers.

Recently established species may persist at low and possibly undetectable numbers before becoming noticeably abundant and invasive years or decades later (Sakai et al. 2001), which may reflect the lag time (i.e. the time between arrival and spread) observed in many species that become invasive (García-Ramos and Rodríguez 2002). This scenario seems likely concerning the invasion of *P. downsi* on Galápagos because the fly was not detected in finch nests and identified until 1997 (Fessl et al. 2001), despite the recent discovery of specimens found in collections made in 1964 (Dudaniec and Kleindorfer 2006 [ Chapter 2]; Fess et al. 2006b). The parasite has since spread successfully and in high numbers across the archipelago (11 of 13 major islands) (Wiedenfeld et al. 2007), indicating that any lag period that took place has passed. Yet it is unknown how recently each island was colonised and thus, whether particular island populations are undergoing a lag period that would favour the success of an immediate eradication effort (discussed in Allendorf and Lundquist 2003).

Ecological (Fessl and Tebbich 2002; Dudaniec et al. 2007 [Chapter 3]; Wiedenfeld et al. 2007) findings do not support the current existence of a lag period and indicate that *P. downsi* has spread successfully in at least 12 avian host species on the Galápagos Islands (Fessl and Tebbich 2002; Dudaniec and Kleindorfer 2006 [Chapter 2]). In the current study, we provide evidence that the *P. downsi* population on Floreana Island has detectable levels of genetic differentiation when compared with two other island populations, which might be the result of a separate introduction event(s) or colonisation pattern. A wider geographic sample of

locations across habitats and islands is needed to examine this more definitively in combination with a larger number of highly polymorphic genetic markers. However, it is clear that *P. downsi* populations generally have high connectivity between islands or high shared ancestry, although variation in population processes (e.g. rates of dispersal, colonisation histories) between particular islands may allow for low levels of inter-island genetic differentiation.

*Absence of local genetic divergence*

Local populations are expected to evolve adaptive differences in response to differing environmental conditions (Allendorf and Lundquist 2003).The lack of genetic structure in *P. downsi* on the Galápagos archipelago may reflect the estimated short time period since the flies' introduction (~40 years ago) (Causton et al. 2005) such that populations have not yet diverged since colonisation. We document no genetic structure according to habitat type across islands, which implies high levels of fly dispersal between the two habitats. Across islands however, differences in host diversity and distribution, ecological variables, or colonisation history may result in genetic divergence due to genetic drift, as was evident from the low genetic differentiation we document on Floreana Island.

Fly populations may show rapid evolution with geographic cline, as shown by Huey et al. (2000) who found increased wing length with latitude in *Drosophila subobscura*, just two decades after its introduction into North America. The evidence we present for high gene flow between habitats implies that morphological variation in *P. downsi* is unlikely, though other insect species on Galápagos show morphological variation and genetic differentiation between habitats and islands of the archipelago (Finston and Peck 1995; Whiteman et al. 2006; Whiteman et al. 2007). Clinal variation in morphology (and evidence for low dispersal) was also found for *Bulimilus* land snails on Galápagos (Parent and Crespi 2006) and Darwin's small ground finch (Kleindorfer et al. 2006).

*Implications for control: the sterile insect technique (SIT)*

The use of SIT to control *P. downsi* on the Galápagos Islands is perhaps the most appropriate method for eradicating an invasive fly within this ecologically fragile island ecosystem. SIT is a non-disruptive method as it does not introduce toxic or foreign chemicals into the environment, it is species specific, and does not introduce new genetic material into

populations because the released organisms are not self-replicating (Krafsur 1998; Hendrichs et al. 2002).

The effectiveness of SIT is affected by population genetic differentiation within the target species because the occurrence of undetected sub-species or strain differentiation across geographic populations can be detrimental to widespread sterile male release (Hendrichs et al. 2002). Reinfestation of parasitic flies in SIT treated regions have been explained by genetic differentiation in the target species among allopatrically separated populations that may be experiencing reproductive isolation (e.g. Sunnucks et al. 1997). It is therefore of great advantage to use molecular genetic techniques for species characterisation and to examine population genetic structure prior to establishing large-scale sterile male release programs. We show that gene flow in *P. downsi* within and between three islands of the Galápagos is high, and unlikely to result in reproductive isolation. Thus, release of a single sterile strain of *P. downsi* could effectively suppress and eradicate the fly across the archipelago. Captive breeding experiments of adult *P. downsi* from multiple island populations are necessary to determine this with high confidence.

**Conclusion**

The wide habitat range and high dispersal capacity of *P. downsi* highlights the significant threat that this parasite poses to the Galápagos endemic avifauna. Our findings are concordant with the prediction that parasites with low host specificity (Dudaniec et al. 2007 [Chapter 3]), good dispersal ability and horizontal transmission will show low population genetic structure and low population differentiation (Whiteman et al. 2007). Ideally, it is best to eradicate invasive species before they become adapted to the local environment in which they have colonised and prior to repeated invasions, with the aid of strict quarantine practices (Frankham 2005). For *P. downsi*, this window of opportunity appears to have passed, prompting the need for a long-term eradication program combined with sustained quarantine and monitoring practices.

**Acknowledgements**

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**CHAPTER 7:**

**Microsatellite analysis reveals multiple infestations and female multiple mating in an invasive parasitic fly of Galápagos birds.**

Rachael Y. Dudaniec, Michael G. Gardner, Sonia Kleindorfer

*Biological Invasions* (submitted)

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**Abstract**

The natural reproductive behaviour of invasive insect parasites is difficult to examine using standard ecological or laboratory methods, yet is pivotal knowledge for managing and controlling species of ecological or economic concern. Using nine microsatellite loci, we examine female multiple mating and the occurrence of multiple nest infestations in the introduced fly, *Philornis downsi*, which parasitises nestlings of Darwin's finches, and other endemic birds on the Galápagos Islands. *Philornis downsi* is considered a major threat to the persistence of vulnerable Galápagos bird populations. We analyse fly larvae and pupae within 57 nests collected over three years (2004-2006) on Santa Cruz and Floreana Islands in both the highland and lowland habitats. Using a maximum likelihood method for sib-ship reconstruction of offspring without parental genotypes, we found that a single nest may be infested up to five times during a single nestling period. Remating in females was inferred in 65 % of the reconstructed maternal genotypes, with an average of 1.97 (± 0.08 s.e.) males per female. Genetic relatedness (*R*) of *P. downsi* within nests was low overall, though within Floreana nests *R* was much higher in the lowlands than the highlands. This study has particular relevance to a potential sterile insect technique program to eradicate the threat of *P. downsi* from the Galápagos Islands.

**Introduction**

Molecular genetic approaches have allowed the oviposition, mating, and dispersal behaviour of insects to be characterised rapidly, which otherwise would require long-term demographic and experimental studies (Roderick 1996; Atkinson *et al.* 2002). An increased understanding of the reproductive behaviour of invasive insect parasites is central to the study of their population dynamics, life-history evolution and genetic structure, and may lead to more effective management strategies (Hendrichs *et al.* 2002; Chevillon *et al.* 2007; Song *et al.* 2007). Two aspects of reproductive behaviour in parasitic insects that can be elucidated using molecular techniques is female multiple mating and host infestation behaviour. Female multiple mating has implications for effective population size, rates of gene flow and maintaining genetic variation (Bonizzoni *et al.* 2002; Song *et al.* 2007). Oviposition behaviour in parasitic insects may vary with host density and distribution and determine parasite intensity and impact among hosts (Minkenberg *et al.* 1992; Cronin & Strong 1999). Genetic studies of parasitic, myiasis-causing flies have focused primarily on species of agricultural relevance, or have been mainly concerned with taxonomy and phylogenetics (Otranto & Stevens 2002; Azeredo-Espin & Lessinger 2006). Molecular genetic data on the reproductive biology and behaviour of myiasis-causing flies of birds (e.g. *Protocalliphora* and *Philornis* species) are absent (Otranto & Stevens 2002; Criscione *et al.* 2005; Azeredo-Espin & Lessinger 2006), despite the high fitness costs in nestlings of numerous bird species that these parasites cause (reviewed in Dudaniec & Kleindorfer 2006).

