Unravelling the dynamics of hybridisation and its implications for ecology and conservation of Darwin's tree finches

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Degree of Doctor of Philosophy

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In memory of my beloved grandmother Oma Martha,
who would have been so proud.

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Thesis Summary

The significance of hybridisation for biodiversity has been the subject of a long-standing debate. Hybridisation has been characterised as being detrimental for biodiversity and speciation as it can blur the borders between distinct species. Contrastingly, hybridisation has also been described as a creative evolutionary process generating increased genetic variation and facilitating adaptation. Only few study systems enable us to observe hybridisation in real time, which has limited our knowledge of its consequences for the ecology and conservation management of contemporary species. This study investigates current hybridisation between two species of Darwin's finches (small tree finch *Camarhynchus parvulus* and medium tree finch *C. pauper*) on Floreana Island, Galápagos, Ecuador, and tests key variables related to foraging ecology, song, gene flow, and parasitism in hybrids and their two parental species.

The current ecological positions of hybrids in relation to parental species are important to identify possible selection pressures that could favour different phenotypes across vertical or horizontal clines. I examined foraging behaviour in relation to vertical habitat use in Darwin's tree finches and hybrid birds as the proportion of hybrids increased across the decade. Both parental species changed foraging height or behaviour with increasing hybrid density, while hybrid foraging behaviour was consistent across years. These findings suggest that parental species and hybrids may be experiencing different selection pressures, and the increasing hybrid abundance could be influencing the foraging behaviour of their parental species. Given the importance of rapid assessment for regular biodiversity monitoring, I investigated if hybrid birds could be acoustically identified, by comparing their song with song of the two parental species. While *C. pauper* had a distinct song, hybrid birds and *C. parvulus* song was indistinguishable and their respective populations could therefore not be surveyed individually. Acoustical surveys

across the decade 2004–2013 showed 52 % decline of the critically endangered *C. pauper*, highlighting the need for targeted conservation actions. Next, I examined the role of female choice as a driver of the hybridisation using a powerful combination of pairing observations and genetic analysis with nine microsatellite markers. I found that *C. pauper* females did not discriminate heterospecifics and frequently paired with *C. parvulus* males, while *C. parvulus* females were never observed to pair with *C. pauper* males. Hybrid females paired predominantly with hybrid and *C. parvulus* males, resulting in asymmetrical introgression with gene flow skewed towards *C. parvulus*. These findings support the formation of a hybrid swarm comprising *C. parvulus* and hybrids of various generations while *C. pauper* retains most of its genetic purity.

Reproductive success is a key measure of biological fitness. I analysed nesting success in *Camarhynchus* and *Geospiza fuliginosa* and identified parasite intensity due to larvae of the introduced fly *Philornis downsi*, whose parasitic larvae have been identified as the primary cause of nestling mortality. Hybrid birds had lowest in-nest *P. downsi* numbers, providing the first evidence of hybrid fitness in this system.

This thesis uses a combination of behavioural, genetic and monitoring methods to assess the survival of hybrids in a rapidly evolving vertebrate system. Under conditions of extreme natural selection from the recently introduced fly *P. downsi*, hybrid fitness was higher than that of the parental species as measured by fewer parasites per nests. I have identified the role of sexual selection in forming the hybrids via female choice of heterospecific males, and the role of natural selection in maintaining the hybrid offspring, It is my hope that the findings of this thesis will encourage conservation efforts of the Darwin's finch species complex including the hybrid birds.

Declaration

'I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.'

Katharina Johanne Peters

24.12.2015

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Statement of Authorship/Contribution

Chapters 1 & 6: KJP

Chapter 2:
Data collection: SK (2005, 2006, 2010), KJP (2013)
Statistical analyses: KJP, SK
Manuscript writing: KJP, SK

Chapter 3:
Data collection: SK (2004), JO'C (2008), KJP & SK (2013), KJP (2014)
Statistical analyses: KJP & SK
Manuscript writing: KJP & SK

Chapter 4:
Data collection: JO'C (2005, 2006, 2010), KJP (2012, 2013, 2014)
Laboratory analysis of DNA samples: KJP, JO'C
Statistical analyses: KJP, SM, RD, SK
Manuscript writing: KJP, SK, SM, RD, JO'C

Chapter 5:
Data collection: JO'C (2005, 2010), KJP (2012), KP & CE (2012, 2013, 2014)
Statistical analyses: KJP, SK
Manuscript writing: KJP, SK, CE

All research procedures reported in this thesis follow the guidelines for the use of animals in research (Flinders University, Charles Darwin Research Station, Directorate of the Galápagos National Park), 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.

Publications associated with this Thesis

Information from this thesis has been published in or submitted to peer reviewed journals as followed:

Peters, K.J., Kleindorfer, S., 2015. *Divergent foraging behaviour in a hybrid zone: Darwin's tree finches (*Camarhynchus *spp.) on Floreana Island.* Current Zoology **61**: 181-190.

Peters, K.J., Kleindorfer, S., in review. *Acoustic surveys cannot detect hybrids of Darwin's tree finches (*Camarhynchus *spp.) but confirm medium tree finch (*C. pauper*) decline.* Submitted to Bird Conservation International.

Peters, K.J., Myers, S., Dudaniec, R.Y., O'Connor, J.A., Kleindorfer, S., in review. Beggars can't be choosers: *Females drive asymmetrical introgressive hybridisation from rare to common species in Darwin's tree finches.* Submitted to Proceedings of the Royal Society B.

The manuscript based on Chapter 4 is currently in preparation to be submitted to the Journal of Evolutionary Biology.

Related publications (included in appendix):

Kleindorfer, S., **Peters, K.J**., Hohl, L., Sulloway, F.J., in press. *Flight behaviour of an introduced parasite affects its Galápagos Island hosts:* Philornis downsi *and Darwin's finches.* In: Biological Invasions and Animal Behaviour. *J. Weis, D. Sol (Eds.). Cambridge University Press, Cambridge, New York.*

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Chapter 1

Introduction

Anthropogenic effects on biodiversity

Biodiversity, defined as the overarching term comprising species diversity as well as genetic diversity, is understood to maintain ecosystem functioning by acting as an insurance in changing systems (Yachi and Loreau, 1999). In fluctuating environments, high levels of species diversity facilitate steady ecosystem functions as different species react differently to changing environmental conditions, and some can compensate for the failure of others, which provides a buffer and stabilises ecosystem processes (Chapin III et al., 2000; Yachi and Loreau, 1999). Similarly, high genetic diversity has evolutionary value as it increases a species adaptive capacity and facilitates persistence in changing conditions (Jump et al., 2009).

Humans are continuously altering the earth's environment on both local and global scales affecting climate (Oreskes, 2004), species distributions (Benning et al., 2002) and habitats (Watson et al., 2010), just to name a few. Human expansion and the accompanied alteration to the physical characteristics of the planet earth have led to an era of species extinctions, and the current global biodiversity loss is one of the most significant problems for humankind (Cardinale et al., 2012; Díaz et al., 2006; Duffy, 2003; Smith, 1994).

Hybridisation and biodiversity: a changing perspective

The biological species concept defines species as 'groups actually or potentially interbreeding natural populations that are reproductively isolated from other such groups' (Mayr, 1963). Since this concept rests on reproductive isolation as the

separating force between species, hybridisation, defined as the interbreeding of two species has had a negative reputation as being detrimental to biodiversity by blurring the barriers between species and thus affecting species integrity (Barton, 2001). Nevertheless, botanists have early on suggested hybridisation to be a potent driver of evolution (Anderson and Stebbins Jr, 1954; Stebbins Jr, 1950).

Hybridisation occurs across taxa and is particularly frequent in plants (Ellstrand et al., 1996), fish (Hubbs, 1955), and birds (Grant and Grant, 1992). The causes and consequences of hybridisation are species and situation specific, but some general patterns have been observed (Randler, 2002). For example, hybridisation seems to be more frequent when at least one of the involved species is rare, likely due to the lack of available conspecifics (Hubbs, 1955). Furthermore, hybridisation has been observed in environmentally disturbed systems following habitat alteration or fragmentation and the introduction of alien species (Anderson and Stebbins Jr, 1954; Seehausen et al., 2008a). Consequences of hybridisation largely depend on hybrid fitness, hybrid sterility or outbreeding depression. To date, various effects of hybridisation on biodiversity have been documented (Abbott et al., 2013). Species have been lost due to genetic swamping causing the disappearance of one or both paternal species (Levin et al., 1996; Rhymer and Simberloff, 1996; Roberts et al., 2010), novel hybrid species have arisen (Amaral et al., 2014; Hermansen et al., 2011), and existing species have increased their genetic diversity and adaptive potential via the introgression of genes from one species to another (Eroukhmanoff et al., 2013; Hamilton and Miller, 2015; Rieseberg et al., 2003). The latter especially has led to a shift in thinking about hybridisation: what was once considered detrimental to biodiversity and speciation is now understood to function as a vital evolutionary mechanism in many cases (Grant et al., 1996; Mallet, 2008).

Hybrids and conservation

Conservation of species rests on the assumption that the species in question are reproductively separated and distinguishable (Fitzpatrick et al., 2015). This classification is not necessary always reflected in nature, as the evolution of species is a gradual process. Species diverge, new species form, but boundaries between species can also dissolve and two species can merge into one. The presence of hybridisation makes conservation legislation and management difficult in many cases (Allendorf et al., 2001; Edmands, 2015; Fitzpatrick et al., 2015). The need for conservation of hybrids has often been dismissed in the past, especially when the hybridisation developed between a native and an introduced species (Allendorf et al., 2001). The increasing recognition of the evolutionary significance of hybridisation has stimulated a rethinking of conservation management of hybrids and hybridising species (Fitzpatrick and Bradley Shaffer, 2007; Garnett et al., 2011; Stronen and Paquet, 2013).

Challenges in researching hybridisation

Before the development of molecular analysis techniques in the 1960s, the detection of hybrid individuals was completely based on morphological characteristics (Allendorf et al., 2001). This was extremely limiting, as it had to be generally assumed that hybrids display intermediate morphological traits to their parental species, which is not always the case (Smith, 1992). Introgressed individuals in particular, are often indistinguishable from the parental species (Leary et al., 1996). Even when employing molecular techniques using highly polymorphic markers such as microsatellites, distinguishing between F1, F2 and backcrossed individuals requires large numbers of loci (> 40, Vähä and Primmer, 2006). Analyses of this scale are not always possible as they require considerable resources, and for some species numbers of available loci are much lower (< 20, Koskinen et al., 2004; Petren, 1998).

Advances in molecular techniques have facilitated the detection of hybrids (Sanz et al., 2008) and consequently, the records of hybridising species have increased substantially throughout the past years. Nevertheless, hybridisation is often only detected in retrospective and systems with currently undergoing hybridisation are rarely observed. This restricts opportunities for the study of ecological dynamics of hybridising species, such as how hybridisation affects the ecology and behaviour of the involved species (Chapter 2).

Although advances in the field of molecular biology have improved methods of hybrid detection, they usually require high levels of invasive sampling effort. This makes them problematic for the regular monitoring of endangered species that require rapid population assessment. Avian species are frequently surveyed using vocal cues (e.g. Dvorak et al., 2012; Dvorak et al., 2004; O'Connor et al., 2010c). Since hybrid song often resembles the song of one paternal species (Grant and Grant, 2014a), it is not clear whether it can be used as a cue to survey their abundance. Understanding how hybridisation impacts population assessment methods is crucial for an accurate estimation of abundance and population size, especially when endangered species are involved. I compared song characteristics of hybrids and parental species in an avian system to investigate options for rapid population assessment using acoustical surveys (Chapter 3).

Hybridisation in Darwin's tree finches

In birds, hybridisation is a relatively widespread process, being evident in at least 30 of the 39 orders (16 %) of known bird species in nature (Ottenburghs et al., 2015). Especially in species groups that diverged relatively recently, hybridisation is a common phenomenon as reproductive barriers had less time to solidify and prevent interspecific matings (Grant et al., 2005a).

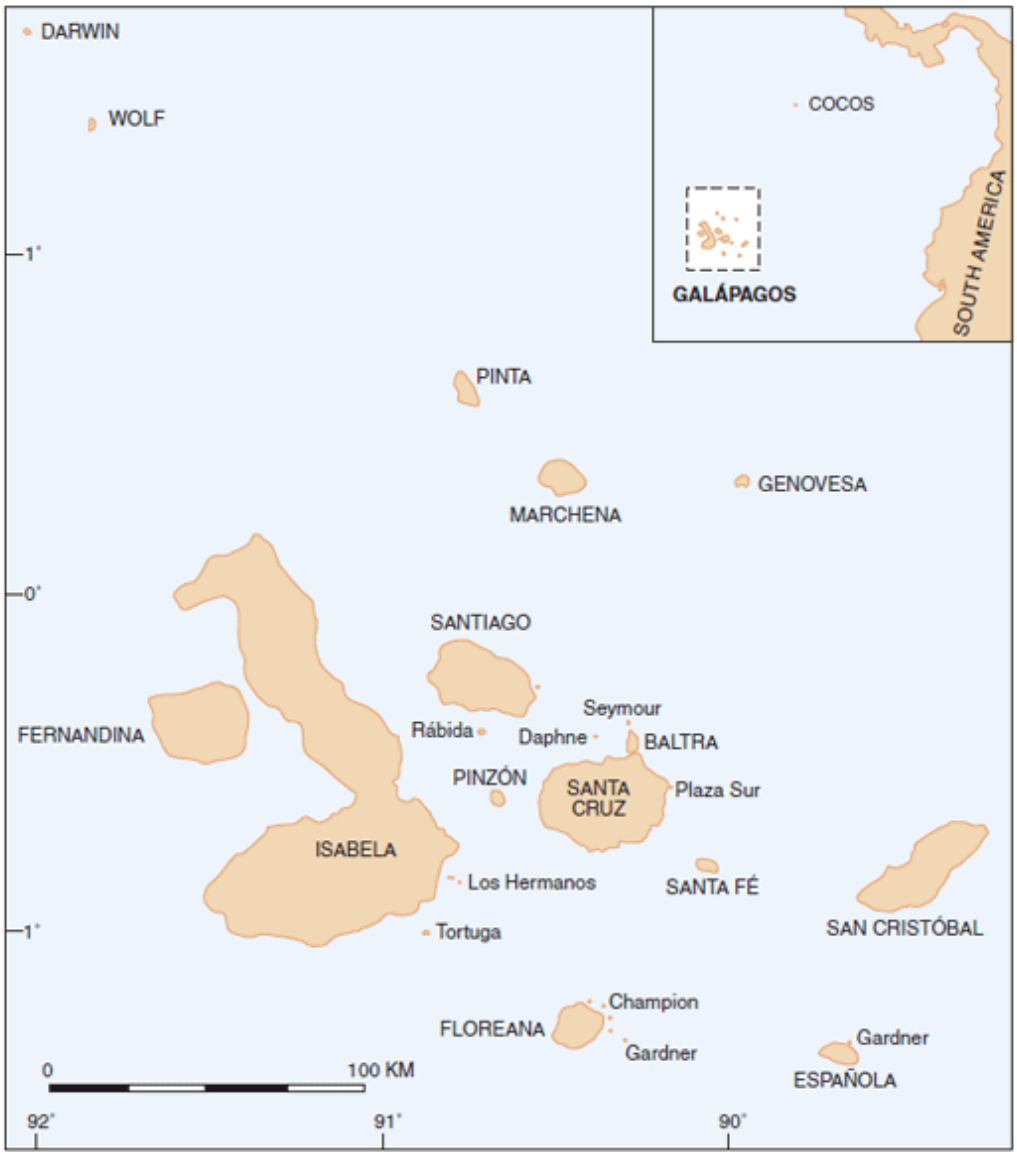

Figure 1.1 Map of Galápagos. Figure 1.1 from *How and Why Species Multiply: The Radiation of Darwin's Finches* by Peter R. Grant and B. Rosemary Grant. Copyright © 2008 by Princeton University Press. Reprinted by permission.

Darwin's finches (Passeriformes: Thraupidae) are considered model species for evolution, speciation and adaptive radiation. They are endemic to the Galápagos Islands, Ecuador (hereafter Galápagos) (Figure 1.1), which are often referred to as a natural laboratory, due to their isolation from humans, oscillating climate and only very few predators (Grant and Grant, 2008b; Schluter, 2000). Here the common ancestor of Darwin's finches arrived only ~ 2.3 million years ago (Sato et al., 2001)

and evolved into the 14 species of small passerines we know today (Grant, 1986). Due to these closely related species having diverged only recently, they still hold much adaptive potential for evolutionary mechanisms such as hybridisation.

Some degree of hybridisation is suspected for all Darwin's finch species (Grant et al., 2005a), and has been extensively documented for ground finches and cactus finches (*Geospiza* spp.) (Grant and Grant, 2014a). In tree finches (*Camarhynchus* spp.) it had only been assumed (Grant, 1986; Grant et al., 2005a; Lack, 1983) until the first case study by Kleindorfer et al. (2014a) who identified hybridisation between the small and medium tree finch (*C. parvulus*, *C. pauper*, Figure 1.2) on Floreana Island (Figure 1.1).

Camarhynchus parvulus and *C. pauper* inhabit the humid highland *Scalesia* forest (300–400m elevation, Figures 1.3 and 1.4) (O'Connor et al., 2010c). Nests are frequently predated by owls and rats, and recently infested by parasitic flies (discussed below) (O'Connor et al., 2010a). While the small tree finch is also found on other islands, the medium tree finch is endemic to Floreana (Lack, 1983). The IUCN conservation status of this species has recently been upgraded from 'vulnerable' to 'critically endangered' since its population is small, endemic and declining due to low nesting success which is strongly influenced by parasitism (O'Connor et al., 2010c).

Understanding the characteristics of hybridisation including its drivers and mechanisms is crucial for predicting the evolutionary and possibly demographic outcome for the involved species. Kleindorfer et al. (2014a) hypothesized that the hybridisation between *C. parvulus* and *C. pauper* on Floreana Island was driven by females of the rare *C. pauper* choosing males of the common *C. parvulus*. I used a powerful combination of molecular, morphological and pairing data collected over eight years to identify the dynamics of this hybridisation (Chapter 4).

Hybridisation in a parasitised system

Geographically isolated environments such as island ecosystems are often extremely vulnerable to introduced parasites since they usually have low genetic variability and lack coevolved defences since they have not been exposed to a variety of parasites before (Hochberg and Møller, 2001; Murray, 2001; Wikelski et al., 2004).

Figure 1.2 Small tree finch (*Camarhynchus parvulus*) female (upper left), medium tree finch (*Camarhynchus pauper)* male (upper right) and hybrid tree finch (lower). Photos by Katharina J. Peters.

Figure 1.3 Cerro Pajas Volcano on Floreana Island, Galápagos. Photo by Katharina J. Peters.

Figure 1.4 *Scalesia* forest at the base of Cerro Pajas volcano on Floreana Island, Galápagos. Photo by Katharina J. Peters.

Figure 1.5 Darwin's small tree finch male (*Camarhynchus parvulus*). This male is at least 5 years of age, recognizable by its black plumage coloration. Photo by Katharina J. Peters.

Figure 1.6 Small tree finch female (*Camarhynchus parvulus*) incubating in her beautifully build dome-shaped nest on Floreana Island, Galápagos. Photo by Katharina J. Peters

Species like the medium tree finch (*C. pauper*) are especially at risk due to their small range and endemism to only one island (Causton et al., 2006).

Multiple introductions of exotic species worldwide have demonstrated how a single species can have large-scale implications and strongly influence ecosystem functioning (Fessl et al., 2006b; Kenis et al., 2009; Mooney and Cleland, 2001; Strayer et al., 2006). The Dipteran fly *Philornis downsi* (Diptera: Muscidae, Figure1.7) was first discovered in nests of Darwin finches in 1997 (Fessl and Tebbich, 2002), although it has been introduced to the Galápagos before 1964 (Causton et al., 2006). *Philornis downsi* is currently considered one of the greatest threats to Galápagos land birds, in particular to the Darwin's finches (Cunninghame et al., 2012; Fessl et al., 2006b). Although adult flies are non-parasitic, they oviposit in bird nests where larvae hatch to feed internally on the nestlings (Fessl et al., 2006a; Fessl et al., 2006b; O'Connor et al., 2010b), causing blood loss, wounds and infections, deformed nares, reduced growth rates and reduced haemoglobin concentrations (O'Connor et al., 2010b) (Figures 1.8 and 1.9). As a consequence, brood mortality has been reported to range from 19 % up to 100 % (Dudaniec et al., 2006; Fessl et al., 2006a; Fessl et al., 2006b; Galligan and Kleindorfer, 2009; Kleindorfer et al., 2014b).

Hybridisation can serve as an evolutionary tool by increasing genetic diversity within a population and raising its adaptive potential (Tompkins et al., 2006). Furthermore, environmental change, habitat disturbance, and introduced species can accelerate the occurrence of hybridisation (Seehausen et al., 2008a). I investigated evidence for hybrid fitness and local adaptation in this young host-parasite system comprising Darwin's finches and P. downsi (Chapter 5).

Figure 1.7 *Philornis downsi* adult (left, photo by Katharina J. Peters) and larvae in finch nest (right, photo by Jody A. O'Connor).

Figure 1.8 Small ground finch nestling (*Geospiza fuliginosa*) dead in its nest due to *Philornis downsi* infestation. Damaged and blood crusted naris clearly visible. Photo by Katharina J. Peters.

Figure 1.9 Fledglings of small ground finch (*Geospiza fuliginosa*) (left) and tree finch (*Camarhynchus* spp.) (right) with deformed naris due to *Philornis downsi* infestation. Photos by Katharina J. Peters.

Thesis scope and objective

This study investigates the dynamics and consequences of the recently discovered hybridisation between two species of Darwin's finches (small tree finch *Camarhynchus parvulus* and medium tree finch *C. pauper*) on Floreana Island, Galápagos, Ecuador. My thesis aims to identify molecular and behavioural characteristics of this hybridisation and its implications for Darwin's tree finch behavioural ecology, and their conservation management.

Organisation of the thesis

This thesis consists of a series of manuscripts that are published, submitted or in preparation for publication in scientific, peer-reviewed journals. Because each data chapter is presented as an individual publication, some repetition of content has been unavoidable. The thesis contains one published paper (Chapter 2), two papers currently in review for publication (Chapter 3 & 4) and one paper in preparation for

submission (Chapter 5). I conclude the thesis with a general discussion of main findings, implications for conservation and suggestions for future research.

Chapter 2

Divergent foraging behaviour in a hybrid zone: Darwin's tree finches (*Camarhynchus* spp.) on Floreana Island

Katharina J. Peters and Sonia Kleindorfer

Current Zoology (2015) **61**(1): 181-190

Abstract

Hybrid speciation is increasingly recognized as a mechanism for novel evolutionary trajectories. However, we know very little about the ecology of a contact zone that has arisen in sympatry. This study examines the foraging behaviour and fitness of two species of Darwin's tree finches (*Camarhynchus parvulus* and *C. pauper*) and hybrid offspring on Floreana Island. Previous study showed that the percentage of hybrids in the tree finch population increased from 19 % in 2005 to 41 % in 2010, and their body and beak size increased by ~ 5 % (parental phenotype did not change). In 2005–06, all three tree finch groups (two parental species and hybrid birds) used the same foraging substrate, technique, and height. By 2010–13, the small tree finch (*C. parvulus*) had changed its foraging technique and the medium tree finch (*C. pauper*) had changed its foraging height. Both parental species had higher body condition when foraging at (divergent) mean foraging heights per species but hybrid birds did not. We discuss the implications of conserving forest to facilitate vertical niche expansion and the role of hybridisation for genetic persistence.

Introduction

The 14 species of Darwin's finches on the Galápagos Islands are a textbook example of adaptive radiation that have provided compelling evidence for both the magnitude and direction of selection in rapidly changing environments (Grant and Grant, 2014a; Grant and Grant, 2002; Grant and Grant, 2008b). Other well-known examples of adaptive radiations that have produced morphologically distinct descendent species include the ~ 150 species of Anoles lizards in the Caribbean (Losos, 2009), ~ 250 species of cichlid fishes in Lake Tanganyika, Tanzania (Burress, 2014; Takahashi and Koblmüller, 2011), and ~ 52 species of Hawaiian Honeycreepers (Pratt, 2005). While these case studies have greatly increased our understanding of the processes underpinning divergence and speciation, the ecology at the time of the divergence has rarely been observed directly. Therefore, there is little direct information about the ecological context of hybridisation events that occurred in the distant past.

Ecological speciation "involves the generation of reproductive isolation between populations as a result of ecologically based divergent selection pressures" (Price, 2008). When phenotypes adapt to local niches in heterogeneous environments, ecological divergence and speciation are favoured (Schluter, 2000). The biological species concept defines species as populations with little or no gene flow as the result of reproductive barriers (Price, 2008). Hybridisation occurs because the reproductive barrier between two species becomes porous or breaks down altogether (Mallet, 2005). Since hybridisation may cause species to collapse into a single swarm, it can lead to reduced species richness and "reverse speciation" (Grant and Grant, 2014b; Seehausen, 2006; Seehausen et al., 2008b; Taylor et al., 2006b). On the other hand, hybridisation may be a valuable source of genetic introgression that increases variance and gives rise to novel favourable genetic combinations (Barton, 2001; Burke and Arnold, 2001;

Grant and Grant, 1992, 1994; Grant et al., 1996). Viewed in this light, hybridisation can be the start of a new evolutionary lineage (Grant and Grant, 2014c).