Since the identification of the parasitic fly, *Philornis downsi*, in nests of Darwin's finches (Fessl *et al.* 2001), the majority of studies have documented the impacts of parasitism among hosts and ecological variation associated with nestling parasitism (Fessl & Tebbich 2002; Dudaniec *et al.* 2006; Fessl *et al.* 2006a,b; Dudaniec *et al.* 2007; Wiedenfeld *et al.* 2007). This research has shown severe fitness costs of *P. downsi* parasitism in Darwin's finches, such as reduced haemoglobin concentration, decreased nestling growth rate, and reduced fledging success (Dudaniec *et al.* 2006; Fessl *et al.* 2006a,b). These impacts are feared precursors to the extinction of vulnerable finch species (e.g. *Camarhynchus heliobates*: Dvorak *et al.* 2004; *Certhidea fusca*: Grant *et al.* 2005). Despite the recognised significance of this parasite for the conservation of endemic Galápagos birds (Wikelski *et al.* 2004; Causton *et al.* 2005), the dispersal and reproductive behaviour of *P. downsi* is little known (Fessl *et al.* 2006b), while information on the reproductive biology of the entire genus is lacking in detail (reviewed in Teixeira 1999; Dudaniec & Kleindorfer 2006).

Potential methods to control *P. downsi* on the Galápagos Islands include the use of insecticides (e.g. Fessl *et al.* 2006a) or pheromone attractants (Witzgall 2001), but these techniques are often not effective in the long term and may be unsuitable for sensitive island ecosystems. The sterile insect technique (SIT) however, is a non-invasive, targeted control method with a high success rate achieving eradication or suppression of agricultural dipteran pests in numerous countries (e.g. Argentina, Australia, Mexico, South Africa, the USA) and islands (e.g. the Netherland Antilles, Okinawa Islands of Japan) (Cayol *et al.* 2002; Vreyson *et al.* 2006). SIT involves the release of laboratory-reared, sterile male flies in to the wild population, causing females to produce infertile offspring (Hendrichs *et al.* 2002). The mating success of released sterile males is dependent upon female remating frequency, whereby the probability of sterile-male/female matings decreases with increasing numbers of mates per female (e.g. in Mediterranean fruit flies: Bonizzoni *et al.* 2002; Kraaijeveld *et al.* 2005). Highly variable molecular genetic markers (e.g. microsatellites) can uncover the occurrence of female remating and patterns of oviposition behaviour using offspring genotypes alone (Chapman *et al.* 2003; Wang 2004; Song *et al.* 2007; Zavodna *et al.* 2007). Molecular genetic methods therefore offer the opportunity to examine within-host genetic structure and mating behaviour of avian parasitic flies, while allowing the efficiency of control methods such as SIT to be assessed prior to, or in aid of, laboratory and field-based experiments (Bonizzoni *et al.* 2002; Song *et al.* 2007).

Using eight polymorphic microsatellite loci we investigate the genetic structure of *P. downsi* individuals within nests of Darwin's finches to examine multiple maternity and paternity of progeny. The methods we implement also allow a concurrent examination of multiple infestations by *P. downsi* (i.e. the number of females infesting each nest). We compare patterns across two islands of the Galápagos (Santa Cruz and Floreana) that each contains two ecologically contrasting habitats: arid lowlands and humid highlands. Specifically, we aim to answer the following questions regarding the largely unknown ecology of this high impact ectoparasite: (1) Is there evidence for female remating (e.g. multiple paternity within families)? (2) Are nests infested by multiple female *P. downsi*? (3) Does within-nest genetic structure and relatedness of *P. downsi* show variation between islands?

**Methods**

*Study species*

Larvae of the dipteran *Philornis downsi* (Muscidae) are semi-haematophagous parasites that are exclusive to birds. The non-parasitic adult fly lays eggs inside the nares of newly hatched nestlings, which hatch into first instar larvae (Dudaniec & Kleindorfer 2006; Fessl *et al.* 2006b; Muth 2007). Second and third instar larvae attach to nestlings externally and feed on blood and tissue over four to six days (Dudaniec & Kleindorfer 2006). At the time of host fledging, most larvae reach their third instar phase, and pupariate at the base of the nesting material. Pupariation is for approximately two weeks before adult flies emerge (Teixeira 1999; Dudaniec & Kleindorfer 2006).

*Field collection and samples*

*Philornis downsi* was collected from two islands of the Galápagos: Santa Cruz (total area: 986 $km^2$; 0º37´S, 90º 21´W) and Floreana (total area: 173 $km^2$, 1º28´S, 90º 48´W). *Philornis downsi* samples were collected from bird nests during the January to March finch breeding season across three years (2004, 2005 and 2006) and two habitats (humid highlands, Santa Cruz: 300-600 m asl; Floreana: 300-400 m asl; arid lowlands, 0-100 m asl on both islands) (Table 7.1). The lowlands are an arid rocky environment with low rainfall and finch nesting is predominantly in trees such as *Bursera graveolens, Croton scouleri,* and most commonly, *Opuntia* cacti (Dudaniec *et al.* 2007; Kleindorfer 2007). The highlands are characterised by a closed-canopy forest with abundant moss and lichen and high rainfall (Dudaniec *et al.* 2007; Wiedenfeld *et al.* 2007) and finches predominantly nest in the endemic tree *Scalesia pedunculata,* or *Zanthoxylum fagara*. Finch nesting is at a higher density and much more consistent across years in the highlands, where resources are more readily available and rainfall is higher (Kleindorfer 2007; Grant & Grant 2008). We collected parasite samples from one site in each habitat on Floreana (lowlands, adjacent to the town of Puerto Velasco Ibarra: 1° 16´S, 90° 29´W; highlands: 1° 17´S, 090° 27´W), while on Santa Cruz we collected from one site in the highlands (Los Gemelos: 0º 37´S, 90º 22´W) and three sites in the lowlands: (1) Garrapatero: 0º 39´S, 90º 28´W; (2) Itabaca: 0º 29´S, 90º 17´W; (3) Punta Estrada, near Puerto Ayora: 0 º 45´S, 90º 18´W. Samples were only collected from the highlands of Floreana in 2005 due to drought conditions and a subsequent lack of breeding in the lowlands (Table 7.1). All sample sites were approximately 2000-4000 $m^2$. The topographic variation across the two habitats was such that sites were much closer together on Floreana (3-5 km) while on Santa Cruz, the distance between all four sites (one highland, three lowland) varied between 15 and 27 km.

**Table 7.1**

The number of host nests (i.e. infrapopulatons) sampled for genetic analyses and total *P. downsi* intensity (mean ± s.e.) in each habitat (highland/ lowland) on Santa Cruz and Floreana in 2004, 2005 and 2006. The total number of individual *P. downsi* analysed are in parentheses.

| | | **# Broods (# individuals) analysed at 9 microsatellite loci** | | **Total *P. downsi* intensity (mean ± s.e.)** | |
|---|---|---|---|---|---|
| **Year** | **Island** | lowland | highland | lowland | highland |
| 2004 | Santa Cruz | 17 (359) | 3 (150) | 32.6 (3.4) | 54 (4.2) |
| | Floreana | 5 (66) | - | 28.8 (9.3) | - |
| 2005 | Floreana | 1 (15) | 8 (82) | - | 28.9 (5.2) |
| 2006 | Santa Cruz | 2 (46) | - | 38.5 (8.5) | - |
| | Floreana | 10 (115) | 11 (157) | 17.4 (4.4) | 35.5 (5.5) |

*Philornis downsi* larvae, puparia and empty puparia cases were opportunistically collected from 57 nests of five Darwin finch species (*Geospiza fuliginosa*, n = 23, *Geospiza fortis*, n = 15, *Camarhynchus parvulus*, n = 2, *Camarhynchus pauper*, n = 4; *Cactospiza pallida*, n = 1); one nest was opportunistically sampled from each of the yellow warbler (*Dendroica petechia aureola*) and the Galápagos mockingbird (*Nesomimus parvulus*), while 10 recently fledged nests were sampled where the finch species was unknown. GPS coordinates were obtained at each nest location (Garmin 12 XC). Following fledging or predation, inactive nests were collected, sealed in individual plastic bags and later dismantled for counting *P. downsi* individuals, which were subsequently preserved in 95 % ethanol. Ethanol was removed from all collection tubes prior to air transportation and replenished three days later.

*Molecular genetic methods*

DNA was extracted from *P. downsi* larvae and puparia using the salting out procedure described in Miller *et al.* (1988), but was not successfully extracted from empty puparia cases. In total, 990 *P. downsi* individuals were genotyped (Table 7.1) for eight *P. downsi* microsatellite markers: Pd1, Pd2, Pd4, Pd6, Pd7, Pd8, Pd9, Pd10 (Dudaniec *et al.* 2008a). Multiplex PCR conditions were followed as described in Dudaniec *et al.* (2008a). Samples were genotyped on an ABI 3730 capillary electrophoresis DNA analyser (Applied Biosystems). A fluorescently labeled size standard (GS500 (-250) LIZ) was run with the samples and alleles were scored using GENEMAPPER version 3.7 (Applied Biosystems). To minimise and estimate genotyping error, 66 individual samples (6.7 % of all samples genotyped) were re-amplified and genotyped at least once, which was achieved by repeating eight samples and one control sample for each run of the DNA analyser.

*Genetic relatedness and sib-ship reconstruction analyses*

Allele frequencies for the eight *P. downsi* microsatellite loci are reported in Table 7.2. Futher characteristics of the microsatellite loci used can be found in Dudaniec *et al.* (2008), while population genetic characteristics (e.g. genetic diversity, heterozygosity) of *P. downsi* from our collection sites are reported in Dudaniec *et al.* (2008b). For this study we use the term 'infrapopulation' to refer to all *P. downsi* parasites collected from a single host nest (rather than a single host). Allele frequencies for genetic relatedness (*R*) and sib-ship reconstruction analyses were calculated in RELATEDNESS 5.0.8 (Queller & Goodnight 1989) using one randomly selected individual per infrapopulation (n = 57) to eliminate the possibility of including related individuals. Only individuals genotyped at all eight loci were used for

calculating allele frequencies. Exact tests were performed for each microsatellite locus to test deviation from Hardy-Weinberg equilibrium using GENEPOP (Raymond & Rousset 1995). All loci were in Hardy Weinberg equilibrium after sequential Bonferroni correction (Hochberg 1988) and these allele frequencies were used for all further analyses. *R* was calculated for each infrapopulation using RELATEDNESS 5.0.8 (Queller & Goodnight 1989). We used ANOVA to examine variation in *R* within *P. downsi* infrapopulations across highland and lowland habitats of each island.

Errors in family reconstruction may arise when a subset of offspring is analysed and individuals of additional family memberships are missed or incorrectly assigned due to an insufficient number of genotyped offspring (Molbo *et al.* 2004). To validate the consistency of our genetic findings with varying sample sizes across infrapopulations, we used linear regression to examine *R* within infrapopulations in relation to the following variables: the percent of the total *P. downsi* infrapopulation that was genotyped from each nest, total *P. downsi* intensity (the total number of parasites per nest, defined by Bush *et al.* 1997), and the number of individuals analysed from each nest.

For sib-ship reconstruction within nests, we used a maximum likelihood method that partitions individuals into pure full-sib families (i.e. monogamous female parent), or full-sib families nested within half-sib families (i.e. polyandrous female parent) using progeny genotypes without known parental genotypes (Chapman *et al.* 2003; Wang 2004). The program COLONY 1.2 was used to implement this procedure, which groups progeny in to putative maternal and paternal half or full sib-ships while accounting for typing errors (Wang 2004). Class II errors are broadly defined as an observed genotype that could result in incorrect relationship inference and may arise from mutations, false alleles, allele identification error, or contaminant DNA, whereas Class I errors generally refer to allelic dropouts (Wang 2004). For each run, all eight loci were used, and a conservative Class II error rate of 5 % was implemented based on evidence from the re-genotyping of 66 individuals, in which genotyping error ranged from 0-5 % across loci.

The method we used for sib-ship reconstruction requires the assumption that one parental sex is multiply mated within each infrapopulation (Wang 2004). It was therefore necessary to assume that all male flies contributing to a single infrapopulation did not sire offspring of more than one female parent in the same nest. *Philornis* species have not been observed to

mate inside bird nests (Teixeira 1999; Dudaniec and Kleindorfer 2006), thus reducing the likelihood of shared paternity of offspring from multiple females contributing to one infrapopulation. The sexual behaviour of *Philornis* species is, however, largely unknown. For each infrapopulation, three runs were performed with different random seed numbers (12, 80, and 243) to ensure data convergence. The reliability of the parental genotypes inferred by COLONY 1.2 is influenced by the number of offspring assigned to each family (lower numbers are less reliable), and the number of mates assigned to the polygamous parent (least reliable in the case of monogamy) (Wang 2004). Thus, reconstructed full-sib families comprising less than four offspring require cautious interpretation, particularly where a low percentage of the infrapopulation is genotyped. Therefore, we also examine the frequency of maternal genotypes per infrapopulation when excluding families comprising one to three offspring. To further validate the results of the sib-ship reconstruction, *R* was examined in relation to the number of putative parental genotypes per nest using linear regression.

## Results

*Total* P. downsi *intensity and genotyping effort*

Total *P. downsi* intensity varied between 5 and 65 parasites per nest (mean = 30.8 ± 16.5) (larvae, pupae and puparia cases altogether) across habitats and islands. Between two and 56 individuals were genotyped from each infrapopulation. For 18 out of the 57 nests sampled, 85-100 % of the individuals within each nest were genotyped, while for 36 nests, over 50 % of the infrapopulation within each nest was successfully genotyped. The majority of individuals were successfully typed at all eight loci (76 %, 785/1020). The percentages of individuals genotyped between three and seven loci were as follows: 7 loci = 7 %; 6 loci = 8 %; 5 loci = 2 %; 4 loci = 1 % and 3 loci = 2 %. The occurrence of missing data is suspected to be partially explained by poor DNA preservation.