Many extant species are the result of hybrid speciation that occurred thousands or millions of years ago (Johnston, 1969; Mallet, 2007; Price, 2008; Weir and Schluter, 2004). The ecology of previous hybridisation events is largely unknown, but range expansion and contraction of parental and hybrid lineages are often invoked to explain current distribution patterns of species of hybrid origin. There are at least 200 known hybrid zones, but there is no compelling evidence for a contact zone that has arisen in sympatry (Price, 2008). It is not known if hybrid offspring use different resources compared with the parental species at the time of genesis, and whether the subsequent genetic and morphological divergence of the novel hybrid genotype can occur in situ. The study of Darwin's tree finches (*Camarhynchus* spp.) on Floreana Island offers a rare opportunity to witness contemporary hybridisation and to test for ecological niche divergence in the contact zone in situ.

From 2005 to 2010, Darwin's tree finches on Floreana Island increased the proportion of hybrids from 19 % to 41 % (Kleindorfer et al., 2014a). During that same time period, hybrid body size increased by 5 % while body size in both parental species remained similar (Kleindorfer et al., 2014a). Given the increase in relative abundance of hybrids and their body size, we ask if hybrid and parental tree finches occupy novel ecological space in sympatry. We compare foraging behaviour (substrate, technique), foraging height, and fitness surrogates (body condition, time to successfully feed) to answer three questions. (1) Do hybrids and parental species occupy different foraging niches? (2) How have the foraging parameters changed over time comparing 2005–06 and 2010–13? (3) Is there evidence for fitness costs (lower body condition, longer time to success) related to foraging behaviour in parental species versus hybrid individuals?

Darwin's finches feed on a variety of foods depending on the species; the finches are renowned for having morphological adaptations that enhance fitness for processing different dietary items (Grant, 1986). Darwin's tree finches are insectivorous, but their diet includes vegetable matter (Christensen and Kleindorfer, 2009; Tebbich et al., 2004). Diet composition is further affected by rainfall because this influences food availability (De León et al., 2014; Tebbich et al., 2004). In a comparative study of foraging behaviour in arboreal Darwin's finches on Santa Cruz Island, Tebbich et al. (2004) found evidence for different foraging technique and substrate use among four Darwin's finch species including small tree finch (*C. parvulus*) and large tree finch (*C. psittacula*). Therefore, we predict that *C. parvulus* and *C. pauper* on Floreana Island will differ in foraging technique and substrate.

Methods

Study location

This study was carried out on Floreana Island in 2005, 2006, 2010, 2012, and 2013. Different fieldwork activities were conducted during the months January to March, which coincides with the onset of peak breeding activity in Darwin's finches. Birds were mist-netted and observed in the *Scalesia* forest at the base of Cerro Pajas volcano (1°17S, 90°27W, elevation 250–350m) (O'Connor et al., 2010c). The highland forest is dominated by the endemic trees *Scalesia pedunculata*, *Croton scouleri*, and *Zanthoxylum fagara*. Other main plant species are *Phoradendron henslowii* (Mistletoe), the shrub *Macraea laricifolia*, as well as several introduced fruit species (*Citrus limetta*, *Passiflora edulis*, *Psidium guajava*). Rainfall varies significantly across years on the Galápagos Islands (Grant and Boag, 1980), and is measured daily by the Galápagos National Park using rain gauges on Santa Cruz Island. The years 2005 and 2006 had lower rainfall from January to March (332mm, 118 mm respectively) while 2010, 2012, and 2013 had

higher annual rainfall (635mm, 672mm, 429mm respectively) (CDF Meteorological Database, http://www.darwinfoundation.org/datazone/climate/).

Study species across two study periods (2005–6 and 2010–13)

All guidebooks refer to three Darwin's tree finch species on Floreana Island: small tree finch, medium tree finch, and large tree finch (*C. parvulus*, *C. pauper* and *C. psittacula*, respectively). But a recent study by Kleindorfer et al. (2014a) found only two genetic groups and one hybrid cluster of tree finches on Floreana Island. The hybrid offspring were the result of pairings between females of the critically endangered *C. pauper* and males of the common (IUCN status: least concern) *C. parvulus* (Kleindorfer et al., 2014a). At present we do not know whether hybridisation extends beyond the F1 generation. Some level of introgression with *C. parvulus* is inferred due to ~ 15 % higher genetic diversity compared to *C. pauper* (Kleindorfer et al., 2014a). Unpublished data show that hybrid birds are fertile and offspring are viable, but more genetic analyses are required to gain insights into introgression patterns. The generation time for Darwin's finches is usually one year, but following extreme rainfall periods shorter generation times have been observed (Grant, 1986).

In this study we refer to the two parental populations (*C. parvulus*, *C. pauper*) and the hybrid birds. Hybrid birds increased in body size from 2005 to 2010 and were of intermediate body size between the parental species (Table 2.1) (Kleindorfer et al., 2014a). In the statistical analyses, the variable to denote the three groups is referred to as "genetic group".

Tree finch genetic assignment and morphology

We mist-netted, colour-banded and measured 47 *C. parvulus*, 61 hybrid finches, and 48 *C. pauper* (Table 2.1). We collected a blood sample for genetic analysis using

microsatellite markers and assigned all birds to their respective genetic populations (for details see Kleindorfer et al., 2014a). Given the difficulty of assessing a Floreana tree finch using morphology, we only included colour-banded birds with genetic assignment in this study. All foraging observations were done on colour-banded and genetically assigned birds. Using callipers, we measured the following morphological traits per bird: (1) beak length naris (mm), (2) beak depth (mm), (3) tarsus length (mm) and (4) wing length (mm). Body condition was calculated as the unstandardized residuals of mass against tarsus length (Brown, 1996). Since the mass of eggs significantly alters the body mass of female finches, we only calculated body condition for male birds.

Foraging behaviour, height, and time to foraging success

Foraging behaviour was recorded for 156 colour-banded birds observed during two weeks each in February (2005, 2006, 2010, 2012, 2013). SK collected all data on foraging behaviour and foraging height by walking a 1 km transect from 7–9 am and scoring every colour-banded bird observed to forage. There were four different transects (one per study plot), each transect was traversed once per day across four days and repeated once (8 days sampling). Most observations were made at close range (< 8 m) due to the generally tame character of the finches. To avoid pseudo-replication in the data, only the first foraging observation per bird was included for analysis (Kleindorfer et al., 2006; Myers et al., 2010). Table 2.2 lists the definitions for foraging substrate and technique used in this study. For analysis, we combined the foraging techniques "pick/glean" and "chip/pry". Additionally, SK visually estimated the height (m) at which the individual was foraging, and measured the time taken to successfully obtain a food item [time to success (s)] using a stop-watch from the moment a bird was first observed until it consumed a food item. The data collection was similar to

methods described in Christensen and Kleindorfer (2009) for foraging *Camarhynchus* tree finches on Floreana Island during the years 2005 and 2006. For this paper, the data analysis differs from Christensen and Kleindorfer (2009) because we only use colour-banded birds that have been assigned to a genetic population (Kleindorfer et al., 2014a); Christensen and Kleindorfer (2009) refer to the large tree finch (*C. psittacula*) while Kleindorfer et al. (2014a) make a case that the *C. psittacula* is locally extinct on Floreana Island. Christensen and Kleindorfer (2009) refer to 2005 as a dry year (< 30mm) but 2006 (~ 150mm) as a wet year given reports for rainfall in the local study site near Cerro Pajas (pers. comm. E. Egas, Galápagos National Park Service). In this study, we refer to lower rainfall conditions during 2005 and 2006 compared with 2010, 2012 and 2013. Although we do not explicitly analyse the effects of rainfall on foraging behaviour, it is an important factor for resource abundance, especially in Darwin's finches. For this reason, we had a short timeframe for foraging data collection, to control for rainfall and resource distribution and to be able to compare foraging in the three genetic populations at the same time of year.

Statistical analysis

We used Chi-square tests to compare foraging technique and substrate use across genetic population and year. We used analysis of variance to test for differences in foraging height, time to success and body condition across genetic population and year, and multivariate analysis of variance to test if foraging height and foraging time to success differed in relation to genetic population and foraging technique. Once we had identified that foraging height differed across tree finch groups, we wanted to test if birds had lower body condition above and below their mean foraging height. Visual inspection of the pattern between foraging height and body condition showed a curvilinear relationship between the two variables. Therefore, we used quadratic

regression within each genetic population to test if body condition was higher at a certain foraging height and lower for other foraging heights. All statistical analyses were performed using IBM SPSS Statistics 22.

Results

Morphological data

Table 2.1 presents the morphological data for male birds with known genetic assignment for the years 2005 and 2010. Hybrid birds became significantly larger within the five-year study period, while *C. parvulus* and *C. pauper* did not change significantly in morphology.

Comparison of foraging between tree finch groups

Combining the data across all years, the three tree finch groups did not differ significantly in foraging technique ($\chi^2 = 7.279$, df = 6, N = 157, $P = 0.296$, Table 2.3), foraging substrate ($\chi^2 = 9.461$, df = 8, N = 157, $P = 0.305$, Table 2.3) or foraging "time to success" (ANOVA: $F_{2,153} = 1.338$, $P = 0.265$, Table 2.3). However, they differed significantly in foraging height (ANOVA: $F_{2,154} = 5.353$, $P = 0.006$, Table 2.4, Figure 2.1).

Given the increase in proportion of hybrids from 2005 to 2010 (Kleindorfer et al., 2014a), we compare changes in foraging behaviour and height across these two time periods below.

Changes in foraging from 2005–06 to 2010–13

Foraging technique differed significantly across sampling period in *C. parvulus* (Likelihood Ratio: $\chi^2 = 11.08$, df = 3, N = 38, $P = 0.011$) but not *C. pauper* (Likelihood

Ratio: $\chi 2 = 7.581$, df = 3, N = 33, $P = 0.056$) or hybrid birds (Likelihood Ratio: $\chi 2 = 0.174$, df = 3, N = 86, $P = 0.98$), (Table 2.3). Comparing 2010–13 with 2005–06, *C. parvulus* used the foraging technique "pick/glean" more often (Fisher's exact test < 0.010) and "probe" less often (Fisher's exact test < 0.011).

Foraging time to success did not differ significantly across year or genetic group (ANOVA, all $P > 0.3$). But time to success differed significantly in relation to foraging technique ($F_{3,154} = 14.577$, $P = 0.001$, see also Table 2.4). Therefore, to test if the genetic groups differed significantly in foraging efficiency, we compared time to success per foraging technique. Correcting for year, only "pick/glean" differed significantly across genetic groups (ANOVA: $F_{2,76} = 3.583$, $P = 0.033$), and was fastest in *C. pauper* (2.9 ± 0.3 sec) compared with *C. parvulus* (3.5 ± 0.6 sec) or hybrid birds (3.6 ± 0.5 sec). Foraging time to success was longest for birds using the foraging technique "chip/pry" (14–26 sec), Table 2.5.

Substrate use per genetic population did not differ significantly across study periods. We found the same pattern of substrate use across the decade in *C. parvulus* (Likelihood Ratio: $\chi 2 = 5.73$, df = 4, N = 38, $P = 0.220$), hybrid birds (Likelihood Ratio: $\chi 2 = 4.85$, df = 4, N = 86, $P = 0.303$), and *C. pauper* (Likelihood Ratio: $\chi^2 = 5.69$, df = 4, N = 33, $P = 0.223$) (Table 2.3).

Foraging height showed different patterns across study years comparing 2005–06 and 2010–13 (Figure 2.1). There was no significant change in foraging height across years in *C. parvulus* or hybrid birds (ANOVA: *C. parvulus*: $F_{1,36} = 0.00$, $P = 0.993$; hybrid: $F_{1,84} = 0.273$, $P = 0.603$) but *C. pauper* foraged higher in the canopy in 2010–13 compared with 2005–06 ($F_{1,31} = 4.715$, $P = 0.038$).

Body condition and foraging height

Body condition differed significantly between genetic groups (ANOVA: $F_{2,207} = 7.377$, $P < 0.001$) but not year ($F_{1,207} = 0.147$, $P = 0.702$). The interaction term genetic group × year was not significant ($P > 0.8$). *Camarhynchus pauper* had higher body condition than *C. parvulus* or hybrid birds (Table 2.1).

We compared body condition and foraging height of individual birds observed to forage in 2010–13 (sample size was too small for 2005–06) (Figure 2.2). The quadratic regression was significant for *C. pauper* ($R^2 = 0.490$, $P = 0.009$) and *C. parvulus* ($R^2 = 0.411$, $P = 0.009$) but not hybrid birds ($R^2 = 0.027$, $P = 0.537$). The peak body condition occurred at the mean foraging height in each parental species, with no discernible pattern between body condition and foraging height in the hybrid birds (Figure 2.2).

Discussion

Given the recent contemporary hybridisation, Darwin's tree finches on Floreana Island provide a timely opportunity to test for changes in sympatric foraging niche in hybrid birds and parental species. The percentage of hybrid birds increased from 19 % in 2005 to 41 % in 2010 and they became ~ 5 % larger in body size (Kleindorfer et al., 2014a, Table 1). In this study, we compared foraging behaviour during 2005–06 and 2010–2013. Across these study periods, both parental species changed their foraging niche while the hybrid birds did not. In 2005–06, all three genetic groups (two parental species and hybrid birds) used the same foraging substrate, technique, and height. By 2010–13, the *C. parvulus* had changed its foraging technique and *C. pauper* had changed its foraging height. Both parental species had higher body condition when foraging at (divergent) mean foraging heights per species but hybrid birds did

not have higher body condition at any foraging height. Because of morphological overlap between the two parental species and hybrid birds, we restricted our analyses to birds with known population genetic assignment (Kleindorfer et al., 2014a). This decision limited our sample size and lowered the power of our analyses, but provided high confidence data. The rare opportunity to observe changes in ecological niche occupation at the time of increasing hybridisation is the justification for our decision to proceed with data analysis using the smaller sample size. Aware of the limitations of small sample size, our findings suggest that different selection pressures could be operating along (possible) adaptive fitness peaks in a vertical niche distribution.

Variation in resource distribution is predicted to promote trait divergence across environments (Jeffries and Lawton, 1984; Schluter, 2000). In this way, natural selection acts on phenotypes. It is often difficult to identify if local phenotypes occur as the result of habitat-phenotype matching via dispersal, for example, or local selection (Galligan et al., 2012; Galligan and Kleindorfer, 2010; Sulloway and Kleindorfer, 2013). The advantage of this study is that the hybrid birds were born into the study area, and hence we can rule out habitat-phenotype matching as the mechanism for the intermediate hybrid phenotype. Once a given phenotype occurs in a particular environment, what is its fitness? There is much debate about relative hybrid fitness (e.g. Arnold and Martin, 2010), especially for ecological analyses of the novel genetic introgression. Here, we use body condition and time to foraging success as measures of fitness, which we compare for the different foraging domains of the sympatric genetic groups. If local phenotypes are adapted to their current resource domain, we predict that they will extract resources with greater efficiency (shorter "time to success") and therefore have higher body condition. Animals with higher body condition are expected to have higher survival and more success rearing their young because they can more easily incur the high costs of reproduction (Golet and Irons, 1999; Reid,

1987). We found no evidence for higher hybrid fitness using these fitness surrogates. *Camarhynchus pauper* had the fastest foraging time to success, and both parental species had higher body condition when foraging within their species-specific mean foraging height.

Adult males of the critically endangered *C. pauper*, which are the largest birds in the Floreana tree finch group (see Table 2.1), had higher body condition than *C. parvulus* and hybrids. Since large-bodied animals can competitively exclude smaller individuals from foraging in preferred resource patches (Rowland, 1989; Schoener, 1983), it is possible that *C. pauper* expanded their foraging into the upper forest because they encountered more insects and more inflorescences from *S. pedunculata*. Our findings of higher body condition and faster foraging success support this interpretation, but more work needs to be done to quantify the (possible) mechanism of competitive exclusion in sympatry (Abbott et al., 1977; Diamond, 1978; Grant and Grant, 1982). Data on vertical resource distribution within the study site are needed to more fully understand vertical patterns of resource use.

Hybrid tree finches did not have higher body condition and were not more efficient at extracting resources than their parental species. Perhaps we chose poor measures of fitness indicators (body condition and time to success), which is why we did not detect a pattern in hybrid birds. Other fitness variables could include clutch size, hatching and fledging success, parasite intensity, recruitment, annual survival and population abundance. From previous survey data and population estimates on Floreana Island, we know that the critically endangered *C. pauper* population plummeted by 61 % from 2004 to 2008 (O'Connor et al., 2010c) but here we show that this species had the highest body condition. This finding seems counter-intuitive. Nesting success has been very low in medium tree finch, with 0 % fledging success since 2012 (Kleindorfer et al.,

2014b). In-nest chick mortality was caused by flesh-eating larvae of the introduced parasitic fly *Philornis downsi* (Dudaniec et al., 2010; Kleindorfer and Dudaniec, 2009; Kleindorfer et al., 2014b; O'Connor et al., 2010d). *Camarhynchus pauper* nests had more *P. downsi* larvae compared with most other Darwin's finch species (Dudaniec et al., 2007; Kleindorfer et al., 2014b). Although higher body condition is often linked with fewer parasites or higher survival under conditions of parasitism (Brown et al., 2000; Møller et al., 1998), this does not directly apply here because the parasite *P. downsi* consumes the blood of nestling birds and not adults (Dudaniec and Kleindorfer, 2006; Dudaniec et al., 2006; Fessl et al., 2006b; Huber, 2008). Therefore, even if adult *C. parvulus* and *C. pauper* had better body condition and higher adult survival than the hybrid birds, their numbers could still be declining due to low nesting success. Likewise, even if adult hybrids have lower body condition, population numbers could still increase if they have higher nesting success due to lower parasite infestation. These ideas need to be tested with longitudinal data. Future research on host-parasite dynamics (including hybrid nesting success in relation to parasite infestation) and the ecology of host and *P. downsi* interactions are needed to better understand possible trade-offs between signalling environment, foraging environment, and fitness (Endler, 1993, 1992; Endler and Basolo, 1998).

Phenotypes that are close to the adaptive fitness peak should have higher fitness (Benkman, 2003; Price, 2008; Schluter and Grant, 1984). We found highest body condition per parental species aligned with their mean foraging height, whereas this was not the case for hybrid birds. Using the logic of local adaptedness to fitness peaks, these findings suggest the possible existence of only two adaptive peaks for foraging – each occupied by one parental species. This reasoning could help to explain the local extinction of the large tree finch (*C. psittacula*) (Kleindorfer et al., 2014a) if its foraging niche was destroyed due to human impacts, for example. In the case of contemporary

hybrid fitness when both parental species occupy the adaptive fitness peaks, selection should not favour the intermediate phenotype of the hybrid birds and consequently the intermediate-sized hybrids might not persist for long.

Rainfall has a significant influence on resource availability and distribution (Grant and Grant, 1980), which influences foraging behaviour. A study on ground finches (*Geospiza* spp.) showed that during years with higher rainfall, the different *Geospiza* species largely overlapped in diets, while during years with lower rainfall (when food was less abundant), each species had a more specific "private" diet (De León et al., 2014). In our study period, we classified the years 2005 and 2006 as 'dry years' and 2010, 2012 and 2013 as 'wet years'. Therefore, our findings of foraging differences across time periods could reflect the underlying effect of rainfall. However our findings were opposite to those of Grant and Grant (2014a) and De León et al. (2014): we found foraging overlap during the low rainfall years (2005–06) and foraging differences during the high rainfall years (2010–2013) in *C. parvulus* and *C. pauper* but did not observe changes in the hybrid birds.

Conservation implications

The outcomes of this study are relevant to conservation biology for several reasons. First, there is widespread debate about the conservation value of hybrids if they dilute evolutionary significant units but generate biodiversity through genetic introgression and hybrid speciation (Allendorf et al., 2001; Barton, 2001; Fitzpatrick and Bradley Shaffer, 2007; Mallet, 2005; Soltis and Gitzendanner, 1999). Second, little is known about the ecology of hybridisation events, which hampers conservation management of such processes. More is known about the ecology of extant hybrid zones (Price, 2008). Third, there is growing evidence that anthropogenic influences may increase hybridisation rates through the introduction of competitor species, pathogens, and

increasing habitat destruction and fragmentation (Allendorf et al., 2001; Seehausen et al., 2008b). Therefore, understanding ecological resource use of hybrids in changing environments will generate insights into biological responses to a range of human-induced impacts. The forest on which Darwin's tree finches depend is highly threatened. On Santa Cruz Island, only 1 % of the *Scalesia* forest remains; it has been virtually cleared from San Cristobal and Isabela Islands (Watson et al., 2010). Floreana Island harbours the last significant remnant of endemic *Scalesia* forest supporting the observed increase in tree finch hybridisation over the past years. The remnant *Scalesia* forest is vulnerable to introduced plant and pest species (Watson et al., 2010). Besides habitat destruction and fragmentation, other significant threats for Darwin's tree finches include the spread of introduced species like *P. downsi* (Causton et al., 2006) and avian poxvirus (Kleindorfer and Dudaniec, 2006; Zylberberg et al., 2012). Our study shows that Darwin's tree finches use the entire vertical range of *Scalesia* forest (Kleindorfer, 2007; Kleindorfer and Dudaniec, 2009), and clearly, drastic efforts are needed to protect this vulnerable habitat.

The novelty of this study is the observation of sympatric habitat use in first generation hybrids and their two parental species. Despite a change in hybrid phenotype across the years, we did not find any evidence for different vertical habitat use by hybrid birds. We observed changes in the foraging behaviour of both parental species as well as higher fitness in relation to their species-specific mean foraging height. These findings could support the idea of two adaptive fitness peaks each occupied by one parental species.

Even though we did not find evidence for contemporary hybrid fitness exceeding parental fitness (using our crude measures of body condition and time to foraging success), the emergence of the hybrid birds raises several conservation issues linked to

fitness. Given the rapid population decline of the critically endangered *C. pauper*, the hybrid birds could a) serve as a valuable genetic reservoir for the species, and b) replace the ecological role of the declining *C. pauper*. Study is required to identify the risk of one or both of the parental species being swamped into a hybrid swarm.

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Table 2.1 Morphological trait values and body condition (mean ± SE) for males with genetic assignment to either parental species (small tree finch *Camarhynchus parvulus*, medium tree finch *C. pauper*) or hybrid birds on Floreana Island, Galápagos, during the years 2005 and 2010. The hybrid birds significantly increased in body size while the parental species had comparable body size across study periods, with the exception of beak depth in *C. parvulus*. The *P*-values of independent t-tests are shown as **P*-value < 0.05; ** *P*-value < 0.01; ****P*-value < 0.001.

	Small tree finch *Camarhynchus parvulus*		**Hybrid**		**Medium tree finch** *C. pauper*	
Trait	2005 (N = 28)	2010 (N = 19)	2005 (N = 18)	2010 (N = 43)	2005 (N = 22)	2010 (N = 26)
Beak length (mm)	7.4 ± 0.1	7.5 ± 0.1	7.5 ± 0.1	7.8 ± 0.1**	8.8 ± 0.1	8.8 ± 0.1
Beak depth (mm)	7.1 ± 0.0	7.4 ± 0.1***	7.4 ± 0.1	7.7 ± 0.1**	8.4 ± 0.1	8.5 ± 0.1
Tarsus length (mm)	20.3 ± 0.1	20.4 ± 0.2	20.5 ± 0.2	21.0 ± 0.2**	22.3 ± 0.2	22.4 ± 0.2
Wing length (mm)	62.0 ± 0.2	61.7 ± 0.0	63.0 ± 0.0	64.0 ± 0.3*	67.0 ± 1.0	68.0 ± 0.5
Body condition	-0.37 ± 0.3	-0.46 ± 0.3	-0.41 ± 0.5	-0.87 ± 0.2	1.22 ± 1.0	1.06 ± 0.3

Table 2.2 Definitions used for foraging substrates and techniques

	Name	Definition
Technique	Glean	Removing prey from foliage surface
	Bite	Ingesting part of food item
	Probe	Inserting beak into substrate
	Pick	Removing prey from non-foliage surface
	Chip off	Downward thrust of beak
	Pry off	Using beak to lift substrate
Substrate	Foliage	Live and dead foliage
	Bark	Live and dead bark, branches
	Flower	Live or dead flower, flower bud
	Moss	Moss and lichen
	Fruit	Berries
	Other	Ground, seed heads

Table 2.3 Foraging technique and foraging substrate in three genetic populations of Darwin's tree finches (*Camarhynchus* spp.) on Floreana Island, Galápagos, for two sampling periods (2005–06, 2010–13). Data are shown as percentage of observations (N).

		Small tree finch *C. parvulus*			**Hybrid *Camarhynchus***			**Medium tree finch *C. pauper***		
		All years	05/06	10–13	All years	05/06	10–13	All years	05/06	10–13
Technique	Pick/glean	59.5% (22)	33.3% (5)	77.3% (17)	46.5% (40)	40% (2)	46.9% (38)	45.5% (15)	0% (0)	53.6% (15)
	Bite	18.9% (7)	26.7% (4)	13.6% (3)	19.8% (17)	20% (1)	19.8% (16)	24.2% (8)	60% (3)	17.9% (5)
	Chip/pry	2.7% (1)	0% (0)	4.5% (1)	19.8% (17)	20% (1)	19.8% (16)	12.1% (4)	20% (1)	10.7% (3)
	Probe	18.9% (7)	40.0% (6)	4.5% (1)	14.0% (12)	20% (1)	13.6% (11)	18.2% (6)	20% (1)	17.9% (5)
Substrate	Bark	15.8% (6)	6.7% (1)	21.7% (5)	31.8% (27)	20.0% (1)	32.5% (26)	21.9% (7)	20.0% (1)	22.2% (6)
	Foliage	65.8% (25)	73.3% (11)	60.9% (14)	40.0% (34)	80.0% (4)	37.5% (30)	50% (16)	20.0% (1)	55.6% (15)
	Flower	10.5% (4)	13.3% (2)	8.7% (2)	11.8% (10)	0% (0)	12.5% (10)	9.4% (3)	20.0% (1)	7.4% (2)
	Fruit	5.3% (2)	0% (0)	8.7% (2)	15.3% (13)	0% (0)	16.3% (13)	15.6% (5)	20.0% (1)	14.8% (4)
	Other	2.6% (1)	6.7% (1)	0% (0)	1.2% (1)	0% (0)	1.3% (1)	3.1% (1)	20.0% (1)	0% (0)

Table 2.4 Multivariate analysis of variance in foraging behaviour of Darwin's tree finch groups on Floreana Island, Galápagos. The model tests the effects of genetic group (small tree finch *C. parvulus*, hybrid birds, medium tree finch *C. pauper*) and feeding technique on foraging height and time to foraging success.