*Within-nest sib-ship reconstruction*

Results were consistent among the three COLONY runs using different random number seeds for each infrapopulation, which assured that the method converged for the data set. Using all data (57 nests), the number of putative parents reconstructed per infrapopulation did not vary with total *P. downsi* intensity (female: $b = 0.20$, $P > 0.4$; male: $b = -0.33$, $P > 0.1$), or the percent of the infrapopulation genotyped from each nest (female: $b = -0.13$, $P > 0.6$; male: $b = 0.42$, $P > 0.1$). The number of putative male parents increased with the number of individuals

**Table 7.2**

Allele frequencies for eight *P. downsi* microsatellite loci calculated from 57 individuals sampled from 57 infrapopulations (bird nests) on Santa Cruz (n=) and Floreana (n=) Islands. All loci were in Hardy-Weinberg equilibrium.

| | | **Allele frequency** |
|---|---|---|
| *Pd1* | 315 | 0.267 |
| | 323 | 0.216 |
| | 325 | 0.112 |
| | 327 | 0.405 |
| *Pd2* | 230 | 0.319 |
| | 236 | 0.500 |
| | 240 | 0.181 |
| *Pd4* | 252 | 0.379 |
| | 254 | 0.284 |
| | 256 | 0.052 |
| | 258 | 0.284 |
| *Pd6* | 241 | 0.328 |
| | 251 | 0.293 |
| | 261 | 0.121 |
| | 263 | 0.259 |
| *Pd7* | 201 | 0.353 |
| | 207 | 0.233 |
| | 210 | 0.397 |
| | 213 | 0.017 |
| *Pd8* | 349 | 0.138 |
| | 353 | 0.095 |
| | 357 | 0.103 |
| | 365 | 0.664 |
| *Pd9* | 194 | 0.345 |
| | 200 | 0.414 |
| | 217 | 0.241 |
| *Pd10* | 189 | 0.233 |
| | 191 | 0.216 |
| | 193 | 0.552 |

analysed from each infrapopulation (ANOVA: male: $F_{57} = 2.60$, df = 13, $P < 0.02$) but not the number of maternal parents (female: $F_{57} = 2.43$, df = 5, $P = 0.057$). *R* decreased significantly with increasing numbers of putative male and female parents per infrapopulation (ANOVA: female: $F_{57} = 15.41$, df = 5, $P < 0.001$; male: $F_{57} = 9.67$, df = 13, $P < 0.001$) (Figure 7.1), which validates the ability of COLONY to detect fine-scale genetic structure within nests.

We present results for within-infrapopulation genetic structure in two separate analyses that each contained four data subsets: (1) including all reconstructed families (no data excluded), and (2) including reconstructed families comprising ≥ 4 individuals. Class II errors were found in 9 % of the reconstructed nested half-sib and full-sib families (16/173) among 13 infrapopulations (22 % of all infrapopulations). Results are also presented without these 16 infrapopulations to eliminate uncertainty in the sibship reconstructions due to typing errors. Furthermore, we have examined variables within each analysis for infrapopulations with ≥ 85 % of individuals genotyped, also with and without typing errors (Table 7.3). A two-way ANOVA with post-hoc tests (Tukey HSD) was conducted to examine statistical similarity of the results between the data subsets (Table 7.3).

*Multiple infestations by female* P. downsi

When including all data (with typing errors), the number of female infestations per infrapopulation estimated by COLONY was 3.04 (±0.18) (range = 1-5 female infestations). Post-hoc tests showed that this value was not significantly different from the other data subsets, except for when including families with ≥ 4 individuals (Table 7.3) (Mean difference with typing errors = 1.30, without typing errors = 1.35, $P < 0.001$). Based on the above, we conservatively conclude that the most common number of ovipositing *P. downsi* females per nest is between two and five (Figure 7.2). When excluding families with ≤ three individuals, 38.6 % of all infrapopulations (n = 22) were assigned to a single female genotype, which increased from 14.3 % (n = 8) when including all families. These results suggest frequent rates of nest infestation by one female fly when excluding small families. However, to avoid biasing results for the number of parents contributing to each infrapopulation and the variance in family sizes within infrapopulations, we include all reconstructed families in subsequent analyses.

**Table 7.3**

Results of sib-ship reconstruction analyses are presented for (1) all data (1a: infrapopulations with ≥85% of all individuals genotyped) and (2) data for families comprising four or greater individuals (2a: infrapopulations with ≥85% of all individuals genotyped). Values are presented as means (± s.e.) for data with, and without class II typing errors (TE). Sample sizes of infrapopulations for each data subset are shown in square brackets. Variables are defined as follows: Number of female infestations = mean number of reconstructed maternal genotypes per infrapopulation; Number of paternal genotypes = mean number of reconstructed paternal genotypes per infrapopulation; Number of males per female = mean number of males assigned to the offspring of each female per nest; % total offspring per female = the mean percentage of the total offspring contributed by each female per infrapopulation.

| | **Number of female infestations** | **Number of paternal genotypes** | **Number of males per female** | **% total offspring per female** |
|---|---|---|---|---|
| ***1:All Data*** | | | | |
| with TE [57] | 3.04 (0.18) | 6.07(0.44) | 1.97(0.08) | 46.60(4.26) |
| without TE [54] | 2.87(0.18) | 5.44(0.45) | 1.91(0.08) | 43.64(4.38) |
| **1a:≥85% genotyped** | | | | |
| with TE [18] | 3.4(0.32) | 8.11(0.78) | 2.17(0.14) | 46.61(7.58) |
| without TE [16] | 3.0(0.34) | 6.44(0.83) | 2.02(0.15) | 39.94 (8.04) |
| ***2:Families ≥4 individuals*** | | | | |
| with TE [38] | 1.74(0.22) * | 2.32(0.54)* | 1.29(0.09) * | 46.31(5.22) |
| without TE [35] | 1.69(0.23) * | 1.80(0.56)* | 1.06(0.10)* | 43.85(5.44) |
| **2a:≥85% genotyped** | | | | |
| with TE [15] | 2.47(0.35) | 3.67(0.86) | 1.54(0.15) | 48.15(8.30) |
| without TE [13] | 1.92(0.37) | 2.46(0.92)* | 1.28(0.16) * | 40.17(8.92) |

* indicates values that were significantly different from the value obtained when including all data with typing errors (i.e. first line of table) (Tukey HSD test: $p < 0.05$).