	Dependent Variable	**SS**	**d.f.**	**MS**	**F**	**P**
Genetic group	Height	39.017	2	19.509	5.353	0.006
	Time to success	21.969	2	10.985	0.245	0.783
Technique	Height	35.334	3	11.778	3.232	0.024
	Time to success	1958.587	3	652.862	14.577	0.001
Genetic group * technique	Height	25.094	6	4.182	1.148	0.338
	Time to success	165.369	6	27.561	0.615	0.718

Table 2.5 Time to foraging success per foraging technique in three genetic groups of Darwin's tree finches (*Camarhynchus* spp.). The data are shown as mean ± SE (N). The F and *P*-values are shown for ANOVA tests per foraging technique with time to success as the dependent variable and genetic population as the fixed factor.

	Small tree finch *C. parvulus*	**Hybrid** *Camarhynchus*	**Medium tree finch** *C. pauper*	**F**	**P-value**
Pick/glean	3.5 ± 068 (22)	3.6 ± 0.5 (40)	2.9 ± 0.3 (15)	3.583	0.033
Bite	5.9 ± 3.2 (7)	3.5 ± 1.1 (17)	3.8 ± 1.6 (8)	0.254	0.777
Chip/pry	14 (1)	23.1 ± 3.3 (17)	26.0 ± 11.4 (4)	0.384	0.687
Probe	6.5 ± 1.3 (7)	4.9 ± 1.6 (12)	3.7 ± 0.6 (6)	0.203	0.818

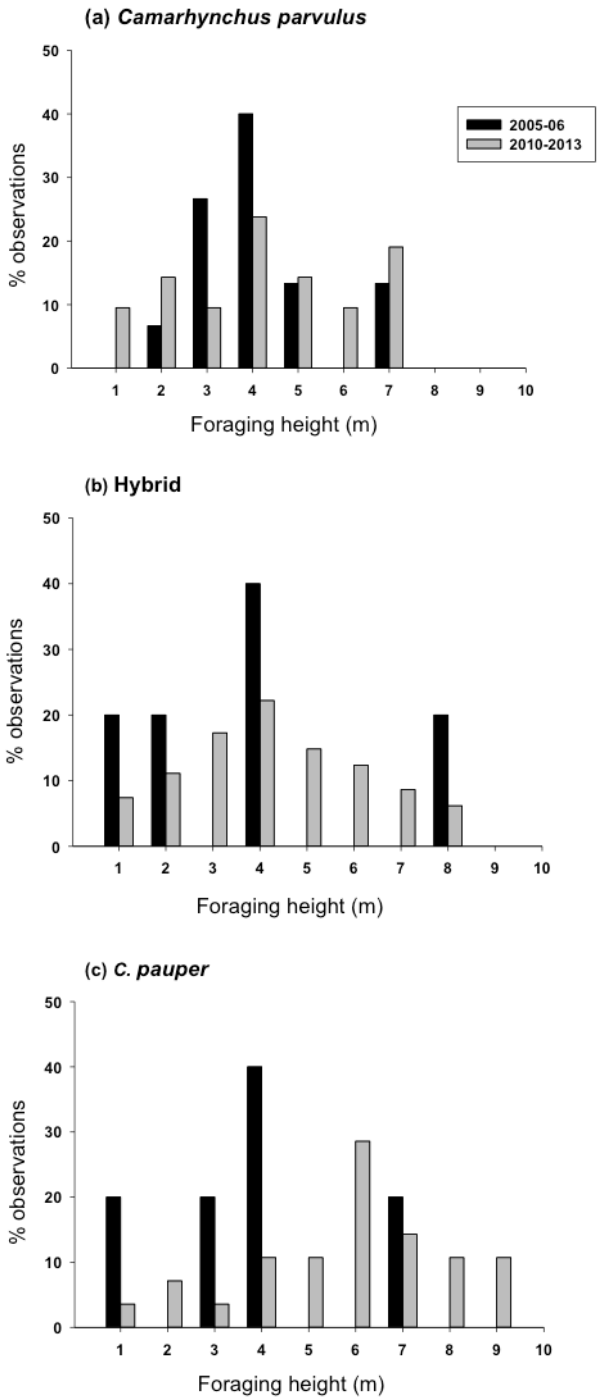

Figure 2.1 The percentage of observations per foraging height (m) for three genetic groups of Darwin's tree finches (small tree finch *Camarhynchus parvulus*, hybrid birds, medium tree finch *C. pauper*) on Floreana Island, Galápagos, across two study periods (2005–06, 2010–13). Mean foraging heights (m) were: *C. parvulus* (2005–06: 4.4 ± 0.4, 2010–13: 4.3 ± 0.4), hybrid (2005–06: 3.9 ± 1.1, 2010–13: 4.4 ± 0.2), *C. pauper* (2005–06: 3.6 ± 0.9, 2010–13: 5.9 ± 0.4).

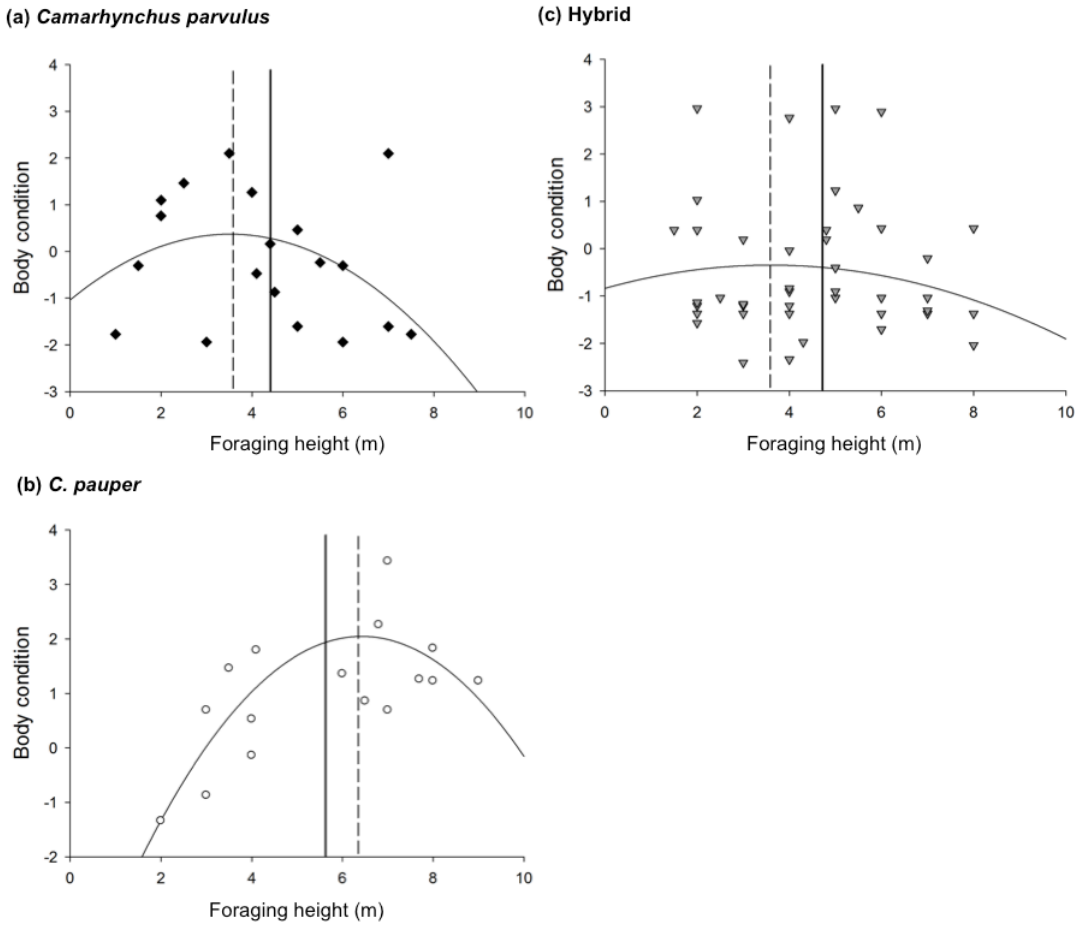

Figure 2.2 Body condition plotted against foraging height for (a) small tree finch *Camarhynchus parvulus*, (b) hybrid birds, and (c) medium tree finch *C. pauper*. Dotted line represents the peak of the regression curve and solid line indicates the mean foraging height. There was a significant quadratic regression in *C. parvulus* and *C. pauper* but not hybrid birds (see results).

Chapter 3

Acoustic surveys cannot detect hybrids of Darwin's tree finches (*Camarhynchus* spp.) but confirm medium tree finch (*C. pauper*) decline

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Abstract

Common species may be genetic reservoirs for the rare alleles of threatened species, and therefore hybridisation may contribute to maintaining biodiversity. While surveys often focus on endangered species, there is need to monitor changes in population status of common species currently undergoing hybridisation. Here we show that a traditional acoustical survey method was unable to distinguish hybrid birds from their common paternal species, but could be used to identify their critically endangered maternal species. The study species were the common small tree finch (*Camarhynchus parvulus*), the critically endangered medium tree finch (*C. pauper*), and the newly discovered hybrid group on Floreana Island, Galápagos. Song differed significantly between *C. pauper* versus *Camarhynchus parvulus* and hybrid birds, but not between *C. parvulus* versus hybrid birds. *C. pauper* declined by 52 % across the decade of study and *C. parvulus*/hybrid increased by 45 %. The findings highlight the need for new approaches to survey contemporary systems with hybridisation, also given the conservation risk of overlooking patterns of decline in "pure" species.

Introduction

Given the current rate of worldwide habitat and biodiversity loss (Baillie et al., 2004; Hails, 2008) and the associated rise in the need for population status information, rapid population assessment techniques are becoming increasingly important. Declining populations in vulnerable ecosystems are especially in need of strategic monitoring to make informed decisions about conservation actions (Sinclair et al., 2009). Given financial and time constraints, non-invasive survey techniques using reliable signals are a preferred option for population assessment in threatened species. Surveys demand the observed species to be distinguishable, which can be near impossible in closely related species, and when cryptic species or hybrid individuals are present (Dawson and Efford, 2009; Lambert and Mcdonald, 2014). In such cases, individuals can often only be identified to species using genetic analyses, which require sampling and sequencing and hence are costly and time consuming procedures (Hebert et al., 2004). Therefore, it is highly desirable to identify traits by which individuals of a species can be clearly and efficiently classified in the field, especially for endangered species that require regular monitoring.

Animal vocalisations are widely used to estimate their abundance given the benefit of sound travelling across water and vegetation with little attenuation. For this reason, both the presence and distance of an animal can be scored by an observer from a distance (Scott et al., 1981). Animal vocalisations have been used in ecological surveys assessing species abundance across taxa including amphibians (Driscoll, 1998), cetaceans (Marques et al., 2009) and birds (Dawson and Efford, 2009; Dvorak et al., 2012; O'Connor et al., 2010c). Among land birds, a survey can be conducted over a relatively vast and/or densely vegetated area even with modest effort since most birdsong can be heard from a distance (Dawson and Efford, 2009). Although in some

cases, two closely related species may have different vocalisations but be indistinguishable morphologically (e.g. Toews and Irwin, 2008), in other cases vocal differences may not be pronounced enough to justify their use as a species indicator. Given that 10-16% of bird species regularly hybridise (Grant and Grant, 1992; Ottenburghs et al., 2015), hybridisation is an additional factor that increases challenges associated with acoustical monitoring.

Darwin's finches are a model system for evolutionary biology with evidence for evolution and speciation by natural selection in the wild (e.g. Grant and Grant, 2014c), but also for species and population decline due to anthropogenic impacts (Dvorak et al., 2012; O'Connor et al., 2010c). Human activity on the Galápagos has resulted in introduced species and pathogens (Causton et al., 2006; Fessl et al., 2001; Fessl and Tebbich, 2002; Kleindorfer and Dudaniec, 2006; Schofield, 1989) as well as habitat loss from increasing human population and agricultural activity (Taylor et al., 2009; Watson et al., 2010). There is consensus about the importance of surveys to monitor endemic populations (Dvorak et al., 2012; Dvorak et al., 2004; O'Connor et al., 2010c), but Darwin's finches are difficult to identify at the best of times given shifts in morphology from interspecific competition (Schluter et al., 1985) and rapid evolution (Grant and Grant 2014). Darwin's finches also regularly hybridise (McKay and Zink, 2014), as has been shown in ground finches (*Geospiza* spp.) (Grant and Grant, 1997a; Grant et al., 2003; Grant et al., 2005a) and tree finches (*Camarhynchus* spp.) (Kleindorfer et al., 2014a). Identifying changes in gene flow between Darwin's finch species and populations is both challenging and necessary to inform our understanding of evolutionary dynamics in this rapidly evolving system.

This study assessed the performance of acoustical survey techniques in a species group with hybridisation. We analyse song in the common small tree finch (*C. parvulus*), the

critically endangered medium tree finch (*C. pauper*), and the recently discovered hybrid group (Kleindorfer et al., 2014a) on Floreana Island, Galápagos Archipelago. We compare song characteristics between the three groups to evaluate species identification based on song, and then apply the established techniques to assess changes in avian abundance from 2004 compared with 2008 and 2013. In addition to comparing population trends in Darwin's finches, we analyse population trends in other highland bird species.

Methods

Study site

The study site is situated at the base of the Cerro Pajas volcano on Floreana Island, Galápagos (173 km², 1° 28'S, 90° 48'W, Figure 3.1) and consists of humid highland forest dominated by the endemic tree *Scalesia pedunculata*. While 62% of the *Scalesia* forest remains intact, by 2010, 38% of this habitat on Floreana Island has been degraded through clearing for human settlement and agricultural purposes (Watson et al., 2010). Our on-going observations of Darwin's finches in this *Scalesia* habitat reveal growing threats from introduced plants, including creeping vines, which could destabilise the system and require careful monitoring. Despite these challenges, this remnant *Scalesia* forest on Floreana Island is the largest on the Galápagos Islands; it has virtually disappeared from San Cristobal and Isabela Islands and only 1-2 % remains on Santa Cruz Island (Dvorak et al., 2012; Watson et al., 2010). The *Scalesia* forest appears to be the preferred habitat for Darwin's tree finches (Kleindorfer et al., in press; Peters and Kleindorfer, 2015), though tree finches have been observed to sing from tall (>10m) non-*Scalesia* trees both within and adjacent to *Scalesia* habitat.

Study species

We analysed song characteristics of male Darwin's tree finches from Floreana Island in small tree finch (*C. parvulus*), medium tree finch (*C. pauper*), and the recently identified hybrid group, which are the result of pairings between the small tree finch and medium tree finch (Kleindorfer et al. 2014a). We aimed to record a comparable number of songs for each of the three genetic groups (for details on genetic analyses see Chapter 4); this balanced sampling was not achieved because we could only determine genetic assignment after data analysis and after song recordings had been made in the field. In total, we analysed morphology and song recordings from 9 *C. parvulus*, 19 *C. pauper*, and 49 hybrid birds. While *C. parvulus* exists on several other Galápagos Islands, the critically endangered *C. pauper* only occurs on Floreana Island (Grant, 1986; Lack, 1983).

Comparing song between Darwin's tree finches

We recorded the song of birds that had previously been colour-banded, measured and that were later genetically assigned to a genetic group using analysis of nine microsatellite loci following Kleindorfer et al. (2014a) and Peters et al. (in review). Darwin's finches do not appear to change their behaviour in the presence of human observers, and we were able to record song at close range (< 10m) using either a Sony DCD-100 DAT recorder or a Sony WMD6 Cassette Recorder with Sennheiser ME 80 directional microphone in 2006, and either a Telinga Twin Science parabolic microphone or a Bøse shotgun microphone from 2010 onwards. All recordings were made during the start of the breeding season between 07h00 and 10h00, which is the time of peak singing activity (Christensen et al., 2006). We recorded up to 15 songs of each individual bird from which we selected up to five of the best quality recordings per bird. From these recordings we calculated the mean of each song parameter for

each individual bird for subsequent analysis with Raven Pro 1.4 for Mac OS X (http://www.birds.cornell.edu/raven). We measured and analysed the following song parameters: song duration (s), minimum frequency (Hz), maximum frequency (Hz), frequency bandwidth (Hz; calculated by subtracting the minimum frequency from the maximum frequency), dominant frequency, number of syllables and trill rate (number of syllables/s). Spectrograms were created using a -24dB cut-off criterion relative to the peak power of the vocalisation with visual adjustment, following Podos (2001) and Goodale and Podos (2010).

Morphological analysis

Birds of the hybrid group have been reported to have intermediate body size between the smaller-bodied *C. parvulus* and the larger-bodied *C. pauper*, but there is much overlap in morphology (Kleindorfer et al., 2014a). We examined the following morphological variables across genetic groups: beak-head (mm), culmen length (mm), beak-naris (mm), tarsus length (mm) and wing length (mm). We compared morphology across genetic groups using analysis of variance (ANOVA).

Survey methods

We conducted point count surveys in February 2004, 2008 and 2013 using the variable circular plot method (for details see Martin et al., 1997; O'Connor et al., 2010c), which has been widely used to census Galápagos' birds (e.g. Dvorak et al., 2012; Dvorak et al., 2004; O'Connor et al., 2010c). We used a total of 15 point counts separated by 200m along the walking trail to the inner crater of Cerro Pajas volcano. At each point we conducted a 5-min survey during which we recorded the following: GPS co-ordinates, species, estimated distance of bird from observer (to the nearest 5m). During the survey the observers changed orientation from 0° to 90°, 180° and 270°. All surveys were conducted early in the breeding season between 06h00 and 12h00.

Due to the dense vegetation of the *Scalesia* forest habitat, visual census data are unreliable. Therefore, records of birds were included in the analysis only if they were heard, which also avoided the counting of non-singing females. In 2004 and 2008 small numbers of large tree finches (*C. psittacula*) had been recorded on Floreana (13 and 1, respectively) (O'Connor et al., 2010c), but recent genetic and morphological analyses suggest that *C. psittacula* did not occur on Floreana Island in 2004 and is likely locally extinct (Kleindorfer et al., 2014a). We therefore reanalysed the survey data from 2004 and 2008, and reclassified the records of *C. psittacula* as *C. pauper* as these two species both produce song with slower trill rate, and previously recorded *C. pauper* were likely identified incorrectly as *C. psittacula* (Bowman, 1983). Following song and morphology analysis (see results) we treated the *C. parvulus* and birds of the hybrid group as one entity (referred to as *C. parvulus*/hybrid group) for demographic analysis, given that it is not possible to distinguish these groups by song or morphology. Observers were Kleindorfer (2004), O'Connor (2008) and Kleindorfer and Peters (2013) who are all familiar with the resident bird species and their songs. In 2013, both observers conducted the survey at the same time.

Male population density calculation

Male population density estimates (number of birds/km^2) and detection probability estimates were explored using DISTANCE 6.0 (Thomas et al., 2006) but our dataset did not meet the assumptions required for analysis. Furthermore detection numbers were low for all species (all < 60) due to sampling restrictions inflicted by patchy and inaccessible habitat, and minimum detections recommended for calculating reliable density estimates using DISTANCE are 60–100 (Buckland et al., 2001). Since we were particularly interested in temporal abundance trends, we wanted to ensure comparability across years and use the same method for all years. We therefore

calculated male population density estimates (number of birds/km^2) using the inflection-point-per-species method following Reynolds et al. (1980). We obtained the number of birds/km^2 by dividing the total number of birds observed by the total observation area, and then dividing the result by 15 (number of survey points). Because two observers were used in 2013, we calculated bird densities for this year using the average of their two values per species. Inflection points varied across years, species and observers as specified in Appendix 1, and only birds observed within these ranges were included in population density and size estimate calculations. The density of the Galápagos flycatcher (*Myiarchus magnirostris*) needs to be interpreted with caution; due to their curious nature, these birds often follow observers and can be easily double counted (Dvorak et al., 2012).The singing activity of the dark-billed cuckoo (*Coccyzus melacoryphus*) can be very low and is not considered a reliable cue to detect cuckoo presence (Dvorak et al., 2012), and therefore our calculated density could be an underestimate. We are aware of the large group size differences of the smooth-billed ani (*Crotophaga ani*) which cause problems using point count surveys (Dvorak et al., 2012). Given that *C. ani* is a predator of Darwin's finches (Connett et al., 2013; O'Connor et al., 2010a) we included this species in our analyses but interpreted results with caution.

Avian population size estimates

We estimated the maximum male population size based on the maximum size of the available suitable habitat: 22.5 km^2 (O'Connor et al., 2010c). This area comprises Floreana's entire highland habitat (25 km^2) and excludes 2.5 km^2 that have been cleared for agriculture. Of the 22.5 km^2 non-agricultural highland area, about 3.71 km^2 are dominated by *Scalesia*, including the study site at Cerro Pajas (2.4 km^2) (O'Connor et al., 2010c). Thus, we sampled from 65% of the remnant *Scalesia* forest. We

conducted the survey from the Cerro Pajas area and used these data to estimate density for the total *Scalesia* and highland habitat (22.5 km^2). We assume that Darwin's finch density will differ across *Scalesia* patches; it is likely that our total density estimate will overestimate rather than underestimate Darwin's tree finch density because *S. pedunculata* dominates just 16.5% of the total highland area.

We only estimated the population sizes for Darwin's tree finches, as their preferred nesting is in *Scalesia* forest, which occurs at Cerro Pajas and Asilo de la Paz, while the other species also breed elsewhere on the island.

Statistical analyses

Male population density estimates were calculated in Microsoft Office Excel following Reynolds et al. (1980); all other statistical analyses were performed using IBM SPSS Statistics 22. Graphs were produced using SigmaPlot 12.0. We examined data for normality before using parametric tests. Because data were collected across years, we first assessed if song and morphology differed across years using multivariate analysis of variance for the interaction effect of 'year' and 'genetic group'. Two variables were transformed to meet assumptions of normality: maximum frequency and trill rate (reflect and square root transformation) and beak width (inverse transformation). We checked the data for homogeneity of variance using Levene's test. The variables for minimum frequency, duration, number of syllables showed homogenous variances and were analysed using ANOVA with Tukey HSD post-hoc test. The variables trill rate, maximum frequency and frequency bandwidth violated the assumption of homogeneity of variance (all $P < 0.03$) and were therefore analysed using Welch's ANOVA with Games-Howell post-hoc tests. The variables number of syllables, dominant frequency, beak length head, beak length nostril and beak depth violated

assumptions of normality and were analysed using Kruskal-Wallis test for independent samples with pairwise comparisons as post-hoc tests.

Results

Species determination based on song

We obtained a total of 325 song recordings from 77 genetically identified Darwin's finches across four years (2006 N = 14, 2010 N = 22, 2013 N = 36, 2014 N = 5, Table 3.1). There was no significant interaction effect of 'year' and 'genetic group' (MANOVA: Pillai's Trace = 0.389, $F_{30,325} = 0.915$, $P = 0.599$) and therefore we pooled the data across years. Tree finch (*Camarhynchus* spp.) song did not differ significantly between genetic groups for the variables maximum frequency, song duration, and number of syllables. However, there were significant differences across genetic groups in minimum frequency, dominant frequency, frequency bandwidth, and trill rate (ANOVA: minimum frequency $F_{2,76} = 16.745$, $P < 0.01$, Welch's ANOVA: frequency bandwidth $F_{2,76} = 8.077$, $P = 0.003$, trill rate $F_{2,19.776} = 12.197$, $P > 0.001$, Kruskal-Wallis test: dominant frequency $F_2 = 21.813$, $P < 0.001$,Table 3.1). Effect size was calculated using eta squared (minimum frequency = 0.31, frequency bandwidth = 0.11, trill rate = 0.12). Post-hoc comparison showed that *C. pauper* had a lower minimum and dominant frequency, a broader frequency bandwidth, and a slower trill rate than *C. parvulus* and hybrid birds (all $P > 0.04$), but there was no significant difference between the song of *C. parvulus* and hybrid birds (all $P > 0.51$). Hybrid birds and *C. parvulus* had significantly larger variance than *C. pauper* for maximum frequency, trill rate and frequency bandwidth (Levene's test for homogeneity of variance, maximum frequency: $F_{2,74} = 3.979$, $P = 0.023$, trill rate: $F_{2,74} = 8.028$, $P = 0.001$, frequency bandwidth: $F_{2,74} = 4.712$, $P = 0.012$, Figure 3.2).

Morphology analysis

There was no significant interaction effect between 'year' and 'genetic group' for morphology (MANOVA: Pillai's Trace = 0.707, $F_{35,310} = 1.458$, $P = 0.05$); therefore we pooled data across years for morphological analysis. *Camarhynchus pauper* was significantly larger in all analysed variables (post-hoc tests all $P < 0.04$, Table 3.2), but *C. parvulus* and hybrid birds were morphologically indistinguishable (post-hoc tests all $P > 0.79$, Table 3.2).