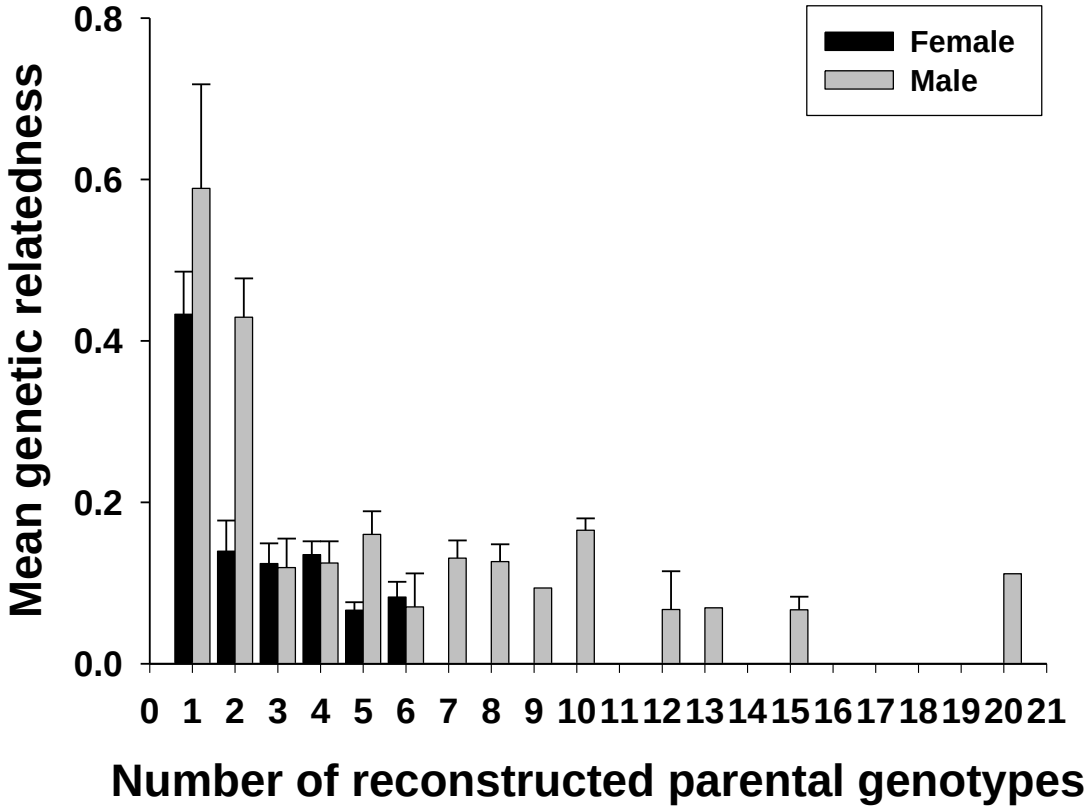


**Figure 7.1**

*P. downsi* genetic relatedness (± s.e.) decreases as the number of reconstructed male (paternal) and female (maternal) genotypes increases within infrapopulations (n = 57).

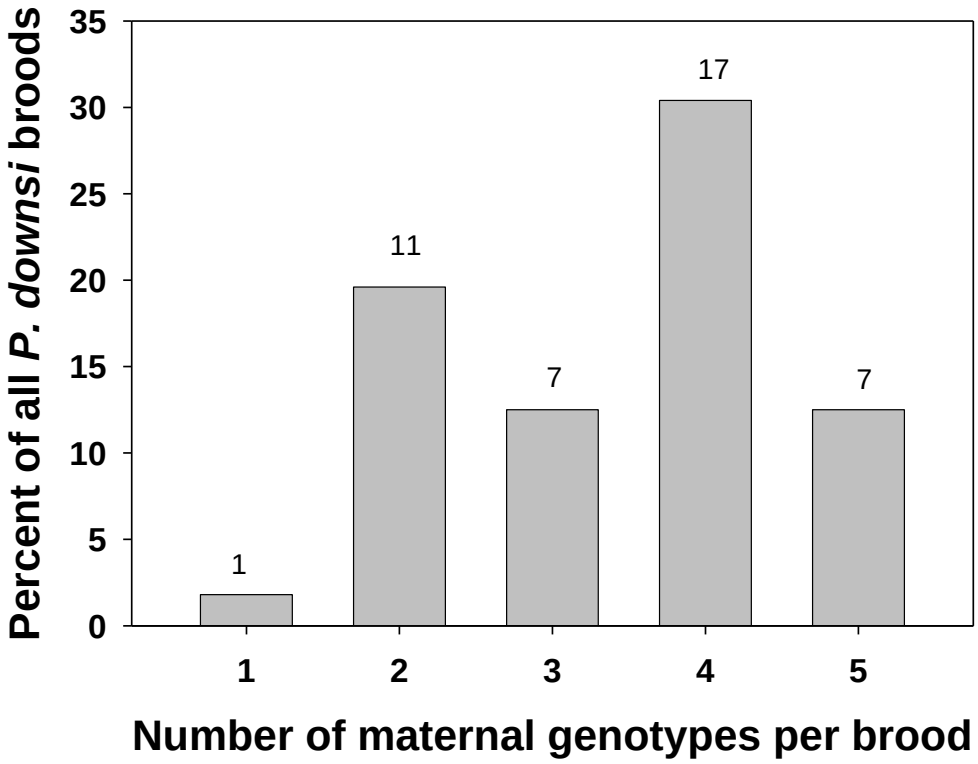


**Figure 7.2**

The percent of *P. downsi* infrapopulations with 1, 2, 3, 4 or 5 infestations, determined by reconstructed maternal genotypes. Numbers of sampled host nests is shown above bars.

*Female multiple mating and paternity of infrapopulations*

We found female multiple mating to be common in *P. downsi*, with a mean of 1.97 (± 0.08) males per female (range = 1-5, n = 57 nests) when including all data with typing errors. This estimate was significantly different from three of the data subsets: ≥ 4 individuals with (mean difference = 0.69) and without (mean difference = 0.92) typing errors (both $P < 0.001$), and ≥85% of the nest genotyped without typing errors (mean difference = 0.69) ($P < 0.004$) (Table 7.3). The highest percentages of reconstructed female parents were estimated to have mated with one (35 %) or two (44.4 %) males, while just 17 %, 2.6 % and 1 % of females were assigned to have mated with 3, 4 and 5 males respectively. Because reconstructed parental genotypes are least reliable in the case of monogamy (Wang 2004), it is possible that the frequency of single matings is overestimated.

The number of putative male genotypes contributing to each infrapopulation ranged from 1-17, with an average of 6.07 (± 0.44 s.e.) when including all data with typing errors. This estimate was significantly different when only including families with ≥ 4 individuals, with typing errors (mean difference = 3.75) and without typing errors (mean difference = 4.27) (both $P < 0.0001$) (Table 7.3). The estimated number of male genotypes also differed when only including infrapopulations with ≥85% of the nest genotyped, with typing errors (mean difference = 3.61) ($P < 0.02$) (Table 7.3).

*Female* P. downsi *contribution to infrapopulations*

The average percentage of offspring contributed by each putative female parent to each infrapopulation did not significantly differ across data treatments ($F_{246} = 0.174$, df = 7, $P > 0.9$). Across all nests, the mean number of *P. downsi* offspring assigned to each putative female parent was only five (± 0.31) (range: 1-24 individuals), though there were three nests where individual female parents contributed 18, 21, and 24 individuals towards the infrapopulation respectively. The eighteen nests for which 85-100 % of the total *P. downsi* infrapopulation was genotyped were examined for equality of female contribution to the individuals within a nest. Putative female parents were found to contribute unequally towards the infrapopulation within a nest, and patterns differed across islands. On Santa Cruz, individual females contributed between 2-49 % (mean: 16.7 ± 1.54 %) of the total progeny within a nest, while on Floreana, the percentage was much higher (range 7.4-100 %; mean: 39.1 ± 6.2 %).

*Genetic relatedness (*R*) of* P. downsi *within nests*

Relatedness was highly variable within *P. downsi* infrapopulations, and ranged from -0.090 to 0.847 (mean: 0.195 ± 0.024). *R* did not vary significantly with the number of individuals analysed from each nest (linear regression: $b = -0.42$, $P > 0.1$), the percent of individuals analysed from each nest ($b = 0.31$, $P > 0.2$), or the total parasite intensity within each nest ($b = 0.09$, $P > 0.7$). Thus, non-sampling error has little influence in our analyses. Therefore, *R* is not biased by variation in genotyping effort between nests (i.e. the number of individuals genotyped per infrapopulation) and is sufficient to detect underlying patterns of genetic structure.