Avian population density and population size estimates

Using the respective inflection points per species and year as a threshold for data inclusion, our avian surveys at Cerro Pajas generated 362 bird records from 9 species across the three survey years (2004 = 133, 2008 = 152, 2013 = 77, Table 3.3). As shown in Table 3.3, *C. pauper* abundance declined by 52 % from 2004 to 2013, and *C. parvulus*/hybrid group numbers increased by 45 %. Two other species showed patterns of decline: medium ground finch (*G. fortis*) (-76 %) and dark-billed cuckoo (*Coccyzus melacoryphus*) (-95 %). Four other highland species showed patterns of increase: Galápagos flycatcher (*Myiarchus magnirostris*) (+11 %), small ground finch (*G. fuliginosa*) (+23 %), yellow warbler (*Dendroica petechia*) (+256 %), and smooth-billed ani (*C. ani*) (+254 %). Neither the warbler finch (*Certhidea fusca*) nor the large tree finch (C. *psittacula*) were detected.

Discussion

Main findings for song analyses and population estimates

Hybridisation created a considerable obstacle for species detection using acoustic surveys in Darwin's tree finches due to the acoustical similarity between hybrid birds and *C. parvulus*. In contrast to the similar song in the common *C. parvulus* and birds of

the hybrid group, the song of the critically endangered *C. pauper* was different in several variables. Therefore, song could not be used to estimate the abundance of *C. parvulus* and birds of the hybrid group separately, but could be used to monitor the abundance of the endemic *C. pauper*. Our acoustic survey results showed that *C. pauper* declined by 52 % over the past nine years, while the *C. parvulus*/hybrid group increased by 45 %. These results underscore the warranted conservation concern for the critically endangered *C. pauper*. Because we cannot distinguish *C. parvulus* from the birds of the hybrid group using song or morphology, only genetic analysis can reveal the population trends for *C. parvulus* relative to the hybrid group.

Differences in song and morphology in Darwin's tree finches

Compared with *C. parvulus* and birds of the hybrid group, song of *C. pauper* had a slower trill rate (fewer syllables per sec), broader frequency bandwidth, lower minimum frequency, and lower dominant frequency. For the variables trill rate, maximum frequency and frequency bandwidth, *C. pauper* song had significantly less variance than song of *C. parvulus* and birds of the hybrid group. The duration of the song, the number of syllables per song, and the maximum frequency was comparable between *C. parvulus* and the hybrid group and *C. pauper*, and therefore these variables should not be used for species identification.

Camarhynchus pauper was significantly larger in all analysed morphological variables, but *C. parvulus* and birds of the hybrid group could not be distinguished morphologically. This poses a further challenge to the population assessment of these two groups, as at present genetic analyses seem to be the only method to determine the group membership of individuals.

The finding that there were no significant differences in song characteristics between *C. parvulus* and hybrid birds has several possible explanations including small sample

size, lack of time or selection for divergence, and the role of vocal tutors for learning of song type. The sample size for song recordings of *C. parvulus* (N = 9) was much smaller than that for hybrids (N = 49). Despite our efforts to sample equally from all three tree finch groups, post-hoc genetic assignment revealed the high relative abundance of birds of the hybrid group on Floreana Island, which explains the higher number of recorded hybrid songs. The skew in sample size could be one factor that explains why we did not detect a difference in song between *C. parvulus* and hybrid birds, especially given the much larger variance in these two genetic groups compared to *C. pauper* (see Figure 3.3). Birds of the hybrid group and *C. parvulus* overlapped in morphology (see also Kleindorfer et al. 2014a). Kleindorfer et al. (2014a) hypothesised that the observed tree finch hybridisation is largely the result of *C. pauper* females pairing with *C. parvulus* males, which was concluded based on the finding that larger-bodied females (presumably *C. pauper*) frequently paired with smaller-bodied males (presumably *C. parvulus*) whereas smaller-bodied females (presumably *C. parvulus*) always paired with smaller-bodied males (presumably *C. parvulus*). Darwin's finches learn song from a male tutor which is usually their father (Grant and Grant, 1996a), therefore it is likely that hybrid sons would have learned their song from their *C. parvulus* fathers and would sing a *C. parvulus* song. A similar pattern of song learning, and hence a possible mechanism for backcrossing in favour of the paternal genetic lineage, was previously shown in *Geospiza* hybrids (Grant and Grant, 1997a; Grant and Grant, 2014d). These possibilities require further investigation.

Survey results for Darwin's tree finches

The survey results showed a 52 % decline in *C. pauper* from 2004–2013; male *C. pauper* density (in birds/km^2) was 5,265 (2004), 2,292 (2008) and 2,537 (2013). The absolute numbers of *C. pauper* differ from those reported for 2004 and 2008 in O'Connor et al.

(2010b); because we only used singing males and allocated any *C. psittacula* (N = 14) (now considered locally extinct) during 2004 and 2008 as *C. pauper*, our estimate of population decline in *C. pauper* between 2004 and 2008 was 56 %, whereas O'Connor et al. (2010b) estimated the decline to be 62 %. In contrast, birds of the *C. parvulus*/hybrid group increased 45 % across the decade with densities of 4,095 (2004), 8,212 (2008) and 5,917 (2013).

According to criteria established by the IUCN, *C. parvulus* is classified as being of 'least concern' (The IUCN Red List of Threatened Species. Version 2014.3, www.iucnredlist.org). However, the hybridisation among tree finches on Floreana Island makes its actual status uncertain. To date we have insufficient information on the makeup of the hybrid group, but unpublished data suggest that the hybridisation extends well beyond F1. Genetic introgression from *C. pauper* to *C. parvulus* has been suspected previously (Kleindorfer et al., 2014a) and most recent analyses show evidence for extensive asymmetrical gene flow towards *C. parvulus* (Peters et al., in review). The acoustic and morphological similarity of *C. parvulus* and the hybrid group presented here supports the scenario that backcrossing has already occurred and the hybrid group does not consist of first generation hybrids but rather comprises later generation hybrids and introgressed individuals (Derégnaucourt et al., 2001). A reliable classification of the conservation status of the Floreana *C. parvulus* population will depend on results of detailed genetic analyses.

Floreana Island has the longest history of human settlement and activity (Lack, 1983; Steadman, 1986; Watson et al., 2010) and the highest number of species extinctions across the Galápagos Archipelago. Three bird species (large ground finch, *G. magnirostris*; sharp-beaked ground finch, *G. difficilis* and Floreana mockingbird, *Mimus trifasciatus*) have become locally extinct over the past 200 years (1835–2005) (Grant et

al., 2000; Grant et al., 2005b; Merlen, 2013a). The warbler finch (*Certhidea fusca*) (Grant et al., 2005b), the vermilion flycatcher (*Pyrocephalus rubinus*) (O'Connor et al., 2010c), and *C. psittacula* (Kleindorfer et al., 2014a) are either currently locally extinct or likely to become locally extinct in the near future (discussed below). The vegetarian finch (*Platyspiza crassirostris*) was only heard once by our group in the highlands in 2010, and hence could also be considered very rare (Kleindorfer pers. observation).

There are multiple possible causes for population declines including habitat fragmentation, habitat loss, introduced species and pathogens (Wiedenfeld and Jiménez-Uzcátegui, 2008), which can be particularly problematic for small and range restricted populations (Simberloff, 1995). Less than 62% of the original *Scalesia* forest persists on Floreana Island given land clearing for human activities. The remaining *Scalesia* habitat is under increasing pressure from introduced flora (Mauchamp, 1997; Rentería et al., 2012) and fauna (Jiménez-Uzcátegui et al., 2008; Whiteman et al., 2005), such as black and Norwegian rats (*Rattus rattus, R. norvegicus*) (Cimadom et al., 2014; Fessl et al., 2010; O'Connor et al., 2010a; Towns et al., 2006), cats (*Felis catus*) (Jiménez-Uzcátegui et al., 2008), smooth-billed anis (*C. ani*) (Connett et al., 2013) and the introduced Dipteran *Philornis downsi.*

Philornis downsi is considered the biggest threat to Darwin's finch survival and to breeding success in Galápagos land birds in general (Cimadom et al., 2014; Dudaniec and Kleindorfer, 2006; Fessl et al., 2006a; Huber, 2008; Kleindorfer et al., 2014b; O'Connor et al., 2010d). Both parasite intensity and Darwin's finch mortality have increased across the past decade (Kleindorfer et al., 2014b; O'Connor et al., 2010d). The available data on impacts of *P. downsi* suggest that low annual recruitment in *C. pauper* is the main explanation for its critical decline (O'Connor et al., 2010d). Given the 45% increase in numbers of the *C. parvulus*/hybrid group, another factor

contributing to the *C. pauper* decline could be selection favouring hybridisation with *C. parvulus*. If hybrid birds have higher fitness (Kleindorfer et al. 2014a) and if hybrid offspring backcross with other hybrids or *C. parvulus*, this will increase recruitment of the *C. parvulus*/hybrid group rather than the *C. pauper* group.

Camarhynchus psittacula has always been rare on Floreana Island (discussed in Grant et al., 2005b; Kleindorfer et al., 2014a). Our repeated survey and nest monitoring efforts support the view that *C. psittacula* is locally extinct on Floreana Island (Kleindorfer, unpublished data). Because we only surveyed at one location, it is possible that this species exists elsewhere on the island. However, we have traversed the island widely for various reasons, and consider that we have not heard *C. psittacula* song, which we tested by comparing our recordings with historical 1960s recordings from Robert Bowman from both Floreana and Santa Cruz Islands (Kleindorfer, unpublished data).

Implications for conservation and survey techniques

The finding that *C. parvulus* on Floreana Island cannot be clearly distinguished from birds of the hybrid group, based on their song, renders acoustic surveys for monitoring their population unfeasible. Given that the majority of songbird species learn song from an adult tutor which is usually their father (Catchpole and Slater, 2003), hybrids are generally likely to sing the song of their paternal species, and therefore other systems with contemporary hybridisation may show the same pattern we present here. High levels of hybridisation in other species have led to genetic and demographic swamping of one or both of the parental species by the hybrids (Rhymer and Simberloff, 1996; Roberts et al., 2010). However, especially rare species can benefit from hybridisation as it increases their often depleted genetic diversity and possibly their fitness and adaptive potential (Baskett and Gomulkiewicz, 2011; Hamilton and Miller, 2015). *Camarhynchus parvulus* could constitute an important source of genetic

variation for the critically endangered *C. pauper*. The hybrid group could serve as a genetic reservoir preserving the genes of an endemic and declining species, in which case all three genetic groups and their habitat should be conserved (López-Pujol et al., 2012).

Because hybridisation usually occurs between already closely related species, detection of hybrids is complicated as it often relies on molecular analyses, and especially backcrosses and later generation hybrids cannot be determined using morphological characters only (Allendorf et al., 2001). In most cases across taxa, hybridisation therefore relies on genetic analyses, for example in wolves (*Canis lupus*) and dogs (*C. familiaris*) (Andersone et al., 2002; Vilà et al., 2003), wild and domestic cats (*Felis silvestris, F. catus*) (Daniels et al., 1998; Randi et al., 2001), greater and lesser spotted eagles (*Aquila clanga* and *A. pomarina*) (Väli et al., 2010) and Hawaiian ducks (*Anas wyvilliana*) and introduced mallards (*A. platyrhynchos*). Hybridisation therefore makes rapid population assessment practically impossible in many species, which is especially problematic when threatened species are involved that require regular monitoring. In the case of the Floreana tree finch group, the distinct song of *C. pauper* means that acoustical identification can be retained for surveys, which is a significant finding given the critically endangered status of this endemic and declining species.

Survey results for other bird species

While this study focussed on the *Camarhynchus* tree finches, we present the findings for other bird species in Table 3.3. We provide comment here on the introduced, and the very rare or possibly locally extinct species known for Floreana Island. *Crotophaga ani* was introduced to Galápagos in the 1960s to consume the ticks off of cattle; but analysis of gizzard contents found Darwin's finch remains instead (Connett et al., 2013; O'Connor et al., 2010a; Olivares and Munves, 1973). Therefore, the increase in *C.*

ani could be a threat to populations of songbirds. The extreme drought across the Galápagos from 2002–2007 (CDF Meteorological Database, http://www.darwinfoundation.org/datazone/climate/) is suspected to have negatively influenced insectivorous and frugivorous species in particular. The vegetarian finch (*Platyspiza crassirostris*) and the vermilion flycatcher (*Pyrocephalus rubinus*) used to be relatively common in the Floreana highland forest, although there is no information about former population size, and abundance has mainly been inferred from statements made by locals and the previously high numbers of collected specimens (*P. crassirostris*: 48 in 1905/06, 3 in 1962, 1 in 1974, *P. rubinus*: 7 between 1888–1891, 133 between 1898–1906 and 10 in 1962) (Merlen, 2013a; O'Connor et al., 2010c; Wiedenfeld, 2006). We have only one sighting of *P. crassirostris* since 2004, and individuals of *P. rubinus* have not been seen since 2008 (person. comm. Walter Cruz, K. J. Peters). In the case of the warbler finch (*C. fusca*), several targeted surveys by Grant *et al.* (2005b) during the breeding season in 1979, 1983, 1997, 1999 and 2004 using species-specific playback to stimulate a response, failed to locate any *C. fusca* on Floreana Island; but O'Connor *et al.* (2010c) reported to have heard a male *C. fusca* singing at Asilo de la Paz in 2008. This species is suspected to be locally extinct or at least extremely rare on Floreana, and the fact that this study did not observe any *C. fusca* supports this view.

Conclusion

Acoustical survey techniques could not reliably detect Darwin's tree finch hybrids. Song was a reliable tool to distinguish the critically endangered *C. pauper*, but song was the same in common *C. parvulus* and birds of the hybrid group. The endemic *C. pauper* population, which only occurs on Floreana Island, is continuing its rapid 52 % decline across the decade. Recent evidence suggests substantial introgression from hybrids into the *C. parvulus* population in the Cerro Pajas Region (Kleindorfer et al.

2014; Peters et al., in preparation). Therefore, our second major finding that the population of *C. parvulus* and birds of the hybrid group has increased across the decade requires further investigation as we cannot ascertain actual size estimates for each respective population without genetic analysis, and it could mask an undercurrent of decline in *C. parvulus*. Repeated bird surveys across the decade show a range of patterns in populations: several species showed a marked increase (including an introduced avian predator), other species showed a noticeable decline (including the locally endemic *C. pauper*). Hybridisation may be a driver of biodiversity and adaptive capacity if alleles from rare species are introgressed into common species, but hybridisation can hamper reliable population estimates of common species when the two groups become acoustically and visually indistinguishable.

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Table 3.1 Male song characteristics in three Darwin's tree finch (*Camarhynchus* spp.) genetic groups. Data are shown as mean ± SE (95 %CI). The sample size per genetic group is shown in brackets. Songs were recorded from colour-banded birds in the field and retrospectively assigned to species/group after laboratory analysis of genetic samples.

Genetic group (N)	Minimum frequency (Hz)	Maximum frequency (Hz)	Frequency bandwidth (Hz)	Dominant frequency (Hz)	Song duration (s)	Number of syllables/song	Trill rate (number of syllables/s)
Small tree finch (*Camarhynchus parvulus*) (N = 9)	2484.3 ± 89.9 (2277.0–2691.6)	5797.6 ± 308.2 (5086.8–6508.3)	3313.2 ± 304.0 (2612.1–4014.3)	4070.3 ± 160.6 (3700.0–4440.6)	1.2 ± 0.1 (1.0–1.4)	7.4 ± 0.1 (5.1–9.7)	6.1 ± 0.7 (4.5–7.7)
Hybrid group (N = 49)	2464.9 ± 33.5 (2397.6–2532.2)	5977.0 ± 111.0 (5753.9–6200.2)	3512.2 ± 117.0 (3277.0–3747.4)	4202.2 ± 86.4 (4028.5–4375.9)	1.2 ± 0.04 (1.1–1.3)	7.8 ± 0.4 (7.0–8.6)	6.6 ± 0.3 (6.0–7.1)
Medium tree finch (*C. pauper*) (N = 19)	2117.9 ± 44.1 (2025.2–2210.5)	6194.2 ± 87.9 (6009.5–6378.9)	4076.4 ± 97.1 (3872.5–4280.3)	3427.0 ± 103.8 (3849.7–4141.3)	1.3 ± 0.1 (1.1–1.4)	6.5 ± 0.4 (5.6–7.3)	5.1 ± 0.1 (4.9–5.4)
df	2, 76	2, 20.440	2, 76	2	2, 76	2	2, 19.776
F	16.745	0.404	8.077	21.813	0.426	4.364	12.197
P	< 0.001	0.673	0.003	< 0.001	0.655	0.113	< 0.001

Table 3.2 Male morphology shown as mean ± SE (95 %CI) per genetic group of Darwin's tree finches (*Camarhynchus* spp.) for which we also analysed song recordings. Statistical results are shown for Kruskal-Wallis test* and ANOVA; post-hoc tests showed that *C. parvulus* and birds of the hybrid group were statistically indistinguishable from each other, but smaller than *C. pauper* (see results).

Genetic group (N)	Beak length head*	Beak length naris*	Culmen length	Beak depth*	Beak width*	Tarsus	Wing
Small Tree Finch (*Camarhynchus parvulus*) (N = 9)	26.6 ± 0.1 (26.3–26.9)	13.3 ± 0.2 (12.9–13.7)	7.5 ± 0.1 7.3–7.7)	7.5 ± 0.1 (7.3–7.7)	6.6 ± 0.1 (6.4–6.7)	20.7 ± 0.3 (20.0–21.4)	64.4 ± 0.9 (62.3–66.6)
Hybrid group (N = 49)	26.9 ± 0.1 (26.6–27.2)	13.5 ± 0.1 (13.2–13.8)	7.6 ± 0.1 (7.4–7.8)	7.5 ± 0.1 (7.4–7.6)	6.6 ± 0.1 (6.5–6.7)	20.5 ± 0.3 (20.0–21.0)	64.0 ± 0.4 (63.3–64.8)
Medium Tree Finch (*C. pauper*) (N = 19)	29.1 ± 0.3 (28.4–29.7)	15.1 ± 0.2 (14.7–15.5)	8.7 ± 0.1 (8.5–9.0)	8.4 ± 0.2 (8.1–8.7)	7.1 ± 0.1 (7.0–7.3)	21.9 ± 0.3 (21.4–22.5)	67.5 ± 0.7 (66.1–69.0)
df	2	2	2, 76	2	2	2, 76	2, 76
F	25.420	26.208	30.745	21.000	16.109	5.306	10.929
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.007	< 0.001

Table 3.3 Estimated male population density of bird species in highland habitat including *Scalesia* forest at Cerro Pajas on Floreana Island in 2004, 2008, and 2013. Data are from singing males monitored using the circular plot method. The total highland male population estimate is given for the two tree finch groups in brackets per year; total population estimates were not calculated for the other species, as they do not predominantly nest in the highlands or in *Scalesia* forest.

	Estimated number of male birds km^{-2} (numbers of birds heard) [estimated population size]			**% Change from 2004 to 2013**
	2004	2008	2013	
Small tree finch *Camarhynchus parvulus*[1] & hybrid group[1]	182 (42) [4,095]	365 (43) [8,212]	263 (43) [5,917]	+ 45
Medium tree finch *C. pauper*[2]	234 (54) [5,265]	102 (12) [2,292]	113 (9) [2,537]	- 52
Large tree finch *C. psittacula*[1]*	0 (0)*	0 (0)*	0 (0)	-
Small ground finch *Geospiza fuliginosa*[1]	136 (23)	272 (32)	167 (10)	+ 23
Medium ground finch *G. fortis*[1]	29 (5)	17 (2)	7 (1)	- 76
Yellow warbler *Dendroica petechia*[1]	189 (8)	623 (47)	483 (21)	+ 256
Galápagos flycatcher *Myiarchus magnirostris*[1]	0 (0)**	477 (9)	531 (3)	+ 11***
Smooth-billed ani *Crotophaga ani*[1]	13 (1)	40 (2)	33 (3)	+ 254
Dark-billed cuckoo *Coccyzus melacoryphus*[1]	0 (0)	43 (4)	4 (1)	- 95**

Current IUCN status: [1] least concern, [2] critically endangered

* O'Connor et al. (2010) noted 13 (2004) and one (2008) singing *C. psittacula*, but findings by Kleindorfer et al. (2014) suggested the *C. psittacula* was locally extinct; in this study, the *C. psittacula* males heard in 2004 and 2008 are considered to be *C. pauper* males.

**Galápagos flycatchers were seen (but not heard) in 2004; therefore the relative increase from 2004 to 2013 is due to the occurrence of vocalising flycatchers.

*** Calculated as change in percentage from 2008 to 2013 given to no individuals heard in 2004.

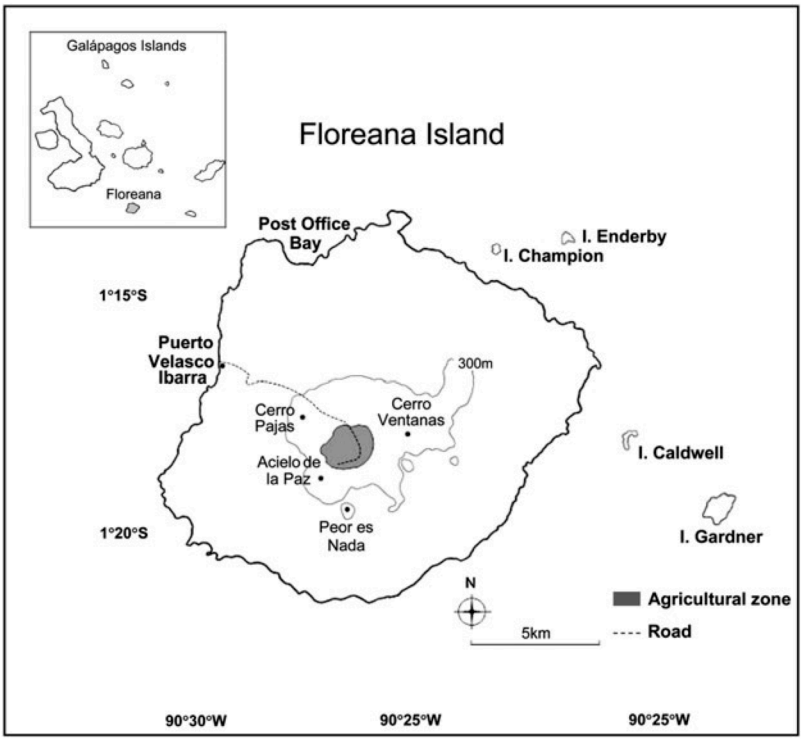

Figure 3.1 Map of Floreana Island, Galápagos Archipelago, Ecuador. The 300m contour line represents boundary of highland area. From O'Connor et al. (2010c).

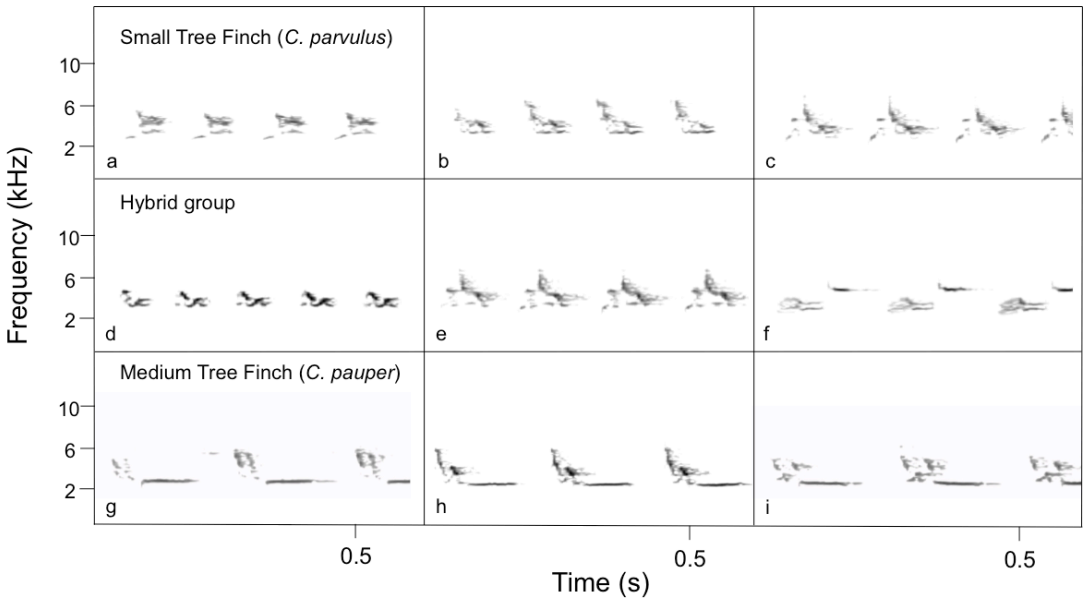

Figure 3.2 Spectrograms of song in: (a-c) Darwin's small tree finch (*Camarhynchus parvulus*), (d-f) hybrid group, and (g-i) medium tree finch (*C. pauper*). Each spectrogram represents the song of one male. The spectrograms are representative of the main traits across genetic groups: the song of *C. parvulus* and hybrid birds could not be statistically distinguished, while the song of *C. pauper* had slower trill rate and lower minimum frequency (see Table 3.1).

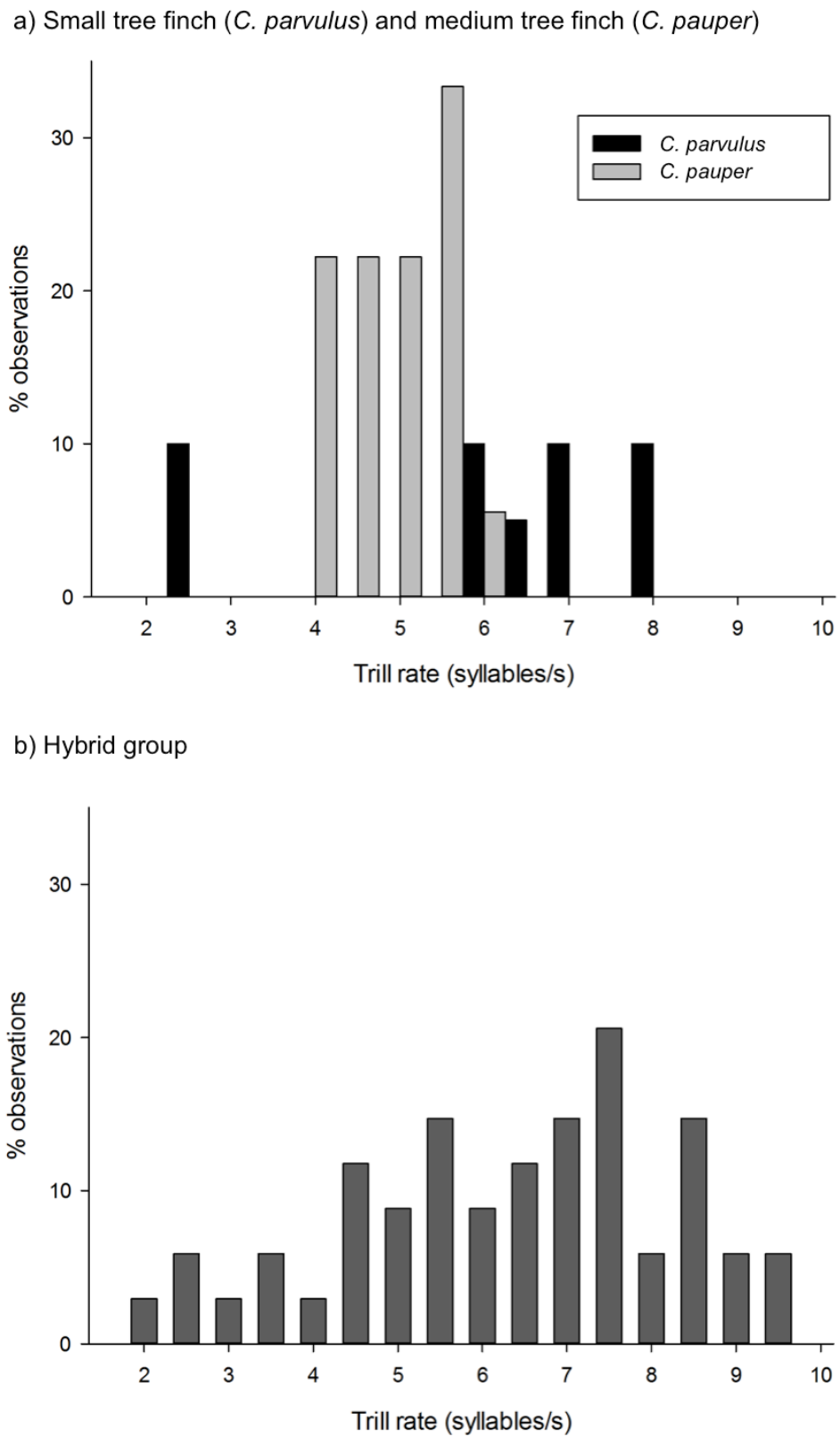

Figure 3.3 Frequency distribution of trill rate (syllables/s) in (a) small tree finch (*Camarhynchus parvulus*) and medium tree finch (*C. pauper*), and (b) the hybrid group derived from pairings between *C. parvulus* and *C. pauper*.

Chapter 4

Beggars can't be choosers: Females drive asymmetrical introgressive hybridisation from rare to common species in Darwin's tree finches

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Abstract

Hybridisation carries both risks and benefits for biodiversity depending on the specific ecological and evolutionary context. To assess the conservation significance of hybridisation scenarios, we first need to identify the dynamics of the individual hybridisation. Here we combine morphological and genetic analysis with pairing observations to investigate the extent, direction and drivers of the recently discovered hybridisation between two species of Darwin's tree finches (*Camarhynchus* spp.) on Floreana Island, Galápagos. We found asymmetrical introgression from the critically endangered, larger bodied *C. pauper* to the common, smaller bodied *C. parvulus*, which appears to be driven by a lack of discrimination against heterospecific males in *C. pauper* females. Examination of pairings showed that *C. parvulus* females paired assortatively while *C. pauper* females showed no such pattern. Our results suggest the existence of a hybrid swarm comprised of *C. parvulus* and hybrid birds, which may function as a reservoir to preserve the genetic diversity found in the critically endangered *C. pauper*. For these reasons we propose that the *Camarhynchus* group on Floreana Island represents a single, interacting source of genetic diversity, and that

conservation management of this pair of Darwin's tree finches should be done jointly rather than on a species-specific basis.

Introduction

The relative risks and benefits of hybridisation for biodiversity vary temporally, spatially, and across biological systems depending on the specific ecological and evolutionary context (Arnold, 1992; Barton, 2001; Seehausen et al., 2008a). Hybridisation can reduce biodiversity when two 'pure' species merge into a hybrid form (Grant and Grant, 2014b; Rhymer and Simberloff, 1996; Roberts et al., 2010; Seehausen, 2006; Taylor et al., 2006a), or when species' fitness is lowered through outbreeding depression (Frankham et al., 2002; Muhlfeld et al., 2009). In contrast, hybridisation can actively increase biodiversity as it can give rise to novel species via hybrid speciation (Amaral et al., 2014; Hermansen et al., 2011; Mallet, 2007; Mavarez and Linares, 2008), increase population genetic diversity (Baskett and Gomulkiewicz, 2011; Grant and Grant, 1994), and may enhance adaptive potential (Hamilton and Miller, 2015; Lewontin and Birch, 1966; Martinsen et al., 2001).

The different effects of hybridisation on biodiversity outcomes make its legal and biological conservation value difficult to evaluate (Allendorf et al., 2001; Fitzpatrick et al., 2015; Stronen and Paquet, 2013). While some studies have dismissed or downplayed the conservation value of hybrids (Giese, 2005; O'Brien and Mayr, 1991), hybridisation has long been suggested to play a vital role in the evolution of species (Anderson and Stebbins Jr, 1954) and is increasingly being recognised as a beneficial factor to foster adaptive potential in changing environments (discussed in Allendorf et al., 2001; Hamilton and Miller, 2015). Because the ecological effects of hybridisation on species are context-specific, hybridisation scenarios need to be assessed individually for their conservation significance. Important considerations include identifying the

drivers of hybridisation, existence and extent (including direction) of introgression, consequences for hybrid fitness (including outbreeding depression), and ultimately, identifying likely outcomes to inform conservation management.

Hybridisation involves a breakdown of reproductive isolating mechanisms between species. It is more likely to occur under particular ecological and/or population level circumstances, such as when at least one of the parental species is rare (Avise and Saunders, 1984; Randler, 2002) or when rapidly changing environments favour enhanced adaptive capacity which can arise through hybridisation as it increases genetic diversity (Arnold and Martin, 2010; Becker et al., 2013; Lewontin and Birch, 1966). 'Hubbs principle', also referred to as the 'desperation theory' (Hubbs, 1955), predicts that hybridising with a heterospecific individual is more likely when the chances of finding a conspecific mate are low (Randler, 2002; Wirtz, 1999). Environmental change, habitat disturbance, and introduced species have been shown to accelerate the occurrence of hybridisation (Anderson and Stebbins Jr, 1954; Seehausen et al., 2008a). Under such conditions, hybridisation may be an adaptive process. Hybrid populations can have greater adaptive capacity due to their enhanced genetic variation, which enables them to respond to novel selection from altered environments (Hamilton and Miller, 2015; Seehausen, 2013). Species that evolved via ecological speciation are particularly susceptible to hybridisation when loss of environmental heterogeneity reduces the availability of ecological niches and starts to reverse the processes that favoured adaptive divergence (Seehausen et al., 2008a).

In systems where females are the discriminating sex, preference for heterospecific males often drives hybridisation among species (reviewed in Randler, 2002; Wirtz, 1999). Examples include indigo and lazuli buntings (Baker, 1996), spadefoot toads (Pfennig, 2007), golden and white-collared manakins (Stein and Uy, 2006) and pied

and collared flycatchers (Veen et al., 2001). Reasons for females choosing heterospecific males may for example be a breakdown of isolating mechanisms (Grant and Grant, 1997a), environmental cues (Pfennig, 2007), or a lack of conspecifics (Grant and Grant, 1997a; Hubbs, 1955; Pfennig, 2007). In the latter case, hybridisation will generally occur by females of the rare species mating with males of the common species, rather than the other way around (Avise and Saunders, 1984; Wirtz, 1999).

Darwin's finches of the Galápagos Archipelago provide an exciting opportunity to investigate contemporary hybridisation given its documented regular occurrence in ground finches (*Geospiza* spp.) (Grant and Grant, 2014a; Grant and Grant, 2014c; Grant et al., 2003; Grant et al., 2005a) and, as recently discovered, also small and medium tree finches (*Camarhynchus parvulus* and *C. pauper*) on Floreana Island (Kleindorfer et al., 2014a). Kleindorfer et al. (2014a) compared morphological data of paired males and females and found that females were either the same size or larger than their chosen males, but never smaller. Here, we investigate dynamics of the contemporary hybridisation among tree finches using a powerful combination of multilocus genetic and morphological data across eight years to test if the hybridisation patterns are driven by asymmetrical female pairing preferences in rare versus common species. We test the prediction that females of the larger critically endangered *C. pauper* pair more frequently with heterospecific males of the smaller and more common *C. parvulus*. We further discuss the implications of this hybridisation in an evolutionary and conservation management context.

Methods

Study species and study site

This study was conducted in the highland *Scalesia* forest on Floreana Island from February to March in 2004–2014 (see also Kleindorfer et al. (2014a)) and focuses on the species complex comprising the common small tree finch (*Camarhynchus parvulus*), the critically endangered medium tree finch (*C. pauper*), and the hybrid birds that result from pairings between these two parental species. Darwin's finches readily hybridise and form viable offspring (Grant et al., 2003; Grant et al., 2005a), and this has been observed directly in *C. parvulus*, *C. pauper*, and their hybrids since 2004 (Kleindorfer et al., 2014a). Tree finches are sedentary and occupy the same territory across several years (Kleindorfer, pers. obs.). Male tree finches build a display nest and sing at the nest to attract a female (Kleindorfer, 2007). A female selects either the male and the nest, or just the male, in which case the pair has been observed to build a nest together (Kleindorfer, 2007). Young finches learn their song from a male tutor, which is usually their father (Grant and Grant, 1996a). The majority of the Floreana tree finch population resides in highland *Scalesia* forest at the base of the Cerro Pajas volcano (1°17S, 90°27W, site area ~ 2.4km^2, elevation 300–400m) (described in O'Connor et al., 2010c).

Sample collection

We captured and measured a total of 368 adult tree finches using 6 x 12m mist-nets between 06h00 and 11h00 from February to April 2004, 2005, 2006, 2008, 2010, 2012, 2013 and 2014. At the time of capture, we measured birds, collected a blood sample for subsequent genetic analysis, and banded each bird with a numbered aluminium band and a distinct combination of coloured plastic bands. We measured the following

morphological variables to the nearest 0.1 mm using a calliper: beak length head, culmen length, beak length naris, beak depth, beak width and tarsus length. Wing length was measured to the nearest mm using a wing ruler. Measurements were taken by S.K., J.O'C. and K.P. (all banders were annually calibrated against S.K. to maintain consistency across years). The blood samples (10µl) were immediately transferred to Whatman Classic FTA® paper for DNA preservation (2004, N = 4; 2005, N = 87; 2006, N = 11; 2008, N = 4; 2010; N = 89; 2012, N = 32; 2013, N = 82; 2014, N = 59). For this study, we only analyse data from adult birds to minimize genetic relatedness between individuals within years.

DNA extraction, genotyping and locus characteristics

We extracted the DNA samples from Whatman Classic FTA paper using a modification (200 µl volumes used for all washes) of method #4 from Smith and Burgoyne (2004). Each individual was genotyped at 11 microsatellite loci designed for *Geospiza fuliginosa*: Gf1, Gf3, Gf4, Gf5, Gf6, Gf7, Gf9, Gf11, Gf12, Gf13, Gf15 (Petren, 1998). We performed PCR amplification following the exact method described in Galligan et al. (2012). Due to lack of sufficient amplification across individuals, we excluded the microsatellite loci Gf9 and Gf15 from further analysis. We also excluded eleven individuals that failed to amplify at more than three loci. We subsequently analysed a total of 357 individuals at nine microsatellite loci. Genotypes were analysed on an ABI 3770 (Applied Biosystems) automated sequencer and scored using Genemapper 4.0 (Applied Biosystems) with manual editing. All samples were scored by K.P. under the supervision of S.M.

Individuals were assigned to one of two putative populations to assist our exploratory analyses based on their differences in beak morphology in accordance with results of O'Connor (O'Connor, 2012). In putative population 1 (*C. parvulus* phenotype) we

included all individuals with beak length naris < 8.2, and in putative population 2 (*C. pauper* phenotype) all individuals with beak length naris ≥ 8.2. We then performed tests of Hardy-Weinberg Equilibrium (HWE) per locus and putative population using GenePop 4.2 with Bonferroni correction. We tested for linkage disequilibrium at each locus using GenePop 4.2 and evaluated significance ($P = 0.01$) after Bonferroni correction (Rice, 1989). Data were checked for neutrality by estimating the mean F_{ST} and calculating the confidence intervals using LOSITAN (Antao et al., 2008; Beaumont and Nichols, 1996).

Population genetic structure and hybridisation

Because the inclusion of directly related individuals may confound genetic analyses, we identified full-sib relationships using the software COLONY 2.0 which applies full pedigree likelihood methods to infer sib-ship and parentage between individuals based on multilocus genotype data (Jones and Wang, 2010).

COLONY can reconstruct sib-ship and paternity while accommodating for class I errors (allelic dropouts) and class II errors (typing errors which could stem from contaminated DNA, allele identification error, false alleles, mutations) and can result in incorrect relationship inference if not accounted for. We used all nine loci for COLONY analysis with locus-specific error rates, which ranged from 1–9 % across loci. We only detected class II errors in our dataset. Error rates were determined by repeated genotyping of 116 individuals. Based on COLONY results, we restructured our original data into three new datasets, aiming to minimize the amount of closely related individuals and retaining only the parents (dataset 1), one offspring of each family (dataset 2) the other offspring of each family (dataset 3). Comparing results of HWE tests and F_{ST} estimates between datasets 1–3 and our initial dataset containing all individuals showed very little difference, suggesting excluding these putatively

related individuals had a negligible effect on results and we therefore used the initial dataset containing all samples (see supplementary materials for details) for subsequent analysis.

Population structure was assessed using a Bayesian model-based clustering method in the program STRUCTURE 2.3.4 (Hubisz et al., 2009; Pritchard et al., 2000). The model assumes that the loci within each cluster are unlinked and in Hardy-Weinberg equilibrium, and assigns individuals to a user-defined number of clusters while minimizing departure form Hardy-Weinberg equilibrium. We ran an initial 10 MCMC iterations for $K = 1–4$ with burn-in of 100,000, chain length 500,000 and allele frequency priors set according to our data: mean $F_{ST} = 0.03$, SD = 0.03, $\lambda = 1$. The results for both the standard admixture and the LOCPRIOR model were identical with respect to optimal *K*. We expected weak genetic structure because of the generally close genetic relatedness of Darwin's finch species, therefore we proceeded with the LOCPRIOR model. LOCPRIOR uses information such as ecotype or sampling location to support clustering if correlated with genetic structure (Hubisz et al., 2009); in our case we used morphology-based putative population assignments (see above and supplementary material for details). We then ran another 20 iterations for $K = 2$, using optimized priors derived from previous runs: initial alpha = 2.35, mean $F_{ST} = 0.07$, standard deviation = 0.029, $\lambda = 1$, burn-in = 100,000, chain length 100,000. We averaged multiple runs for each *K* using the program CLUMPP (Jakobsson and Rosenberg, 2007) and examined most likely *K* using Structure Harvester (Earl, 2012). We evaluated the number of clusters detected using both the mean log likelihood method following Pritchard et al. (2000) and the delta *K* method following Evanno et al. (2005). We then assigned each genotype to a genetic group using the individual membership coefficient (q_i). Following simulation results (see below and results) we used an inclusive threshold of $q_i > 0.80$ to the morphologically smaller cluster (*C.*

parvulus) to assign individuals to three genetic groups: the *C. parvulus* group, hereafter referred to as *C. parvulus* ($q_i \geq 0.80$), the *C. pauper* group, hereafter referred to as *C. pauper* ($q_i \leq 0.20$) and the admixed group ($0.20 < q_i < 0.80$). We compared private (cluster specific) alleles and heterozygosity (Ho), using GenAlEx 6.5 (Peakall and Smouse, 2006, 2012), and allelic richness (AR), using FSTAT (Goudet, 1995)) between clusters (using a q_i threshold of 0.50).

In order to select the most suitable threshold value for q_i, we assessed the accuracy of three potential threshold values using simulations based on real genotypes. Simulations were performed using the software HYBRIDLAB 1.0 (Nielsen et al., 2006), which randomly draws alleles based on their estimated frequency distributions from each of the two user specified populations and creates F1 hybrids, assuming linkage equilibrium among loci, marker neutrality and random mating.

The highest assignment probability for the *C. parvulus* cluster was 0.88, so, assuming there were 'pure' parental individuals in the population, we tested three threshold values below this, 0.75, 0.80, and 0.85. Using each of these values, we assigned our data to *C. parvulus* cluster and *C. pauper* cluster while omitting hybrid individuals, resulting in three datasets containing only 'pure' individuals. In order to avoid pseudo replication, we generated nine times as many genotypes of the *C. pauper* cluster and the *C. parvulus* cluster as were in each respective dataset. Simulated and original individuals were then merged and randomly split into ten separate datasets per tested threshold value, containing the same number of *C. pauper* and *C. parvulus* individuals as the original data. Using these 'parental' datasets, we simulated hybrid genotypes and added them to the dataset of their 'parents', resulting in 30 datasets (three threshold values, ten datasets per value) consisting of a mix of original and simulated *C. pauper* and *C. parvulus* individuals, and their simulated hybrids. We ran these datasets in

STRUCTURE for $K = 2$, using the LOCPRIOR model and the same running conditions, method and replicates as for the original samples. The proportion of incorrect cluster assignments was used to evaluate the threshold values (see Appendix 2).

Compared to our previous study (Kleindorfer et al., 2014a), we have tested and optimised the q_i threshold value for assigning individuals to genetic groups (0.80 for this study, 0.75 for Kleindorfer et al. (2014a)). Low genetic differentiation among parental samples and high admixture among species prevented us from differentiating between hybrid generations, and therefore from using other molecular hybridisation analyses. We thus chose a conservative q_i threshold, which should exclude most of the recent hybrids (F1, F2 and backcrosses thereof) from the 'pure' groups, but likely assigns some individuals from the 'pure' groups to the admixed group, which is why this group is called 'admixed group' rather than 'hybrid group'.

Morphological analysis

We compared the following morphological traits of male and female tree finches across genetic groups: beak length head, culmen length, beak length naris, beak depth, beak width, tarsus length and wing length. Male and female morphology was analysed separately due to known sexual dimorphism (Lack, 1983). Using IBM SPSS Statistics 22, we examined data for normality and homogeneity of variances and used ANOVA with Tukey HSD post hoc test for female tarsus length. All other morphological variables were not normally distributed and were therefore analysed using Kruskal-Wallis test with pairwise comparisons performed using (Dunn's Dunn, 1964) procedure with Bonferroni correction for multiple comparisons as post-hoc test. We used factor reduction via principal component analysis (PCA) to condense the morphological measurements into a reduced set of variables; PCA_beak (beak length

head, beak length, beak length naris, beak depth) and PCA_body (wing length and tarsus length). The derived PCA factor scores for PCA_beak had high factor loadings for beak length head (male: 0.95, female: 0.96), beak length (male: 0.92, female: 0.96), beak length naris (male: 0.94, female: 0.95) and beak depth (male: 0.91, female: 0.92), and explained 87 % (male) and 90 % (female) of the variance. PCA factor scores for PCA_body had high factor loadings for wing length (male: 0.91, female: 0.89) and tarsus length (male: 0.91, female: 0.89) and explained 84 % (male) and 76 % (female) of the variance. We then explored the relationship between beak morphology (PCA_beak_male and PCA_beak_female) and body size (PCA_body_male and PCA_body_female) and individual probability of genetic membership (q_i) using bivariate correlation analysis. Tree finches can be sexed visually when males are > 1 year old due to a gradual change in male plumage coloration (Figure 4.1), but females and yearling males look alike (Kleindorfer, 2007), although males can often be determined due to their protruding cloaca and breeding females by their swollen ventral brood patch (Kleindorfer, pers. obs.). To reduce potential error, we conducted avian molecular sexing using the primers P8 (5'-CTC- CCAAGGATGAGRAAYTG-3') and P2 (5'-TCTGCATC- GCTAAATCCTTT-3') (Griffiths et al., 1998), following standard methods outlined in (Griffiths et al., 1998) with modifications to the protocol as follows. We carried out PCR amplification in a total volume of 24µl with PCR reagents in following final concentrations: 1XµM MRT buffer, 0.2µM of each primer, 0.5 units Immolase and between 10–100ng DNA. PCR conditions were an initial denaturing step at 94°C for 10 min, followed by 35 cycles of 94°C for 45 s, 48°C for 45 s and 72°C for 45 s. The program was completed with a final run of 72°C for 5 min and 25°C for 2 min. We genetically sexed 58 out of 116 birds that could not be confidently identified as either male or female via their plumage coloration. For the remaining 58 individuals we relied on visual sex determination.

Pairing outcome

To identify genetic group assignment among pairs observed, we used data from colour-banded birds for which we also had genetic samples. The data were collected across years with the following sample sizes: 2005 (N = 16), 2010 (N = 15), 2012 (N = 13), 2013 (N = 12) and 2014 (N = 14). Our criterion for inclusion into the pairing data set was the observation of nest attendance by a male and female for a clutch of eggs; nesting contents were verified by inspecting nests visually (using a pole-mounted scope camera for nests higher > 2m). Each pairing event was considered independent as females may re-nest and re-pair across their lifetime (see also Kleindorfer (2007)). Each bird was assigned to a genetic group based on genotype data using q_i as described above. We analysed pairings in two ways: firstly, we used likelihood ratio test (IBM SPSS Statistics 22) to test if tree finch pairs showed the same pattern of species-specific association across the three genetic groups. We tested the null hypothesis that proportions of mixed (male and female from different genetic groups) and pure (male and female from the same genetic group) pairs were comparable across the genetic group of the female. Because the admixed group likely contains a large proportion of *C. parvulus* individuals, and based on their morphological similarity (see results), we then merged the admixed group with the *C. parvulus* group (hereafter referred to as hybrid swarm). This resulted in only two genetic groups (*C. pauper* and hybrid swarm), for which we analysed species-specific pairing using Fisher's exact test (IBM® SPSS® Statistics 22). Secondly, we statistically investigated the relationship between the genetic identity (using the membership coefficient (q_i) derived from STRUCTURE) of the male and female within each pair. We used linear regression analysis (controlling for 'year') (IBM SPSS Statistics 22), while separating the dataset based on genetic identity of the female using all three genetic groups (*C. parvulus, C. pauper*, admixed group).

Results

Microsatellite characteristics and genetic structure

In total, four loci (Gf1, Gf3, Gf4, Gf11) showed significant departure from HWE, but only one (Gf11) departed from HWE in both putative populations. Given that we expected our dataset to include hybrids, we anticipated that this might influence HWE dynamics, and we proceeded with data analysis using all nine loci, as all loci have been used successfully for Darwin's finches in previous studies (Galligan et al., 2012; Kleindorfer et al., 2014a; Petren, 1998; Petren et al., 1999). All loci were unlinked and confirmed to be neutral using LOSITAN. The number of alleles per locus across all individuals ranged from 3–19 (mean 9.2 ± 1.3 SE), expected heterozygosity ranged from 0.06–0.89 (mean 0.54 ± 0.07 SE, Appendix 3). Missing data were 6–21 % across loci. Estimates of the logarithm of probability averaged over 10 MCMC replicates for K = 1–4 were maximal for K = 2 using both the mean log likelihood method (Pritchard et al., 2000) and delta K method (Evanno et al., 2005) for the standard admixture model (F_{ST} between clusters = 0.082) and the LOCPRIOR model (F_{ST} between clusters = 0.084) in STRUCTURE (Appendices 4 & 5). Following morphological analysis (see below), the two clusters are hereafter referred to as the '*C. parvulus* cluster' and the '*C. pauper* cluster'. Individual proportions of membership (qi) for LOCPRIOR model are shown in Figure 4.2. Private alleles can be used as a tool to identify the direction of genetic introgression between two species (e.g. Beaumont et al., 2001; Gottelli et al., 1994). The *C. parvulus* cluster had more private alleles, (31, 32.3 % of all alleles were private), higher heterozygosity (Ho = 0.52) and higher mean allelic richness (AR = 8.87) than the *C. pauper* cluster (5 private alleles (7.1 %), Ho = 0.46, AR = 7.57).

Using an inclusive threshold of $q_i > 0.80$, the LOCPRIOR analysis performed in STRUCTURE was able to correctly recognize 91.3 % of simulated individuals (compared to 83.4 % for 0.75 and 76.8 % for 0.85, Table B, supplementary material) and was therefore selected as the most suitable threshold for our dataset. Individuals of the *C. parvulus* group could be assigned with an accuracy of 82.6 %, hybrids with 92.1 % and individuals of *C. pauper* group had an assignment accuracy of 99.2 %. Mean q_i was 0.83 ± 0.003 for *C. parvulus* group (N = 62), 0.92 ± 0.004 for *C. pauper* group (N = 85), and 0.66 ± 0.009 for the admixed group (N = 210). This difference in mean q_i suggests asymmetrical gene flow between groups, with introgression into the *C. pauper* group being less frequent as membership coefficients are higher and individuals are less mixed.