Overall, *P. downsi* individuals within nests on Santa Cruz had significantly lower *R* (mean: 0.108 ± 0.02) than those from Floreana nests (mean: 0.254 ± 0.03) ($F_{57} = 5.82$, df = 1, $P < 0.01$). Individuals within Floreana nests were much more highly related in the lowlands than in the highlands ($F_{35} = 21.76$, df = 1, $P < 0.001$) (Figure 7.3). This difference was also upheld when including nests from 2006 only ($F_{21} = 9.664$, df = 1, $P < 0.01$), when total parasite intensity was also significantly lower in the lowlands than in the highlands (ANOVA: $F_{21} = 6.37$, df = 1, $P < 0.03$) (Table 7.1). Individuals within Santa Cruz nests had similar *R* across habitats ($F_{22} = 0.14$, df = 1, $P > 0.7$), though we appreciate that sample size was small in the highlands (three nests) (Table 7.1).

**Discussion**

P. downsi *multiple infestations*

The propensity of insects to oviposit on a previously infested host is influenced by a number of factors, including the detection of host-marking pheromone from previous ovipositing females (Arredondo & Diaz-Fleischer 2006), the number of hosts available, host density and distribution (Cronin & Strong 1999; Diaz-Fleischer & Aluja 2003), female egg load (Minkenberg *et al.* 1992), costs of larval competition (Dukas *et al.* 2001), and competition between unrelated conspecifics (Frank 1994; Atkinson *et al.* 2002; Puustinen *et al.* 2004). We confirm that multiple *P. downsi* females frequently infest a single nest, with mainly two to five infestation events over the nestling period (Figure 7.2). This supports previous studies of *Philornis* that have documented or suggest multiple infestations (reviewed in Dudaniec & Kleindorfer 2006), though multiple infestations by *P. downsi* have not previously been reported in nests of Darwin's finches (Fessl *et al.* 2006a). To date, there has been one observation of an ovipositing female *P. downsi* that laid 19 eggs (Muth 2007). We found that

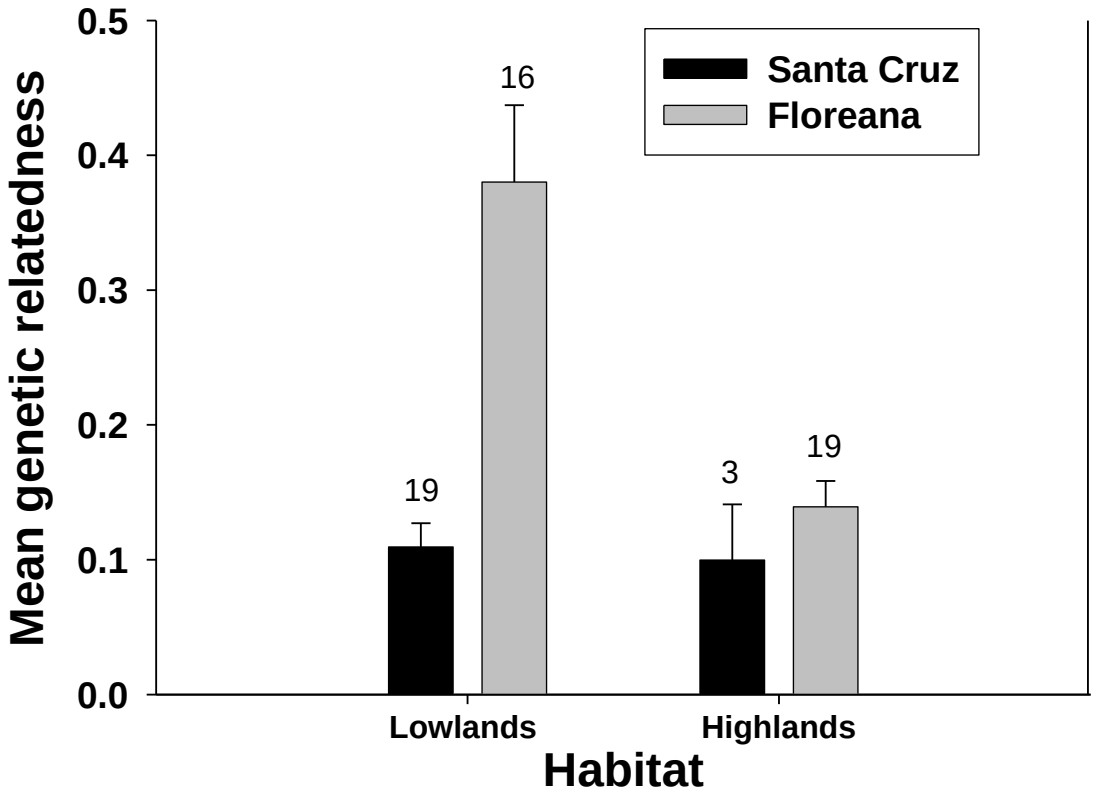


**Figure 7.3**

Mean genetic relatedness (± s.e) of *P. downsi* infrapopulations in nests from Santa Cruz and Floreana across two habitats, the lowlands and the highlands. Numbers of sampled host nests is shown above the error bars.

the average number of offspring per female was just five, yet our study suggests that *P. downsi* females can lay at least 24 eggs at a time, as this was the largest number of offspring assigned to one female in an infrapopulation.

The decision of female flies to infest nests that are already infested may be affected by larval survival trade-offs associated with the temporal spacing of oviposition events. For example, experiments on Mediterranean fruit flies (*Ceratitis capitata*) suggest that older larvae may have a competitive advantage over younger larvae in terms of growth rate and resource use (discussed in Dukas *et al.* 2001). On the Galápagos Islands, almost 100 % of finch nests are infested with *P. downsi* (Dudaniec *et al.* 2007). In combination with the high frequency of multiple infestations we report, there is evidence that *P. downsi* occurs at high density and may be host limited, rendering the costs of reinfesting a nest minimal in comparison with the prospect of zero reproductive success.

*Genetic relatedness within* P. downsi *infrapopulations*

Theoretical models predict that when genetic relatedness between parasites is high, competition among parasites is reduced, favouring increased transmission, colonisation, and low virulence (Frank 1994). Low parasite relatedness through multiple infections is predicted to enhance within-host competition, leading to rapid host exploitation, high virulence, and increased fitness of competitively superior parasite genotypes (Frank 1994). The very low *R* we found in the vast majority of *P. downsi* infrapopulations, combined with the parasite's high impact on nestling survival (Fessl & Tebbich 2002; Dudaniec *et al.* 2006; Fessl *et al.* 2006b) supports the prediction of high virulence with low *R*. Given the large human population on Santa Cruz (~15000) compared with Floreana (~100), the availability of resources for adult flies (e.g. food and water) is presumably greater on Santa Cruz, particularly in the lowlands. In the current study, Floreana nests were found to have lower parasite intensity than nests on Santa Cruz (Table 7.1). Individuals within Santa Cruz nests also had much lower *R* than within Floreana nests, which may reflect higher *P. downsi* densities on Santa Cruz that result in an increase in multiple infestations (or possibly higher female remating frequency) and consequent low *R* within infrapopulations. A thorough examination of inter-island *P. downsi* density is required to examine these relationships in more detail.

*Spatial and genetic distance between infrapopulations: dispersal implications*

Parasite population fluctuations are strongly driven by host abundance and the spatial distribution of both hosts and parasites. Parasitic insects that deposit eggs in discrete resource patches may be limited by spatial or temporal restrictions on the availability of oviposition sites, leading to time or egg-limitation constraints under variable environmental conditions (Diaz-Fleischer & Aluja 2003). Therefore, reproductive investment may be adjusted according to the dispersal distance between hosts, whereby investment per host decreases when hosts are more closely spaced (e.g. in wasps: Cronin & Strong 1999). A recent study of Darwin's finches in the Santa Cruz highlands found that groups of between two and four finch nests within a 20 m radius had significantly higher *P. downsi* intensity than solitary nests (one nest within 20 m radius) (Kleindorfer and Dudaniec in press). This may indicate dispersal-dependent oviposition behaviour, whereby female flies oviposit more frequently among nests in close proximity, resulting in increased parasite intensity. Alternatively, this pattern may represent a strategy to minimise risks that jeopardize offspring survival, such as host predation (Dukas *et al.* 2001).

Greater within-infrapopulation *R* in the Floreana lowlands compared to the highlands suggests a habitat-specific oviposition strategy in *P. downsi* that is potentially governed by differences in host spatial distribution, which may interact with ecological and climatic conditions. Increased *R* in lowland infrapopulations indicates there are less female flies ovipositing per nest, possibly due to increased dispersal distance between hosts compared to the highlands. However given this pattern, the low total *P. downsi* intensity in lowland nests (Table 7.1) is counterintuitive if females are capable of laying over 20 eggs per nest (see Results) and are investing more offspring per host to maximise reproductive fitness. So although *R* is high in lowland nests, this does not predict high parasite intensity within nests, and indicates interactions with other variables that may affect reproductive investment. Factors other than host spatial distribution that may influence the oviposition strategy of mobile parasites include desiccation risk during host searching (which for *P. downsi*, is likely to be higher in the lowlands), availability of nutritional resources (e.g. fruit, organic matter), the number of hosts within a nest, or the risk of predation during host searching (Minkenberg *et al.* 1992; Dukas *et al.* 2001; Atkinson *et al.* 2002).

Oviposition of a greater number of eggs in nests following extended dispersal has been explained as a mechanism to counteract the Allee effect (i.e. local population decline or

extinction due to low population density) by increasing mate availability and successful establishment in isolated host patches (Cronin & Strong 1999; Stephens & Sutherland 1999). One key determinant of the patterns we observe is rainfall, because the density of finch nests on the Galápagos is generally determined by precipitation level, particularly in the lowlands (Grant & Grant 2008). Rainfall also shows high inter-annual variation, resulting in changes in nesting density and high *P. downsi* intensity in years of high rainfall (Dudaniec *et al.* 2007; S Kleindorfer *et al.* unpublished). The interaction between rainfall and the number of nestlings within a nest may also influence *P. downsi* intensity across habitats. Clutch size in Darwin's finches is larger in the lowlands (3-5) than in the highlands (2-3) (Kleindorfer 2007) and varies within habitats according to rainfall, which is much higher and more consistent in the highlands (Kleindorfer 2007; Grant & Grant 2008). Habitat-dependent oviposition behaviour in *P. downsi* on Floreana Island is therefore likely influenced by the climatic conditions of 2006, while an inter-annual and larger-scale study would be required to examine behavioural plasticity in *P. downsi* with regard to oviposition strategy, rainfall, host abundance and host density.

*Female multiple mating and implications for SIT*

We present the first evidence for frequent multiple mating in a wild population of *P. downsi,* providing a significant advance in our understanding of the mating system of this species. Through sib-ship reconstruction analysis, we determined indirectly that most female flies likely mated with two males (44.4 %), though the offspring of 35 % of females were assigned to a single male. Thus female remating was detected in 65 % of all reconstructed females. These results should be interpreted conservatively however, given the absence of certain parental genotypes and low allelic variation. In addition, our findings do not necessarily apply across the entire species range. Our results may provide insight into future laboratory studies of *P. downsi* reproductive biology, given that insect mating behaviour may be affected under laboratory conditions (Zouros & Krimbas 1970). In the future, genotyping of sperm stored in the spermathecae of females may allow a more accurate measure of the number of mates per female (e.g. Fernandez-Escudero *et al.* 2002).

Although we examined *P. downsi* multiple mating indirectly, our results indicate that the impact of multiple mating on the efficiency of a potential SIT program may be significant. High female remating frequencies increase the risk that wild females will mate with one or more resident fertile males (Bonizzoni *et al.* 2002; Kraaijeveld *et al.* 2005). Sterile males are

also often less successful than wild males when competing for mates (Kraaijeveld *et al.* 2005). However, SIT may still be effective in suppressing populations of species in which females remate (e.g. melon fly: Steiner *et al.* 1965), particularly if sterile males are released continuously (Song *et al.* 2007). Our study provides evidence for frequent female multiple mating while revealing inter-habitat patterns of oviposition behaviour in *P. downsi*. These findings represent a pivotal step towards characterising the reproductive ecology of *Philornis* (~50 species), which is a necessary task for understanding the invasive biology of *P. downsi* parasitism, and more crucially, for assessing the viability of SIT as a control method on the Galápagos Islands.

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# CHAPTER 8: General Discussion

This thesis represents the first detailed study of a bird-dipteran ectoparasite interaction that utilises both an ecological and a molecular genetic approach. In doing so, the collection of papers in this thesis significantly advance our understanding of the genus *Philornis*, while providing constructive information for evaluating and mitigating the severe impacts of these parasites, which burden vulnerable avian species in the Neotropical region (Teixeira 1999; Arendt 2000). The invasion of *P. downsi* on the Galápagos Islands is the only documented occurrence of an avian ectoparasitic fly successfully invading an archipelago with such devastating consequences for current and long-term species survival.

In light of this recent threat to one of the most famous bird groups world-wide (i.e. Darwin's finches), in combination with the lack of biological synthesis regarding the genus of parasite in question, a thorough review of the existing knowledge of *Philornis* species was clearly required, and hence, is provided in this thesis. This review shows that *Philornis* species clearly show generalist parasitic behaviour with regard to host species, host size, clutch size, and nest type across the Neotropical region (Chapter 2). The variation in impact (e.g. reduced fledging success, nestling growth, development) caused by the different *Philornis* species across hosts is particularly evident, though high rates of mortality are frequently observed across taxa (Chapter 2). As the majority of *Philornis* studies have focused on sub-cutaneous species, the investigation of ectoparasitic *P. downsi* presented here constitutes a novel contribution to avian parasitology.

***Host behavioural ecology and* P. downsi *impact: Chapters 3-4***

The interrelationship between host, parasite, and climatic conditions is one of ecological complexity, particularly where multiple hosts and habitats are involved (e.g. Gold and Dahlsten 1989; De Lope et al. 1993; Goulson et al. 2005). On Santa Cruz Island, *P. downsi* intensity in nests of six Darwin finch species was found to differ markedly between six, climatically variable years (1998-2005), being highest in years of increased rainfall (Chapter 3). This finding highlights the influence of El Niño events, and more generally, climate change, in regulating parasite populations in response to the availability of hosts. Inter-specific variation in intensity across Darwin's finches reveals possible host selection behaviour in *P. downsi* governed by host size, though fitness costs are high across all species

(40-100 % of nests with parasite-induced mortality) (Chapter 3). However, larger species of Darwin's finches build larger nests, introducing another variable potentially linked with *P. downsi* intensity if space is a limiting factor for parasitic larvae and pupae. The relationship between host size, nest size and parasite intensity is further examined in Chapter 4 to tease these interactions apart, in conjunction with the additional factor of mixed species nesting associations (MSNA).