Morphological differentiation among parental and hybrid birds

Tree finch morphology was associated with genetic assignment for all morphological variables (Table 4.1), as well as in combined beak size and body size (PCA factors, Figure 4.2). For both sexes, birds from the *C. pauper* group were significantly larger than *C. parvulus* and birds of the admixed group for all morphological variables (Tukey HSD/pairwise comparison test all $P < 0.001$), while *C. parvulus* and birds of the admixed group did not differ from each other (Tukey HSD/pairwise comparisons all $P > 0.05$, Table 4.1). For both sexes, beak size and body size were both strongly negatively correlated with membership coefficient (q_i); birds with higher q_i were smaller and vice versa (PCA_beak_male: $\rho = -0.816$, $P < 0.001$, N = 247; PCA_body_male: $\rho = -0.743$, $P < 0.001$, N = 232; PCA_beak_female: $\rho = -0.874$, $P < 0.001$, N = 107; PCA_body_female: $\rho = -0.807$, $P < 0.001$, N = 94, Figure 4.2).

Asymmetrical pairing among genetic groups

The percentage of 'pure' (male and female from the same genetic group) and mixed (male and female from different genetic group) pairings differed between genetic groups (likelihood ratio = 9.115, df = 2, $P = 0.010$, N = 70, Figure 4.3a), as well as when comparing *C. pauper* and the hybrid swarm only (Fisher's exact test, $P = 0.001$, N = 70, Figure 4.3b).

Female *C. parvulus* were never observed to pair with a male *C. pauper* (0/8 nests), but 44.4 % of female *C. pauper* paired with male *C. parvulus* or the admixed group (Table 4.2, Figure 4.3a). Figure 4.3b shows the small percentage of females of the hybrid swarm that paired with *C. pauper* males (7.7 %), compared to *C. pauper* females that paired within (55.6 %) and outside (44.4 %) of their genetic group at comparable percentages.

To further examine whether 'like pairs with like', we compared q_i within each pair. Female *C. parvulus* chose males that had a q_i similar to their own (± 0.02–0.13 (SE), $\rho = 0.817$, $P = 0.025$, Figure 4.4). The mean ± SE difference in q_i within *C. parvulus* pairs was 0.07 ± 0.02, indicating high fidelity for pairing with conspecifics. In contrast, *C. pauper* and admixed females did not pair assortatively for q_i similarity (and thus, by correlation, morphological similarity) (*C. pauper*: $\rho = -0.217$, $P = 0.402$, mean difference in q_i between pairs = 0.25 ± 0.06 (SE), admixed group: $\rho = 0.107$, $P = 0.495$, mean difference in q_i between pairs 0.16 ± 0.03 (SE), Figure 4.4).

Discussion

Understanding the patterns of interspecific gene flow is important for determining evolutionary trajectories for species in systems with hybridisation. The described contemporary hybridisation between *C. parvulus* and *C. pauper* on Floreana Island was

underpinned by three observations: (1) *C. parvulus* and the admixed group have a high proportion of private alleles, while *C. pauper* shares the majority of its alleles with *C. parvulus* and the admixed group, suggesting asymmetrical introgression, (2) females of the common species (*C. parvulus*) paired with males with membership coefficients similar to their own, and (3) females of the rare species (*C. pauper*) and the admixed group did not show a significant pairing preference for males with a similar membership coefficient. Our results support the conclusion that the lack of pairing preference shown by *C. pauper* females is driving asymmetrical introgression from *C. pauper* into *C. parvulus*.

Asymmetrical introgression

Our analysis of nine microsatellite loci identified two genetic clusters ($K = 2$) within the tree finches on Floreana. This is congruent with previous findings by Kleindorfer et al. (2014a) that identified *C. parvulus* and *C. pauper* as different genetic groups with a reduced sample size of N = 201 from two years of data (2005 and 2010) compared to N = 357 from eight years of data collected across 2004–2014 in the current study. Consistent with this, we identified one group of birds with the *C. pauper* phenotype that were unambiguously assigned to one cluster (defined as *C. pauper*), and one large group of smaller birds with mixed assignment, which consisted of *C. parvulus* phenotypes and hybrids of various generations. We assigned these birds to the *C. pauper* genetic group and the admixed group). Admixture proportions were skewed towards the *C. parvulus* cluster (Figure 4.2), which indicates higher rates of backcrossing towards *C. parvulus* than *C. pauper*.

The fact that *C. parvulus* and birds of the admixed group were morphologically similar, yet significantly smaller than *C. pauper*, suggests that *C. parvulus* and hybrids are not readily distinguished and form a hybrid swarm. Recent generation avian hybrids often

display intermediate morphological traits compared to their parental species (e.g. Grant and Grant, 1994; Pierce, 1984; Steeves et al., 2010), as in the New Zealand black stilt (*Himantopus novaezelandiae*) and the self-introduced pied stilt (*H. h. leucocephalus*), where limited introgression retains the option of visual hybrid detection based on plumage characteristics (Steeves et al., 2010). It is therefore likely that the admixed group contains only relatively few recent hybrids (which would have intermediate morphology), but consists mostly of *C. parvulus* and introgressed individuals (offspring from matings between hybrids x *C. parvulus*). If the admixed group received similar levels of gene flow from both the smaller bodied *C. parvulus* and the larger bodied *C. pauper* group, we would expect the individuals of the admixed group to have intermediate morphology. *Camarhynchus pauper* therefore appears to retain most of its genetic purity, while transferring its genes into the hybrid swarm via hybridisation followed by asymmetrical introgression.

The skewed admixture proportions suggest possible asymmetrical introgression from *C. pauper* into the hybrid swarm. A similar scenario has been observed in two species of mulberry (*Morus rubra* and *M. alba*) that also differ in abundance (Burgess et al., 2005). The contrast in proportion of private alleles between the *C. parvulus* and *C. pauper* clusters is congruent with a scenario of asymmetrical introgression and indicates gene flow towards *C. parvulus*, but little gene flow towards *C. pauper*. The greater heterozygosity and allelic richness of the *C. parvulus* cluster is also congruent with asymmetrical introgression. Analysis of genomic data has proven as a valuable tool to enhance our understanding of introgressive hybridisation in other taxa (Baack and Rieseberg, 2007), and could be the next step to confirm patterns observed here.

Female pairing preference as a driver of hybridisation and introgression

This study found that across a decade, *C. parvulus* females never paired with *C. pauper* males. Instead, they paired with conspecifics and hybrids with a high membership coefficient (0.67–0.79). In contrast, *C. pauper* females did not pair assortatively. This observation indicates that female pairing preference drives the asymmetrical introgression observed in this system, as this difference in heterospecific discrimination between *C. parvulus* and *C. pauper* most likely results in increased gene flow into the hybrid swarm. Similarly, female preference for golden-collared males in a hybrid zone of golden- and white-collared manakins (*Manakis vitellinus* and *M. candei*) has been observed to lead to asymmetric introgression of golden-collared plumage traits (Parsons et al., 1993; Stein and Uy, 2006). A comparable dynamic was shown by a study of asymmetrical introgression between two lineages of common wall lizards (*Podarcis muralis*) (While et al., 2015). Interestingly, here the driver was male-male competition with the introgression being skewed towards the more dominant lineage (While et al., 2015). Since in lizards, male reproductive success is driven by male-male competition rather than female choice (Olsson and Madsen, 1995), these findings are in accordance with the aforementioned avian studies, leading to the conclusion that the sex that drives reproductive decisions is also likely to drive the direction of hybridisation and introgression.

Females are regarded as the 'gatekeepers' of reproductive isolation in systems where they are the more discriminating sex (Parker and Partridge, 1998; Willis, 2013). The lack of heterospecific discrimination in mating decisions by *C. pauper* females suggests a partial breakdown of reproductive isolating mechanisms. Male song is an important mating signal that differs between Darwin's finch species and is transmitted via learning (Bowman, 1983; Grant, 1986; Grant and Grant, 1997a; Podos, 2010). Sons

learn the song from their father, and daughters have a preference for the song of their father (Bowman, 1983). Therefore, male hybrid offspring resulting from the mating of a female *C. pauper* with a male *C. parvulus* should sing *C. parvulus* song, and female hybrid offspring should favour *C. parvulus* (their father's) song, which is sung by both *C. parvulus* and hybrid males. In this case, subsequent generations should favour backcrossing to *C. parvulus* (the father's genetic lineage) and hybrids, but not *C. pauper* (Grant and Grant, 2008a; Grant and Grant, 2014c). This will likely maintain high levels of hybridisation and introgression into the hybrid swarm rather than with *C. pauper*. In support of this scenario, almost all observed pairings by females of the hybrid swarm (92.3 %) were with males of the hybrid swarm.

The rarity of *C. pauper* has likely contributed to the development of this extensive hybridisation within the tree finch group. Hubbs principle (Hubbs, 1955) states that hybridisation is more likely when at least one of the involved species is low in numbers, due to restricted mate choice. Because there is a trade off between the costs and benefits of being choosy when it comes to mate selection, animals may decide to mate with heterospecifics when no conspecifics are available as has been shown across taxa (e.g. for indigo and lazuli buntings (Baker, 1996), swordtails (Willis et al., 2011), and western and Clark's grebes (Nuechterlein and Buitron, 1998)). Our findings are consistent with this, as numbers of the critically endangered *C. pauper* have declined by 52 % since 2004 (Peters and Kleindorfer, in review), suggesting that *C. pauper* females mate with the more abundant *C. parvulus* and hybrid males due to a likely struggle to find conspecifics.

Anthropogenic impact and implications for conservation

An increase in human impact on global ecosystems (Vitousek et al., 1997) highlights the importance of a species' ability to rapidly adapt to environmental change

(Hoffmann and Sgrò, 2011; Myers et al., 2012; Reusch and Wood, 2007). Adaptive capacity may be enhanced by hybridisation through the transfer of advantageous alleles across species boundaries (Anderson, 1949; Anderson and Stebbins Jr, 1954; Barton, 2001; Hamilton and Miller, 2015), and it has been demonstrated in a variety of study systems including Italian sparrows (*Passer italiae*) (Eroukhmanoff et al., 2013), monkeyflowers (*Mimulus aurantiacus*) (Stankowski and Streisfeld, 2015) and *Heliconius* butterflies (Pardo-Diaz et al., 2012). Declining species with depleted gene pools can benefit from the transfer of new genes (Baskett and Gomulkiewicz, 2011; Benson et al., 2011; Grant et al., 2003; Ingvarsson, 2001), which stimulates a rethinking of hybrid conservation management (discussed below).

One line of evidence suggests that hybridisation between *C. parvulus* and *C. pauper* is favoured to reduce mortality impacts of invasive *Philornis downsi* fly larvae. Nests of the hybrid swarm contained fewer parasitic larvae, compared to those of *C. pauper* (Peters et al. unpublished data). Individuals of the hybrid swarm seem to make up the majority of the tree finch population on Floreana (77 % of collected samples), which can indicate a high reproductive and/or survival rate compared to *C. pauper*. Adaptation to anthropogenic impact facilitated through hybridisation generating higher fitness in offspring may be the only option for the endemic *C. pauper* to bring its genes in future generations (Baskett and Gomulkiewicz, 2011; Hamilton and Miller, 2015).

Darwin's finches are a young and closely related species group with a history of fluctuation and rapid evolution, and introgressive hybridisation likely played an important part in the evolution of this system (Grant et al., 2005a). Consequently, the hybridisation between *C. parvulus* and *C. pauper* is potentially an adaptive response to

both species' decline as a result of habitat fragmentation and disturbance, as well as parasitism from *P. downsi.*

As described above, this asymmetrical introgression with limited gene flow into the *C. pauper* group retains much of the genetic purity of this species, but simultaneously the gene pool of *C. pauper* may not receive enough foreign alleles to benefit from increased genetic diversity and associated higher adaptive potential. Continued asymmetrical introgression of this nature may contribute to the decline in *C. pauper* numbers (Levin et al., 1996) and in the long term lead to the extinction of the pure form of this already rare and critically endangered species. Galápagos is known for its fluctuating climate and oscillating natural selection (Grant and Grant, 2014c), which requires species to have high adaptive potential in order to persist (Grant and Grant, 2008a). Therefore, the conservation value of the hybrid swarm is raised by the preservation of the endemic *C. pauper*'s genetic variation. An example of a similar case is the Norfolk Island boobook owl (*Ninox novaeseelandiae undulate*) which is now considered 'extinct in pure form' but 'extant in hybrid form', because a hybrid population now persists in low numbers, with individuals harbouring half the nuclear genome and all the mitochondrial DNA of the original taxon (Garnett et al., 2011). Similarly, the hybrid zone between two species of gartersnakes (*Thamnophis butleri* and *T. radix*) in Wisconsin appears to contain relatively ancient genetic variation (Placyk Jr et al., 2012). Placyk et al. (2012) argue that this diversity is potentially of evolutionary importance and hybrids should therefore be conserved at the same level as the threatened parental species.

Becker et al. (2013) argue, that when hybrids and hybridisation have the potential to increase adaptive capacity within a system, the system as a whole should be protected as this will lead to greater preservation of biodiversity in the future. We consequently

advise to consider the species complex on Floreana comprising the hybrid swarm and *C. pauper* as a single conservation management unit. This makes sense in both an ecological and management context as Floreana tree finches share the same highland *Scalesia* forest habitat, where the nesting success of all Darwin's finch species is limited by *P. downsi* (Dudaniec et al., 2010; O'Connor et al., 2010b) and introduced predators such as black and Norwegian rats (*Rattus rattus* and *R. norvegicus*) (Grant and Grant, 1997b), cats (*Felis catus*) (Jiménez-Uzcátegui et al., 2008), smooth-billed anis (*Crotophaga ani*) (Connett et al., 2013) and potentially fire ants (*Solenopsis geminata*) (e.g. Stake and Cimprich, 2003). Conservation of remaining *Scalesia* habitat and management of introduced species will therefore benefit the entire Floreana Darwin's finch population, while the persistence of *C. pauper* will rest on conservation actions taken to mitigate the threats mentioned above. Since other islands of the Galápagos Archipelago also suffer from the above anthropogenic impacts (Santa Cruz Island (Cimadom et al., 2014) and Isabela Island (Fessl et al., 2010)), our recommendation to manage Darwin's finch communities as an interacting entity rather than as individual species may be applied to other islands where hybridisation patterns are yet to be examined.

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Table 4.1 Male (a) and female (b) morphology of three genetic groups of Darwin's tree finches (*Camarhynchus* spp.). For both sexes, *C. pauper* was significantly larger than *C. parvulus* and birds of the admixed group in all variables (Tukey HSD/Games-Howell post-hoc test all $P < 0.001$). *Camarhynchus parvulus* and birds of the admixed group did not differ.

(a) Male	***Camarhynchus parvulus* (Small tree finch)**		**Admixed group**		***C. pauper* (Medium tree finch)**		**Test statistics**		
Measurements (mm)	N	Mean ± SE (CI 95 %)	N	Mean ± SE (CI 95 %)	N	Mean ± SE (CI 95 %)	*P*	df	Kruskal-Wallis test
Beak-head	42	26.4 ± 0.1 (26.2–26.5)	140	26.7 ± 0.1 (26.5–26.8)	65	29.3 ± 0.1 (29.1–29.6)	< 0.001	2	1117.996 (2)
Culmen	42	13.2 ± 0.1 (13.0–13.4)	141	13.4 ± 0.1 (13.3–13.6)	65	15.2 ± 0.1 (14.9–15.4)	< 0.001	2	99.474 (2)
Beak-naris	42	7.4 ± 0.1 (7.3–7.5)	141	7.5 ± 0.04 (7.5–7.6)	65	8.7 ± 0.1 (8.6–8.9)	< 0.001	2	116.612 (2)
Beak depth	42	7.4 ± 0.05 (7.3–7.5)	141	7.4 ± 0.04 (7.4–7.5)	65	8.4 ± 0.1 (8.3–8.6)	< 0.001	2	99.543 (2)
Beak width	42	6.5 ± 0.06 (6.3–6.6)	141	6.5 ± 0.04 (6.5–6.6)	65	7.2 ± 0.0 (7.1–7.3)	< 0.001	2	82.165 (2)
Tarsus length	42	20.5 ± 0.1 (20.3–20.7)	141	20.5 ± 0.1 (20.3–20.7)	64	22.2 ± 0.1 (22.0–22.4)	< 0.001	2	93.753 (2)
Wing length	39	63.1 ± 0.3 (62.5–63.8)	133	63.8 ± 0.2 (63.3–64.2)	60	68.5 ± 0.4 (67.9–69.1)	< 0.001	2	89.349 (2)

(b) Female	*Camarhynchus parvulus* (Small tree finch)		Admixed group		*C. pauper* (Medium tree finch)		Test statistic (df)		
Measurements (mm)	N	Mean ± SE (CI 95 %)	N	Mean ± SE (CI 95 %)	N	Mean ± SE (CI 95 %)	*P*	df	Kruskal-Wallis test, ANOVA*
Beak-head	19	25.7 ± 0.1 (25.4–25.9)	68	26.5 ± 0.2 (26.2–26.9)	20	29.3 ± 0.2 (29–29.7)	< 0.001	2	42.807
Culmen	19	12.8 ± 0.1 (12.5–13.1)	68	13.2 ± 0.1 (13–13.4)	20	15.4 ± 0.2 (15–15.7)	< 0.001	2	43.511
Beak-naris	19	7.2 ± 0.1 (7–7.3)	68	7.4 ± 0.1 (7.2–7.5)	20	8.9 ± 0.1 (8.7–9.1)	< 0.001	2	44.594
Beak depth	19	7 ± 0.04 (6.9–7.1)	68	7.3 ± 0.1 (7.2–7.4)	20	8.2 ± 0.1 (8–8.4)	< 0.001	2	40.282
Beak width	19	6.1 ± 0.1 (6–6.2)	68	6.4 ± 0.1 (6.2–6.5)	20	6.9 ± 0.1 (6.7–7)	< 0.001	2	29.471
Tarsus length	19	19.6 ± 0.2 (19.2–19.9)	68	20 ± 0.1 (19.8–20.3)	20	21.4 ± 0.2 (21.1–21.7)	< 0.001	2, 106	20.097*
Wing length	18	61.1 ± 0.3 (60.5–61.7)	58	62.4 ± 0.4 (61.6–63.1)	18	67.4 ± 0.5 (66.3–68.6)	< 0.001	2	33.478

Table 4.2 Pairings for the three genetic groups of tree finches (*Camarhynchus parvulus*, *C. pauper* and admixed group) across 2005, 2010, 2012, 2013 and 2014. Data are shown as column percentage (N).

		Female genetic group		
		Camarhynchus parvulus	Admixed group	*C. pauper*
Male genetic group	*C. parvulus*	25.0 % (2)	13.6 % (6)	5.6 % (1)
	Admixed group	75.0 % (6)	77.3 % (34)	38.9 % (7)
	C. pauper	0.0 % (0)	9.1 % (4)	55.6 % (10)

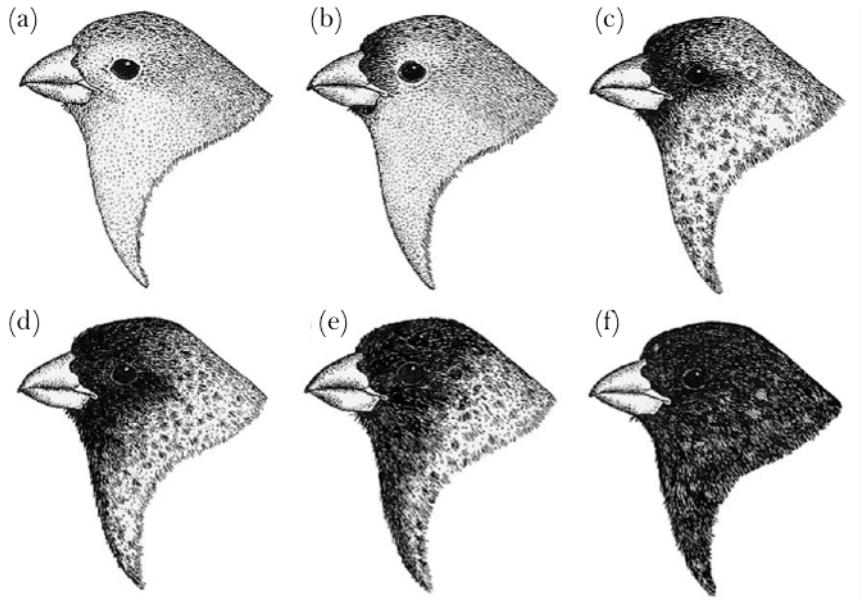

Figure 4.1 Gradual change in male plumage coloration for tree finches (*Camarhynchus* spp.). Males usually require five annual moults to attain a full black head, neck and chest. From Kleindorfer (2007).

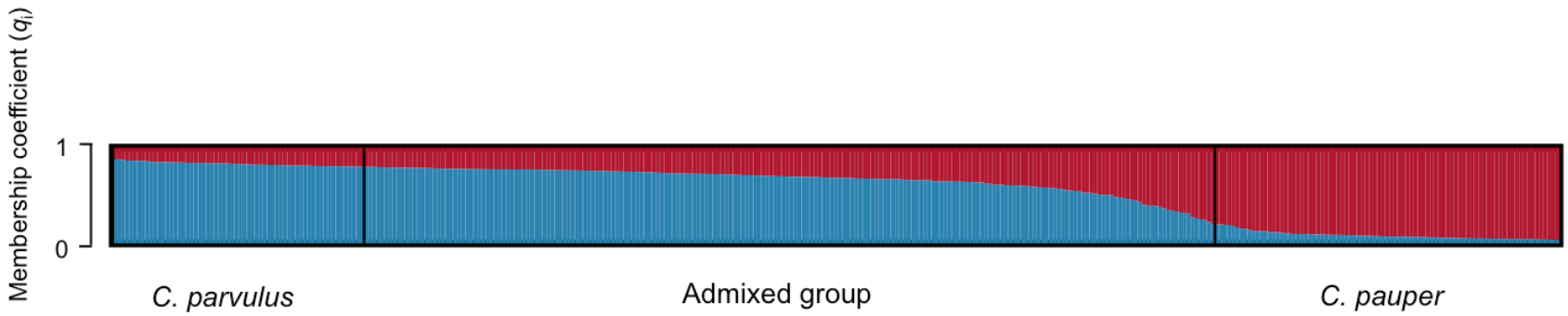

Figure 4.2 Probabilistic assignment to the genetic clusters using individual membership coefficient (q_i) inferred by the Bayesian analysis performed in STRUCTURE with $K = 2$ clusters for Darwin's tree finches on Floreana Island, Galápagos. Each vertical bar represents one individual; membership to *Camarhynchus pauper* cluster (medium tree finch) is shown in red, and membership to *C. parvulus* cluster (small tree finch) in blue. Birds were sorted into three genetic groups using an inclusive threshold of $q_i > 0.80$ (*C. parvulus*) and $q_i < 0.20$ (*C. pauper*) to the *C. parvulus* cluster. Individuals with $0.20 < q_i < 0.80$ were assigned to the admixed group. Black bars show the border between groups.

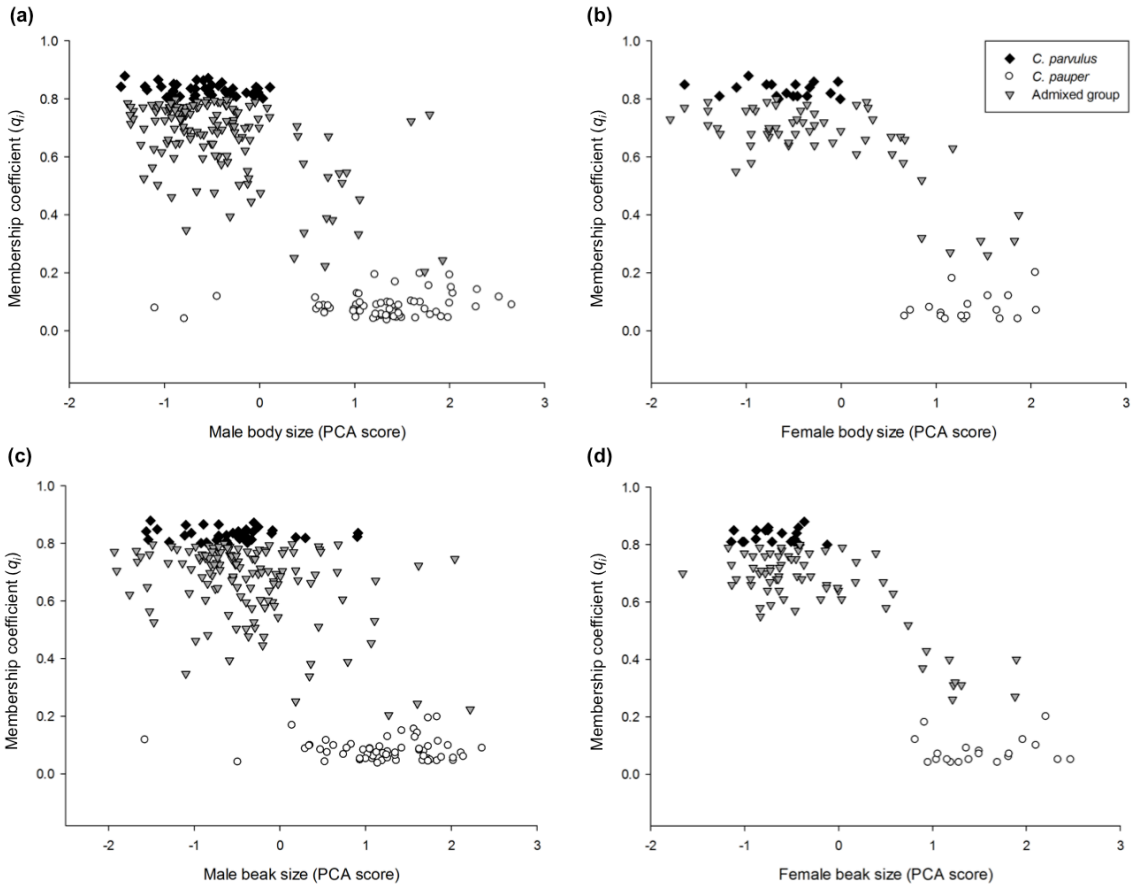

Figure 4.3 Correlation between (a) male and (b) female beak size and (c) male and (d) female body size (PCA score) and the individual membership coefficient (q_i) for birds genetically assigned to *Camarhynchus pauper*, admixed group, and *C. parvulus*. For both sexes beak size and body size were strongly negatively correlated with genetic assignment probability (PCA_beak_male: $\rho = -0.816$, $P < 0.001$, N = 247; PCA_body_male: $\rho = -0.743$, $P < 0.001$, N = 232; PCA_beak_female: $\rho = -0.874$, $P < 0.001$, N = 107; PCA_body_female: $\rho = -0.807$, $P < 0.001$, N = 94).