The effect of host sociality on parasite intensity and impact has inspired research across a variety of avian taxa, resulting in great theoretical advancements in 'behavioural' parasitology (Poulin 1991; O'Donnell 1997; Tella 2002). Small tree finches (*Camarhynchus parvulus*) on Santa Cruz Island exhibit solitary or mixed species nesting (up to four nesting neighbours within 20 m radius), and *P. downsi* intensity was higher in nests within mixed species aggregations (Chapter 4). Notably, heavier finch species with larger nests (i.e. large tree finch and woodpecker finch) were consistently members of MSNA and had higher *P. downsi* intensity. However, no relationship with nest bottom thickness (a spatial limiting factor for larvae and pupae) and *P. downsi* intensity was observed, indicating that host size is a stronger predictor of *P. downsi* intensity than nest structure (Chapter 4). Though as pointed out in Chapter 3, when examining nestling mass-specific parasite intensity, smaller species may experience higher impact, thus host size can not be viewed as a predictor of *P. downsi* fitness costs across host species *per se* (Poulin and George-Nascimento 2007).

***Genetic insights into* P. downsi *ecology: Chapters 5-7***

Despite an understanding of the fitness costs of *P. downsi*, patterns of inter-specific impact across habitats and years, and the influence of host nesting behaviour and nest characteristics on parasite intensity (Chapters 2, 3 and 4), a vital aspect is still missing: the behavioural and reproductive ecology of *P. downsi*. The development of microsatellite markers for *P. downsi* (Chapter 5) enabled questions to be addressed concerning inter-island and inter-habitat dispersal, gene flow, mating and oviposition behaviour. Low genetic differentiation between islands, habitats (highland, lowland) and sites revealed high gene flow (i.e. dispersal) of *P. downsi* within islands and between islands, though not in the absence of fine-scale genetic structure (Chapter 6). Bayesian clustering analyses showed no habitat-specific genetic structure, and evidence of inter-island ancestry among individuals was weak, indicating low rates of dispersal between the three islands examined. Firstly, these findings confirm that *P. downsi* has not significantly diverged on islands of the archipelago, and there is clearly no

evidence for reproductive isolation within populations. Secondly, population bottlenecks on two of the three islands examined are indicative of few introduction events or a founder effect followed by restricted migration. However, broader sampling and a comparative genetic analysis of mainland *P. downsi* populations are necessary for describing the colonisation pathway of *P. downsi* with confidence.

The reproductive behaviour of mobile insect parasites is difficult or near impossible to measure under laboratory conditions, making molecular techniques a vital tool for field-based investigations (Criscione 2005). Low within-brood genetic relatedness (*R*) and sib-ship reconstruction of *P. downsi* within nests revealed a high frequency of reinfestation by female flies throughout the nestling period. Reconstructed parental genotypes of *P. downsi* broods also indicated high female remating frequencies, with between one and five males per female. However, these patterns were non-ubiquitous across habitats and in relation to host nesting density, with lower within-brood *R* in the highlands where host nests were at higher density (Chapter 7). This finding raises the possibility that the successful establishment of *P. downsi* in both the highlands and lowlands is augmented by an adaptive response in oviposition behaviour to changes in host density. The highly variable climate of Galápagos is however, likely to influence such patterns greatly, given the dependence of Darwin's finches on rainfall for breeding (Grant and Grant 2008). Given the short time since *P. downsi* was first collected on Galápagos (~ 44 years ago), this pattern may be a remarkable example of the adaptive strategies used by invasive insects to spread and reproduce successfully within foreign environments (Garcia-Ramos and Rodriguez 2002; Lambrinos 2004).

***Significance for the sterile insect technique***

By advancing our understanding of the impacts and ecology of *P. downsi* on the Galápagos Islands, this thesis provides a platform of knowledge from which to develop plans to control, and ultimately eradicate the undeniable threat of this destructive avian parasite. As an assessment of the viability of the sterile insect technique (SIT) on Galápagos commences (C. Causton, personal communication), information on the genetic structure and reproduction of *P. downsi* has become essential. The lack of evidence for cryptic species (determined by mitochondrial analysis) and relatively high gene flow found in *P. downsi* within and between islands is beneficial for SIT, which could now be implemented with reasonable certainty that *P. downsi* populations on the three islands are reproductively compatible, and would most likely mate successfully with a single sterile strain. Furthermore, now that a high frequency of

multiple mating in female *P. downsi* has been established, plans for SIT must consider this for predictive modelling in relation to sterile-male release effort, expected mating success and rates of population decline (Vreyson et al. 2006). Clearly, mating behaviour and breeding experiments of *P. downsi* sourced from different islands would greatly reinforce and complement the results of the current study.

***Future directions***

This thesis is among the very first attempts at characterising the reproductive biology and behaviour of *P. downsi* and will undoubtedly inform and inspire further investigations in the interest of conserving the endemic Galápagos avifauna. Admittedly, certain limitations of this study that could not be avoided are present and point towards some avenues for improvement. Firstly, the low polymorphism and the moderate number of microsatellites used and developed in this study deserve mention given the reduction in statistical power that this introduces to the genetic analysis (Pritchard et al. 2000). However, repeated and concerted attempts to obtain *P. downsi* microsatellites (Chapter 5) are testament that polymorphic microsatellite markers are not common in the genome of this dipteran.

Drought conditions in 2004 and 2005 resulted in very limited or no breeding in Darwin's finches and hence, an unbalanced sample size of *P. downsi* broods across species, habitats and islands. Unequal duration of time spent in the field on the various islands across years further contributed to the sampling design. For the molecular analysis, this resulted in some constraints on measures of allele frequency within islands across years, yet this was addressed through sub-sampling as best as possible (Chapters 6 and 7). Future sampling would benefit from trapping adult *P. downsi* in multiple geographic locations on each island in order to confirm the allele frequencies reported in the current study, while simultaneously gathering information on population densities. Despite attempts by O'Connor et al. in 2006 to catch *P. downsi* using various baits and trapping techniques, none were successful, though a recent discovery by Muth (2007) found that sweetened milk is an effective attractant, enabling adult trapping to be conducted in the future. Not only should this be on done on the Galápagos Islands, but also in regions of mainland South and Central America where *P. downsi* does or may occur (e.g. Ecuador, Trinidad, Brazil), such that invasion pathways can be reconstructed using genetic data.

Although research on the biology and ecology of *P. downsi* is in its infancy, large gaps in knowledge also exist concerning the impact of parasitism on Darwin's finches, such as effects on parental care, nestling behaviour, nestling immune response and implications for life-history trade-offs and host-parasite co-evolution. Plans to implement SIT on Galápagos will likely instigate a detailed investigation into the viability of this control method that will expand upon the content presented in this thesis. Meanwhile, strict quarantine measures must continue to be enforced between islands, and between the mainland and the Galápagos archipelago to prevent further spread and colonisations of *P. downsi*. The loss of Darwin's finches to parasitism would be an irretrievable loss to the biological sciences. Current declines in vulnerable Darwin finch species under threat from *P. downsi* highlight the significance of this thesis for the conservation of this unique and endemic group of island birds.

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