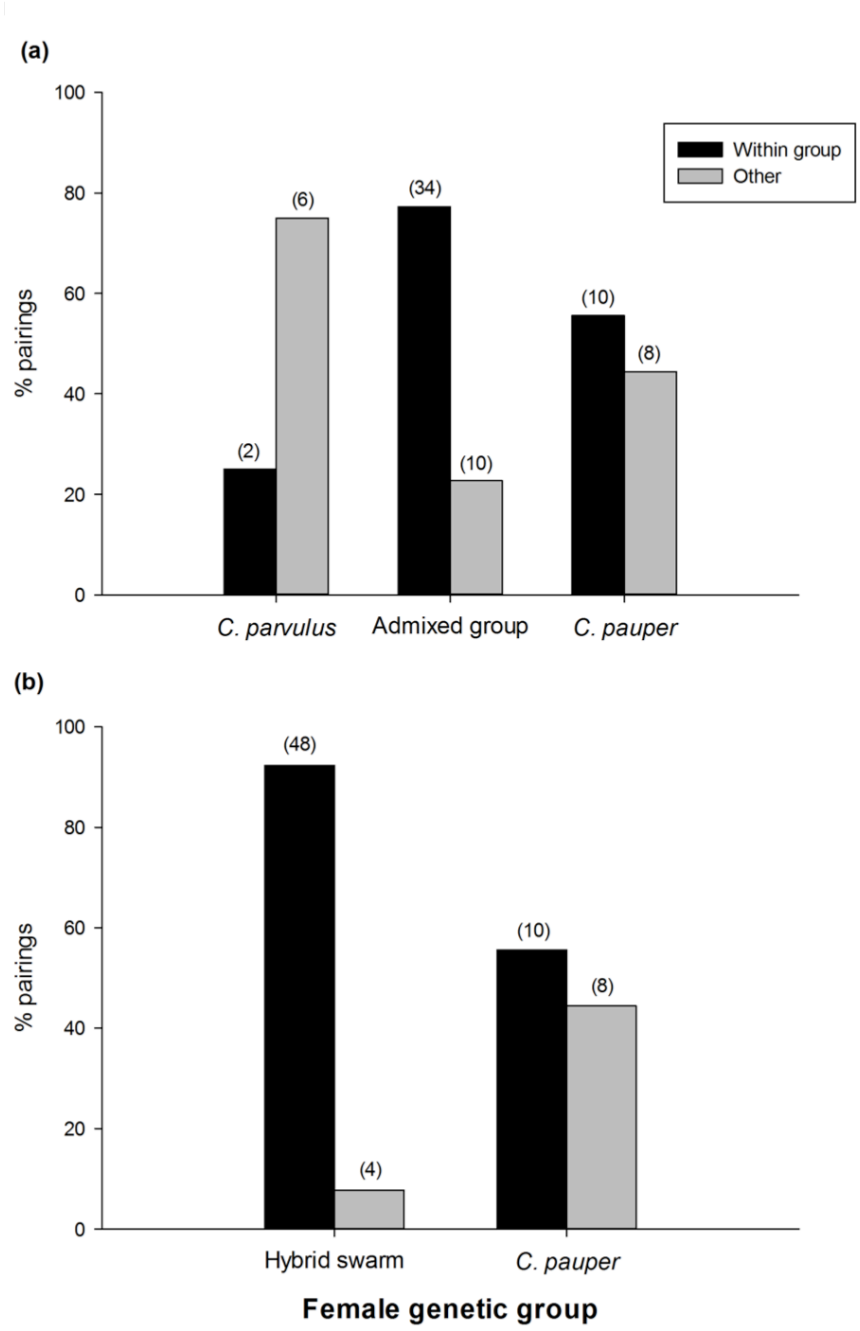

Figure 4.4 Pairings in Darwin's tree finches (*Camarhynchus* spp.) for three (a) and two (b) genetic groups for 2005, 2010, 2012, 2013 and 2014. Bars show the percentage of female pairings with a male of the same ('within group') and a different ('other') genetic group; N is given above each bar. Pairing differed between females of different genetic groups a) likelihood ratio = 9.115, df = 2, $P = 0.010$, N = 70 and b) Fisher's exact $P = 0.001$, N = 70.

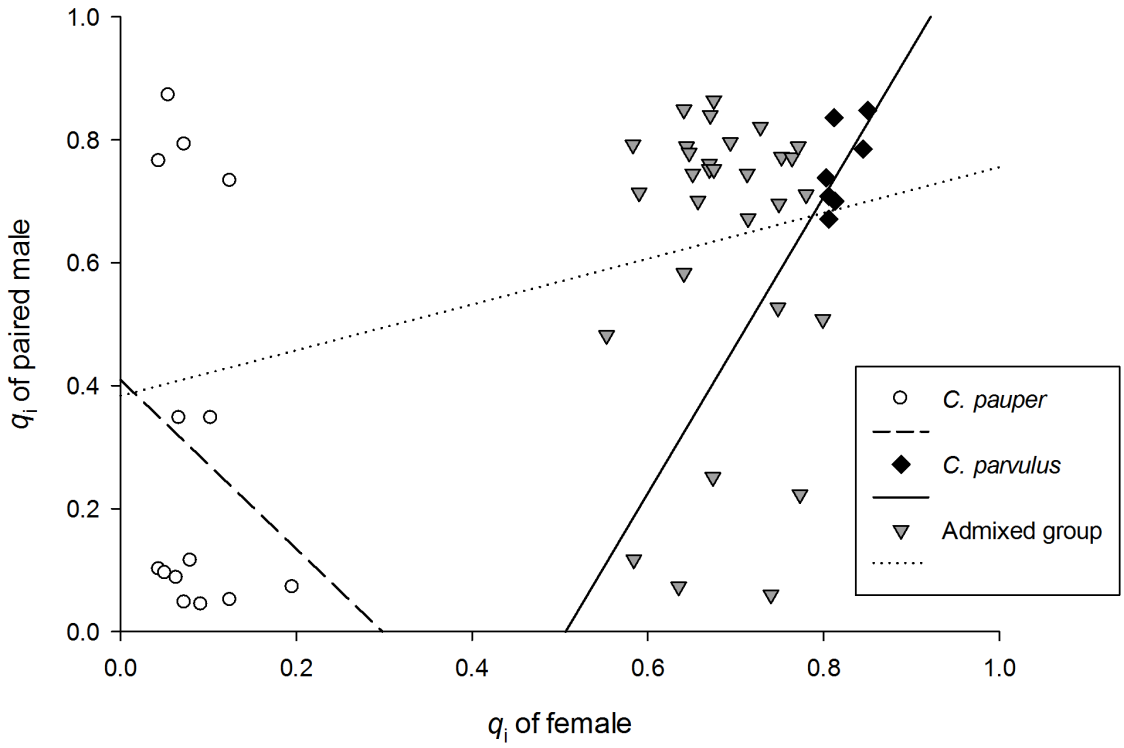

Figure 4.5 The relationship between genetic membership probabilities (q_i) of paired male and female tree finches for females genetically assigned as *Camarhynchus pauper*, admixed group, and *C. parvulus*. Regression lines represent the association between male and female genetic assignment in paired birds, which was significant in *C. parvulus* ($\rho = 0.817$, $P = 0.025$, solid line), but not in *C. pauper* (dashed line) or females of the admixed group (dotted line).

Chapter 5

Winners and losers in a Galápagos host-parasite system: evidence for hybrid fitness in Darwin's tree finches?

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Abstract

Invasive parasite species pose great challenges to naïve hosts that lack coevolved defence mechanisms. Hybridisation has been shown to facilitate adaptation by increasing genetic diversity, providing hybrids with an adaptive advantage to their parental species. Here we present the first evidence of parasite related hybrid fitness in Darwin's tree finches of the Galápagos Islands, which suffer from nest-infesting larvae of the introduced fly *Philornis downsi*. We compared host nesting success, parasite infestation, and parasite success across three species of Darwin's finches (*Geospiza fuliginosa*, *Camarhynchus parvulus*, *C. pauper*) and a recently discovered hybrid group on Floreana Island. We found hybrids to have the lowest *P. downsi* infestation levels of all four genetic groups and a lower nesting height than their parental species. Within the tree finch group, infestation of *P. downsi* per nest decreased with increasing genetic admixture of the nesting adult male. Nests of the critically endangered *C. pauper* contained three times more *P. downsi* larvae compared to hybrid birds. Nesting success was alarmingly low (0–9.1 % of nests had fledglings) across all four genetic groups due to *P. downsi* infestation and nest depredation. *Philornis downsi* was most successful in nests of *G. fuliginosa*, possibly indicating the onset of local adaptation of *P. downsi* to

this host. Our results provide evidence for an adaptive function of hybridisation in relation to parasitism by *P. downsi* in this system. Drastic conservation measures targeting control and eradication of *P. downsi* are urgently needed to protect this iconic ecosystem.

Introduction

Understanding host-parasite evolution is crucial for developing conservation frameworks and species management plans, especially for vulnerable ecosystems (Dybdahl and Storfer, 2003). Parasite virulence can be extreme in environments where parasites have been introduced to naïve hosts that lack defensive anti-parasite mechanisms (Huber et al., 2010). Because parasites are usually considerably smaller than their hosts and have shorter generation times, they generally evolve more quickly. These differences tend to confer parasites with an adaptive advantage. Under conditions of reciprocal selection, parasites are expected to be a step ahead of their host (Kaltz and Shykoff, 1998). One consequence of this life history advantage for coevolutionary dynamics is that parasites can become locally adapted to their host, with higher parasite fitness on a local host vs. a foreign host (Gandon and Van Zandt, 1998; Joshi et al., 2001).

Hosts are not evolutionarily defenceless under conditions of parasitism. Parasitised hosts have developed physical barriers to prevent parasitism (McNabb and Tomasi, 1981), or behavioural and/or immune responses to minimise parasite impact (Huber et al., 2010; Parker et al., 2011). Hosts can increase genetic diversity through hybridisation, and therefore hybridisation can facilitate adaptation to environmental disturbance such as introduced parasites (Hamilton and Miller, 2015; Lewontin and Birch, 1966; Rhymer and Simberloff, 1996). A study of hybridising red-crowned and Forbes' parakeets (*Cyanoramphus novaezelandiae* and *C. forbesi*, respectively) found

increased cell-mediated and innate immunity in hybrids (Tompkins et al., 2006). Recognizing hybrid fitness in parasitised systems is a crucial step towards understanding the extensive occurrence of hybridisation worldwide (Wolinska et al., 2008).

The remote Galápagos Islands are a natural laboratory to observe evolution in action. The iconic Darwin's finches are a model system for adaptive radiation and rapid speciation (Schluter, 2000). Like most ecosystems that have developed in isolation, Galápagos and its biota are extremely vulnerable to human disturbance. Invasive species span all taxonomic groups and now genuinely threaten endemic flora and fauna across the archipelago (Causton et al., 2006; Jiménez-Uzcátegui et al., 2008; Wikelski et al., 2004). The biggest threat to the 29 species of Galápagos land birds is the introduced fly *Philornis downsi* (Causton et al., 2006). The fly likely arrived on the archipelago via cargo boats transporting fruit, though its actual manner of introduction is unknown (Bulgarella et al., 2015; Causton et al., 2006; Kleindorfer et al., in press). First collected in 1964, the impact of *P. downsi* on native land birds was first observed in 1997 (Fessl et al., 2001; Fessl and Tebbich, 2002). Although its adult form is vegetarian, the female fly deposits its eggs in the base of bird nests where the larvae reside and consume nestling blood and tissue, resulting in up to 100 % brood loss per species per year (Dudaniec et al., 2007; Fessl et al., 2006b; O'Connor et al., 2010d). Such massive in-nest mortality caused by introduced fly larvae has caused stark declines in the critically endangered Darwin's mangrove finch (*Camarhynchus heliobates*) on Isabela Island (Fessl et al., 2010) and medium tree finch (*Camarhynchus pauper*) on Floreana Island (O'Connor et al., 2010d; Peters and Kleindorfer, in review).

Given that the strong selection from the introduced *P. downsi*, it is perhaps not surprising that we are beginning to detect what could be the first signs of

'coevolutionary dynamics'. Across a decade of research (2004–2013), Kleindorfer et al. (2014b) found earlier host and parasite in-nest mortality, indicating the potential for strong reciprocal natural selection. As a consequence of this earlier host death there was (1) earlier termination of the parasites' resource, (2) lower parasite success as evidenced by more 1st instar larvae (too young to pupate and hence will die), and (3) fewer pupae (= fewer emergent adult flies) at the time of host death (Kleindorfer et al., 2014b). Kleindorfer et al. (2014b) found this pattern of early host death and elevated in-nest *P. downsi* mortality in tree finches (*C. parvulus*, *C. pauper)*, but much less in nests belonging to the small ground finch (*Geospiza fuliginosa)*. For reasons we do not know, parasite success and host success were both higher in *G. fuliginosa* compared with *C. parvulus* and *C. pauper* (Kleindorfer et al., 2014b). Local adaptation is defined as the evolution of advantageous traits under local environmental conditions (in our case a specific host), resulting in genotypes experiencing higher relative fitness in local environments than genotypes from other environments (Kawecki and Ebert, 2004). The higher success of *P. downsi* in nests of *G. fuliginosa* could therefore be a result of local adaptation of *P. downsi* to this species.

In addition to extremely high in-nest mortality and high parasite intensity from *P. downsi* in Darwin's tree finches, Kleindorfer et al. (2014a) recently detected extensive hybridisation between *C. parvulus* and *C. pauper* on Floreana. The hybridisation is driven by matings between females of the critically endangered *C. pauper* and males of the common *C. parvulus* (Peters et al., in review). This hybridisation provides a timely opportunity to test for hybrid fitness in relation to the novel and lethal *P. downsi*.

We aim to answer two main questions: 1) Is there evidence for hybrid fitness in Darwin's finches on Floreana Island? and 2) is there evidence for local host-parasite assemblages with higher fitness relative to other host-parasite assemblages in the

Darwin's finch-*Philornis* system? We investigate these questions by analysing nesting success, parasite intensity and estimated parasite survival (as the percentage *P. downsi* pupae per nest). This is the first study to provide information on interspecific differences in Darwin's finch breeding biology and nesting success on Floreana Island with genetically confirmed species determination including hybrid birds.

Methods

Host study species and study site

We collected data on breeding biology, nesting success, and *P. downsi* per nest on Floreana Island, Galápagos over three field seasons in 2010, 2013 and 2014. We sampled nests of Darwin's small tree finch (*Camarhynchus parvulus*, ~ 12g), medium tree finch (*C. pauper*, ~ 16g), the recently identified tree finch hybrid (~ 13g) (Kleindorfer et al., 2014a; Peters et al., in review), and Darwin's small ground finch (*Geospiza fuliginosa*, ~ 13g). While *G. fuliginosa* are also found elsewhere on the island, Darwin's tree finches nest almost exclusively in humid highland forest dominated by their preferred nesting tree *Scalesia pedunculata* (Peters and Kleindorfer, 2015). Sampling was conducted in this habitat at the base of the Cerro Pajas volcano on Floreana Island, Galápagos (1°17S, 90°27W, elevation 300–400m) (O'Connor et al., 2010d). Because tree finches have been shown to overlap in morphology (Kleindorfer et al., 2014a), we only included nests of males that have previously (from 2005 onwards) been banded and genetically identified using nine nuclear microsatellite markers (Peters et al., in review); see Kleindorfer et al. (2014a) for detailed methods.

Genetic groups

Previous study showed asymmetrical introgressive hybridisation in the *Camarhynchus* tree finch group, which makes classification of hybrid tree finches difficult because

hybrids frequently backcross with *C. parvulus*, which results in the formation of a hybrid swarm (Chapter 4). Here, we assign adult males to one of three genetic groups (*C. parvulus*, *C. pauper* and hybrids) based on the individual membership coefficient (q_i) derived from Bayesian clustering analysis using STRUCTURE (Pritchard et al., 2000), which rates the probability (0–1) per individual of belonging to the *C. parvulus* cluster. We chose a q_i threshold of 0.80 for *C. parvulus* and 0.20 for *C. pauper* and assigned individuals with $0.80 > q_i > 0.20$ to the hybrid group (Peters et al., in review). This most likely excludes recent hybrids from the *C. parvulus* and *C. pauper* group, but very likely retains *C. parvulus* individuals in the hybrid group. Here we want to separately examine the hybrids and compare them with their parental species as well as with *G. fuliginosa;* therefore we excluded individuals with a q_i of 0.79–0.70 and of 0.30–0.21. By retaining only the hybrid individuals with a *qi* of 0.69–0.31, we can be relatively certain that these are actually hybrids of various generations, but not members of the pure genetic groups *C. parvulus* and *C. pauper*. Because the hybrid tree finches are not a separate species, we refer to them as a genetic group. For the ease of understanding, we refer to *C. parvulus*, *C. pauper* and *G. fuliginosa* also as genetic groups instead of species when reporting our findings.

To analyse parasite intensity across tree finch nests in more detail and with higher resolution of genetic membership, we used q_i to calculate a hybrid index (HI) (as advised in Fritz et al., 1999). The highest HI value was 0.5 (0.5 probability to belong to either of the two clusters = hybrid) and the lowest value 0 (1.0 probability to belong to one of the two clusters = pure individual). To calculate HI we retained the q_i for individuals with $q_i < 0.50$ and used the inverse value ($1–q_i$) for individuals with $q_i > 0.50$. In this analysis we did not exclude any individuals.

Parasite study species

The dipteran fly *P. downsi* is an invasive insect to the Galápagos; adult flies are non-parasitic and feed on organic matter but their larvae consume the blood and tissue of developing nestlings (Dudaniec and Kleindorfer, 2006). Female *P. downsi* oviposit in avian nests where larvae after hatching of host eggs. First instar larvae feed internally on their host through nasal and body cavities, while second and third instar larvae feed externally on hatchling blood and tissue (for detailed information on the life-cycle see Fessl et al. (2006b)). After feeding on hatchlings for ~ 4–7 days, larvae pupate in the base of the nest (O'Connor and Kleindorfer, unpublished data) from where they emerge as adult flies after approximately 7–18 days (P. Lincango and C. Causton, unpublished data). *Philornis downsi* has been identified as the biggest threat to contemporary Galápagos avifauna. Its larvae cause naris malformation (Galligan and Kleindorfer, 2009), blood loss and external and internal wounds (Fessl et al., 2006a) and up to 100 % annual nestling mortality (Dudaniec et al., 2007; O'Connor et al., 2010d). Recent study has shown vertical differences in fly abundance and found a correlation between nesting height (m) and *P. downsi* intensity (Kleindorfer et al., in press). Although the study used birds that had been assigned to species level morphologically, not genetically, and did not include the hybrid tree finches, its findings indicate the relevance of host nesting height for infestation levels (Kleindorfer et al., in press).

Nest searching and monitoring

We conducted daily nest searches within the study area from late January to late March 2010, 2013 and 2014 to record nesting activity (singing males, courtship, breeding behaviour). Each nest was marked and numbered; we recorded the colour bands of the nesting pair in *Camarhynchus* but *G. fuliginosa* birds were generally not

colour-banded. In *G. fuliginosa*, males and females were clearly distinguishable as males had all black plumage and females were grey-brown. Nests were checked at varying intervals; we monitored each nest every three days until day 10 of incubation; thereafter nests were checked every two days. Using a ladder and a pole mounted scope camera, we were able to accurately record the nest contents. We recorded nesting height (m), nesting status (building, incubating, feeding, failed, fledged), clutch size (number of eggs), brood size (defined as number of nestlings that hatched or were present in the nest at the onset of monitoring) and approximate nestling age (calculated based on hatching date, or estimated based on visual observations of nestlings for nests where hatching date was unknown).

Nesting outcome and P. downsi *intensity*

Once the nesting had finished, nesting outcome for each nest was scored as one of the following categories: (1) fledged (at least one chick fledged), (2) abandoned (incubation stopped at egg stage, eggs still in the nest), (3) dead nestling(s) (at least one dead nestling in the nest, total/partial brood loss), (4) empty (previously active nest with no clear sign of depredation, although this cannot be fully excluded), (5) depredated (nest is empty and shows clear sign of depredation such as broken egg-shell) and (6) environmental destruction (e.g. heavy wind breaks tree branch; nest collapses). The failure of nests containing dead nestling(s) (category 3) was attributed to *P. downsi*, although nests that were empty (category 4) could have also failed due to *P. downsi* as parents are known to remove dead nestlings (O'Connor et al., 2010b). We then collected the nest and transported it to the field station in a sealed plastic bag. Back at the field station we dismantled the nest, counted *P. downsi* larvae, pupae and empty puparia and assessed larval stages (1st, 2nd and 3rd instar) using criteria outlined in Fessl et al. (2006b) and Kleindorfer et al. (in press). Fragmented puparia could be

counted by identifying individuals based on the distinct puparium cap, which was always intact (Wiedenfeld et al., 2007). After assessment, larvae and pupae were preserved in ethanol for future analyses; therefore we could not assess observed pupation success for *P. downsi*. After the nestlings had fledged or died, larvae could potentially pupate provided they were in the 3rd instar larval stage; 1st instar larvae will perish (Fessl et al., 2006b). To investigate potential pupation success, we therefore combined numbers of 3rd instar larvae and pupae and calculated their percentage in relation to total *P. downsi* per nest.

We aimed to provide comparable sample sizes across genetic groups, but the rarity of *C. pauper* and *C. parvulus* combined with the greater numbers of hybrids and *G. fuliginosa* resulted in a skewed sample size towards the latter two groups.

Statistical analysis

Darwin's finch pairs can have several nests within the same year if climatic conditions allow, especially when previous nests have been unsuccessful and breeding failed at an early stage. To reduce possible pseudo-replication, we only included the first nesting event observed for each male per year and excluded subsequent nests, resulting in a dataset of 156 nests (2010 = 77, 2013 = 38, 2014 = 30). For analysis of nesting outcome, only nests with known outcome were included (N = 87, 2010 = 45, 2013 = 23, 2014 = 19). Because male territories are small, pseudo-replication of unbanded *G. fuliginosa* pairs within years is unlikely, but cannot be excluded between years. We compared nesting outcome across genetic groups using Chi-squared likelihood ratio tests.

We examined data for normality and homogeneity of variances. The number of days that the nest was occupied by nestlings (number of days nestlings survive) was log transformed to meet assumptions of normality and compared across genetic groups

using ANOVA with Tukey HSD post-hoc test. The variables nest height, clutch size, and brood size violated assumptions of a normal distribution and were analysed using Kruskal-Wallis tests. We performed pairwise comparisons following Dunn's (1964) procedure with Bonferroni correction for multiple comparisons as post hoc tests. Additionally, we analysed nest height using linear regression analysis with focussed contrast coding (*G. fuliginosa* = -2, hybrid = -1, *C. parvulus* = 1, *C. pauper* = 2) to visualise trends that might stay unnoticed when only comparing means. The independent variable was 'genetic group'; dependent variable was 'nest height'.

For analyses of parasite intensity, we only included nests that had produced nestlings (2010 = 23, 2013 = 27, 2014 = 14), as *P. downsi* larvae hatch after host eggs hatch (P. Lincango and C. Causton, unpublished data). Often nestling age is positively correlated with parasite intensity (number of parasites per nestling, defined by Bush et al. (1997)). We therefore use regression analyses to examine parasite intensity with the number of days that the nest was occupied by nestlings (number of days nestlings survive) as an independent variable. The variable "number of days nestlings survive" was scored as the age at death (in days) of the last nestling for failed nests, and age at fledging for successful nests.

We analysed the interspecific differences in total *P. downsi* numbers, *P. downsi* intensity and pupation percentage using focussed contrast linear regression including the independent variables 'year' and 'number of days nestlings survive'. Data were checked for outliers and heteroscedasticity (assessed using Glesjer test for heteroscedasticity (Glesjer, 1969), and one outlier was adjusted for analysis of parasite intensity. The dependant variables were total parasites, parasite intensity, and potential pupation success. Independent variables were genetic group (coded as hybrid = -2, *G. fuliginosa* = -1, *C. parvulus* = 1, *C. pauper* = 2 for analysis of total parasites and

parasite intensity, and *G. fuliginosa* = -2, hybrid = -1, *C. parvulus* = 1, *C. pauper* = 2 for analysis of nest height and potential pupation success), year and number of days nestlings survive.

To investigate the influence of larval competition and potential pupation success we analysed the relationship between total number of parasites in the host nest and the number of pupated and third instar larvae combined (as a measure of potential pupation success). The dependent variable was potential pupation success, independent variables were total parasite numbers, year and number of days nestlings survive.

To assess hybrid fitness in more detail, we used linear regression to test if hybrid tree finches have fewer parasites, using the hybrid index (HI) as a measure of admixture. The dependant variable were total parasites and the independent variable was HI, year, and number of days nestlings survive.

All analyses were performed using IBM SPSS Statistics 22.

Results

Nesting outcome

Only *G. fuliginosa* nests produced fledglings (8.9 %; 5/56). None of the tree finch nests produced a fledgling in any nest monitored by our group since 2010. Statistically, nesting outcome did not differ significantly between genetic groups (χ^2 likelihood-ratio = 16.412, $P = 0.355$, df = 15, N = 87, Table 5.1). Total nesting failure in tree finches was due to *P. downsi* infestation (37.9 % of nests) and nest depredation (31.0 %). Similarly, in *G. fuliginosa* total nesting failure was also mostly due to *P. downsi* parasitism (34.5 %) and nest depredation (20.0 %).

Nesting height and clutch size

Nesting height (m) differed significantly among the four genetic groups (Kruskal-Wallis test: 25.416, df = 3, $P < 0.001$, N = 98, Table 5.2). Nests of *G. fuliginosa* were lower than those of hybrid birds and *C. pauper* (pairwise comparison both $P < 0.03$). Using linear regression analysis, that nesting height of *G. fuliginosa* was lowest, followed by hybrids and *C. parvulus*, with *C. pauper* having highest nests (linear regression: $r_{partial} = 0.477$, t = 5.314, $P < 0.001$, N = 97, Table 5.2, Figure 5.1).

Genetic groups did not differ significantly in clutch size ($P = 0.09$) or brood size ($P = 0.61$) (Table 5.1).

Parasite numbers and intensity

All tree finch nests with hatchlings contained *P. downsi*, but surprisingly two *G. fuliginosa* nests with hatchlings were parasite-free. Four of the five *G. fuliginosa* nests that produced fledglings had high *P. downsi* intensity (51.2 ± 7.2 *P. downsi*/nest, parasite numbers of fifth nest are unknown) and all five nests had partial brood loss.

Total *P. downsi* per nest differed between genetic groups (linear regression, controlling for 'year' and 'number of days nestlings survived': $r_{partial} = 0.540$, t = 3.849, $P < 0.001$, N = 40, Table 5.3). Hybrid birds had lowest total *P. downsi*, followed by *G. fuliginosa* and *C. parvulus*. *Camarhynchus pauper* had the most *P. downsi* per nest of all four genetic groups. We found this same statistical pattern when analysing mean parasite intensity per hatchling (linear regression including 'year' and 'number of days nestlings survived': parasite intensity $r_{partial} = 0.483$, t = 2.589, $P = 0.004$, N = 38, Table 5.3).

Total *P. downsi* per nest was negatively correlated with genetic admixture (linear regression, controlling for 'year' and 'number of days nestlings survived': $r_{partial}$ = -

0.602, t = -2.447, P = 0.001, N = 25, Table 5.3, Figure 5.2). Regardless of the genetic group, total *P. downsi* numbers decreased with increasing genetic admixture (measured using HI), indicating that nestlings suffered less from parasitism when sired by hybrid males.

Local host-parasite assemblages

Linear regression using focussed contrast revealed that the percentage of pupae and 3rd instar larvae was highest in *G. fuliginosa*, followed by hybrid birds and *C. parvulus*, with lowest percentage in *C. pauper* (linear regression, controlling for 'year' and 'number of days nestlings survived': $r_{partial}$ = -0.336, t = -1.819, P = 0.011, N = 30, Table 5.3).

Estimated pupation success decreased with increasing parasite intensity within the host nest (linear regression, including 'year' and 'number of days nestlings survived': $r_{partial}$ = -0.455, t = -3.066, P < 0.001, N = 40).

Discussion

This study found hybrids to have fewer parasites than their parental species *C. parvulus* and *C. pauper*, adding to evidence of the first evidence for increased parasite-related fitness of hybrids in this system. Although we did not observe any successful fledging in hybrid nests, the hybrid nests had 28 % and 68 % fewer *P. downsi* than the two parental species *C. pauper* and *C. parvulus*, respectively. In-nest numbers of *P. downsi* decreased with increasing genetic admixture of the nesting male. This finding points to possible hybrid fitness in this newly evolving system under strong natural selection from *P. downsi*. Since 2010, despite intensive nest monitoring by our team, we can confirm no successful nesting outcome in any *Camarhynchus* tree finch nest monitored by our group on Floreana Island. The main cause of nesting failure in 87 tree finch

nests was parasitism by *P. downsi* (37.9 %) or depredation (31.0 %). Another significant finding was higher pupation success for *P. downsi* in *G. fuliginosa*. Similar to hybrids, *G. fuliginosa* had 21 % and 64 % fewer *P. downsi* than *C. pauper* and *C. parvulus* respectively, as well as highest parasite pupation success.

Hybrid fitness in Darwin's tree finches

Hybrid fitness relative to the parental species may be lower when there are genetic incompatibilities (Mayr, 1963), higher due to enhanced heterozygosity (Grant and Grant, 1992), or comparable. Hybrids in host-parasite systems may differ from their parental species in their susceptibility or resistance to parasites (Fritz et al., 1999). Our study found fewer *P. downsi* in hybrid nests compared to their parental species. Even more compelling evidence for hybrid fitness was the pattern of fewer *P. downsi* with increasing genetic admixture (measured by the hybrid index). These findings suggest selection for hybridisation in this system.

Despite no evidence for nesting success, a previous study by our group found higher annual recruitment among hybrid birds as evidenced by more hybrid yearling birds that we mist-netted than yearlings of the parental species (Kleindorfer et al., 2014a). This indicates that tree finches may nest at other times of the year, in other locations, or at cryptic nests not discovered by our team. Re-nesting is common in Darwin's finches, so increased nest monitoring across the year would give us a more complete understanding of recruitment success. We clearly found fewer *P. downsi* in hybrid nests. Knutie et al. (2015) recently explored the concept of tolerance to *P. downsi* and found differing levels of tolerance between nestlings of Darwin's medium ground finch (*Geospiza fortis*) and the Galápagos mockingbird (*Mimus parvulus*). Unlike *G. fortis*, nestlings of *M. parvulus* were interpreted as being more tolerant to *P. downsi* larvae as they increased their begging intensity and henceforth parental provisioning, which

apparently compensated for parasite damage (Knutie et al., 2015). Low levels of tolerance in hybrid tree finches could help explain their non-existent nesting success despite their lower parasite numbers. Such interspecific differences in adaptive behavioural strategies certainly deserve much future attention and research.

Despite the longstanding perception of prevailing hybrid inferiority (Mayr, 1963, 1992), hybrid fitness is not uncommon: Arnold and Hodges (1995) found that in 55 % of reviewed studies, hybrids were fitter or equally fit in comparison to their parents. Most hybrid studies have been carried out in laboratory settings (e.g. Hawaiian silverswords (Carr and Kyhos, 1981), sockeye salmon (Wood and Foote, 1990) and *Drosophila* (Coyne and Orr, 1989) therefore examining the genetic component of hybrid fitness rather than the ecological one (Hatfield and Schluter, 1999). Hatfield and Schluter (1999) examined hybrid fitness of sticklebacks (*Gasterosteus aculeatus* complex) in a laboratory and in the wild and found high fitness for hybrids in the laboratory, but hybrid inferiority in the wild, suggesting ecological rather than genetic factors limiting hybrid fitness in this system. Fitness in hybrids between medium ground finches (*G. fortis*) and cactus finches (*G. scandens*) for example was shown to be linked to the varying availability of small and large seeds (Grant and Grant, 1996b). These studies highlight the importance of ecological factors in the selection for or against hybrids and emphasize the need for field studies.

The ecological and evolutionary significance of hybridisation in rapidly changing environments is increasingly becoming a focus of conservation approaches (Burke and Arnold, 2001; Hamilton and Miller, 2015; Seehausen, 2004). For example, sunflower hybrid species *Helianthus paradoxus*, had increased fitness in extreme and novel environments compared to the parental species *H. annuus* and *H. petiolaris* (Rieseberg et al., 2003). Hybrid fitness in relation to parasites has been shown to vary across taxa

(reviewed in Fritz et al., 1999). In 10–27 % of analysed plant studies and 50 % of animal studies, hybrids were more susceptible to parasites than their parental species, suggesting that parasites could play an important role in limiting the extent of hybrid zones (Fritz et al., 1999). A study of the house mouse (*Mus musculus*) showed that hybrids between subspecies *M. m. domesticus* and *M. m. musculus* had fewer internal parasites than their parental species (Moulia et al., 1995). Peters et al. (in review) speculated that the hybridisation between *C. parvulus* and *C. pauper* may facilitate adaptation to the introduced *P. downsi* through the increase of genetic variation. Our observation that tree finches had fewer parasites with increasing genetic admixture supports this idea.

Mechanisms of parasite related hybrid fitness in Darwin's tree finches

The question remains of why hybrid nests had fewer parasites than nests of *C. parvulus* and *C. pauper*. Little is known about the ecology, behaviour, and host selection criteria of *P. downsi* on Galápagos. Previous study by Kleindorfer and Dudaniec (2009) found that larger nests and nests in close nesting aggregations had higher *P. downsi* intensity. Quiroga et al. (2012) showed that the choice of nesting material could influence *P. downsi* infestation. Kleindorfer et al. (in press) found that adult *P. downsi* males and females differed in vertical distribution, with most female *P. downsi* trapped at heights of ~ 2 m and ~ 7 m and most male *P. downsi* trapped at ~ 4–5 m. Because the nesting height of *C. pauper* is ~ 7 m, Kleindorfer et al. (in press) proposed that it is the encounter probability with female *P. downsi* at ~ 7 m that could explain higher parasite intensity in *C. pauper*. In support of the nesting height hypothesis for *P. downsi* encounter probability, the genetic groups with the lowest parasite intensity (hybrids and *G. fuliginosa*) also had lowest nesting height. The frequency distribution of nest height (Figure 5.1) shows that most host nests across genetic groups were

distributed between 2–5 m from the ground. If female *P. downsi* predominantly occur at heights of 2 m and 7 m, the flies at 7 m could have fewer host nests available for oviposition, which raises infestation risk for those few host nests. Several studies have documented this 'encounter dilution effect' across taxa (e.g. sticklebacks (Poulin and FitzGerald, 1989), feral horses (Duncan and Vigne, 1979) and wasps (Wcislo, 1984)). In the case of mobile parasites infecting multiple hosts, social nesting aggregations can increase the detectability of host nests and therefore result in higher infestation levels than solitary nests (Mooring and Hart, 1992), as has been shown in this system on Santa Cruz Island (Kleindorfer and Dudaniec, 2009). Future research is needed to examine tree structure and nesting density in *Scalesia* forest on Floreana Island.

Species-specific pupation success: evidence for local adaptation?

Both parasite and host performance was highest for nests of *G. fuliginosa*. The proportion of potentially successful pupae was highest, and only *G. fuliginosa* nests produced any fledglings as observed by our team, whereas none of the monitored *Camarhynchus* nests were confirmed to produce a fledgling. Theory states that parasites should not kill their host too soon as this means an early termination of their resource base (Frank, 1996). In the case of *P. downsi*, larvae pupate after feeding on hatchlings for 4–7 days (P. Lincango and C. Causton, unpublished data). Once the chicks are consumed, larvae can only pupate if they are in the 3rd instar larval stage (Peters, pers. obs.). Dudaniec et al. (2010) found multiple *P. downsi* infestations and estimated that up to six different female flies oviposit in the same host nest. Intraspecific larval competition for resources can increase larval mortality and hence reduce reproductive success (Dukas et al., 2001; Quiring and McNeil, 1984). Our analysis revealed a highly significant negative relationship between potential pupation success and infestation levels. The low potential pupation success in *C. pauper* nests

illustrates that *P. downsi* depleted its resource (the nestlings) too quickly before being able to grow to a sufficient size allowing successful pupation. Our results therefore suggest that decreased larval competition due to lower parasite intensity could explain the high potential pupation success in *G. fuliginosa* and hybrids compared to *C. pauper*. Nevertheless, local adaptation of *P. downsi* to *G. fuliginosa* cannot be ruled out at this stage, as clearly this system holds a magnitude of (co)evolutionary potential (Kleindorfer et al., 2014b; Peters et al., in review). Genetic analyses of *P. downsi* across different host species are needed to test for intrinsic genetic versus ecological factors of pupation success.

Implications for conservation

The chronically low nesting success observed in Darwin's finches infested with *P. downsi* is alarming. Our data clearly show that the majority of nests failed due to *P. downsi* infestation. But depredation was also a key cause of nesting failure, and this is also an area of conservation concern. Previous studies have shown similar patterns with low nesting success due to *P. downsi* and high depredation (Cimadom et al., 2014; Huber, 2008; O'Connor et al., 2010d). Known predators of Darwin's finches include the endemic short-eared owl (*Asio flammeus galapagoensis*), but also introduced species such as the smooth-billed ani (*Crotophaga ani*) and the black rat (*Rattus rattus*). Particular conservation concern is warranted for the critically endangered *C. pauper* as this species only occurs on Floreana Island. Earlier studies have documented steep population declines of up to 52 % from 2004–2013 (O'Connor et al., 2010c; Peters and Kleindorfer, in review), and this study found *C. pauper* nests to have the highest *P. downsi* numbers (2–3 times more than *G. fuliginosa*, *C. parvulus* and hybrids), as has been shown previously (Kleindorfer et al., 2014b; O'Connor et al., 2010d). The introgressive hybridisation between *C. parvulus* and *C. pauper* has earlier been proposed

to function as a mechanism of gene preservation for *C. pauper* (Peters et al., in review). The occurrence of hybrid fitness documented in this study provides further evidence for a fitness benefit of hybridisation, perhaps as an adaptive response to environmental disturbance from an introduced parasite. This study reconfirms the importance of the hybrids in this system for the maintenance of genetic diversity and supports our earlier recommendation to manage the conservation of the Floreana tree finches as one group. Continuation of efforts to eradicate *P. downsi* or at least mitigate its impact is urgently needed.

Acknowledgements

We are grateful to the Charles Darwin Research Station and Galápagos National Park Service for the opportunity to work on the Galápagos, and for logistical support. This work was generously supported by Rufford Small Grants Foundation, Earthwatch Foundation, Club300 Bird Protection, Mohamed bin Zayed Species Conservation Fund, Ecological Society of Australia, and Flinders University. TAME airlines provided reduced airfares. We thank D. Arango, R. Bassi, R. Christensen, S. Gantefoer, D. Gaspard, K. Gavrilchuck, S. Humberto, M. Louter, B. O'Connell, J. Robertson, T. Seebacher, M. Schmidt, R. Schubert and V. Zanollo for field assistance. We thank Jody A. O'Connor for leading the field team in 2010 and for data collection and field work. We extend special thanks to the community of Floreana Island, and the local National Parks team for their invaluable assistance and support.

Table 5.1 Nesting outcome for Darwin's finches on Floreana Island, Galápagos in 2010, 2013 and 2014. Data are shown as percentage per species (N)

	Camarhynchus parvulus	*C. pauper*	**Hybrid**	*Geospiza fuliginosa*
Nests with fledglings (%)	0 (0)	0 (0)	0 (0)	9.1 (5)
Abandoned nests (%)	0 (0)	20.0 (2)	20 (3)	14.5 (8)
Depredated nests (%)	57.1 (4)	10.0 (1)	26.7 (4)	20.0 (11)
Nests containing dead nestlings (%)	28.6 (2)	50.0 (5)	46.7 (7)	34.5 (19)
Failed nests: empty/unknown (%)	14.3 (1)	20.0 (2)	6.7 (1)	14.5 (8)
Other* (%)	0 (0)	0 (0)	0 (0)	7.3 (4)
Total number of nests	7	10	15	55

* environmental causes such as breaking of nesting tree from strong wind

Table 5.2 Breeding variables for four genetic groups of Darwin's finches in the *Scalesia* zone on Floreana Island, Galápagos during 2010, 2013 and 2014. Data are shown as mean ± SE (95 % CI) [N].

	Camarhynchus parvulus	*C. pauper*	Hybrid	*Geospiza fuliginosa*	df	F	*P*
Clutch size	3.1 ± 0.1 (2.8–3.5) [7]	2.6 ± 0.3 (1.9–3.2) [9]	2.9 ± 0.3 (2.3–3.4) [13]	3.3 ± 0.1 (3–3.6) [49]	3	6.542	0.088
Brood size	2.1 ± 0.5 (0.9–3.3) [8]	1.6 ± 0.5 (0.6–2.6) [11]	2.2 ± 0.4 (1.3–3) [11]	2.3 ± 0.2 (2–2.7) [54]	3	1.842	0.606
Nesting height (m)	4.9 ± 0.6 (3.4–6.4) [9]	6.3 ± 0.5 (5.2–7.3) [10]	4.8 ± 0.4 (4–5.5) [16]	3.4 ± 0.2 (3–3.8) [64]	3	25.416	< 0.001
Number of days nestlings survive	7.3 ± 1.5 (1.1–13.6) [3]	9.4 ± 1.4 (5.4–13.4) [5]	6.0 ± 0.6 (4.5–7.5) [8]	6.9 ± 0.6 (5.6–8.2) [30]	3	1.136	0.346

Table 5.3 *Philornis* data for four genetic groups of Darwin's finches in the *Scalesia* zone on Floreana Island, Galápagos during 2010, 2013 and 2014. Data are shown as mean ± SE (95 % CI) [N].

	Camarhynchus parvulus	*C. pauper*	Hybrid	*Geospiza fuliginosa*	R_{part}	t	*P*
Number of *P. downsi* per nest	43.0 ± 15.8 (-0.9–86.9) [5]	95.5 ± 13.7 (60.2–130.8) [6]	30.8 ± 6.7 (15.7–45.9) [10]	34.1 ± 4.0 (26.0–42.3) [29]	0.540	3.849	> 0.001
***P. downsi* mean intensity (total parasites per nest /number of hatchlings)**	18.9 ± 8.8 (-5.5–43.2) [5]	43.9 ± 11.7 (13.9–73.9) [6]	13.1 ± 2.5 (7.6–18.7) [10]	13.1 ± 1.7 (9.5–16.7) [27]	0.483	2.589	0.004
Potential pupation success (% pupae and 3rd instar larvae)	41.1 ± 22.2 (-54.5–136.8) [3]	26.6 ± 9.0 (-2.0–55.2) [4]	50.4 ± 12.4 (22.5–78.3) [10]	60.7 ± 5.7 (48.8–72.7) [20]	-0.336	1.819	0.011

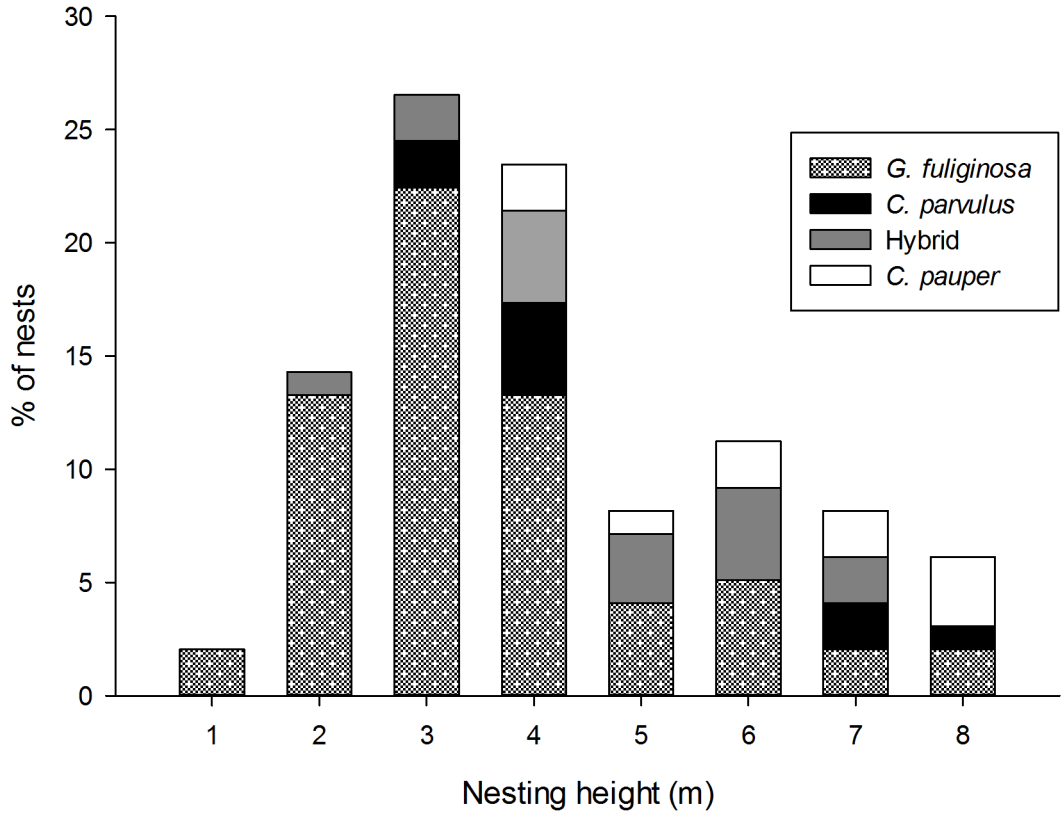


Figure 5.1 Frequency distribution of nesting height across four genetic groups of Darwin's finches (*G. fuliginosa*, *C. parvulus*, *C. pauper*, hybrids) in 2010, 2013 and 2014. Nesting height was lowest in *G. fuliginosa*, followed by hybrids and *C. parvulus*, and highest in *C. pauper* (linear regression: $r_{partial} = 0.477$, $t = 5.314$, $P < 0.001$, $N = 97$).

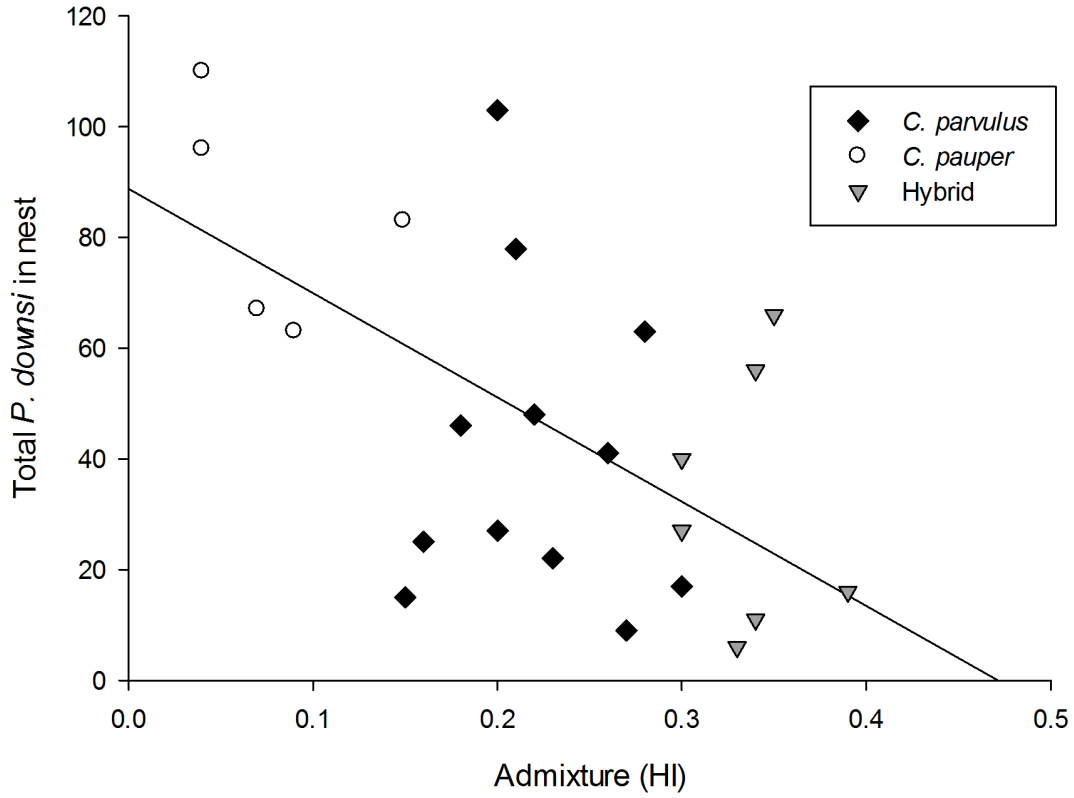


Figure 5.2 The relationship between total parasite intensity per nest and genetic admixture in Darwin's tree finches (*Camarhynchus* spp.) on Floreana Island, Galápagos in 2010, 2013 and 2014. Genetic admixture was measured using the hybrid index (HI), derived from the membership coefficient of microsatellite analysis using Bayesian clustering analyses. Parasite intensity decreased with increasing admixture (linear regression: $r_{partial} = -0.471$, $t = -2.447$, $P = 0.001$, $N = 25$). Graph shows the raw data excluding other independent variables in the regression model.

Chapter 6

Conclusion

Synthesis of findings & future directions

My thesis addresses the biological process of hybridisation and its significance for the preservation of biodiversity. The findings of my thesis provide a contemporary case study of hybridisation between rare and common species in parasitised systems. Identifying the driving forces in evolutionary processes is a crucial step for predicting species' trajectories and managing species conservation. My analysis of pairing observations and genetic data revealed that females of the rare *C. pauper* are driving the hybridisation with the common *C. parvulus* (Chapter 4). In contrast to *C. parvulus* females, who were only observed to pair with conspecifics or hybrids, *C. pauper* females showed no discrimination of heterospecifics and frequently paired with *C. parvulus* males. This has been observed in other studies where one of the hybridising species is rare (Hubbs, 1955). Due to the limited availability of conspecifics, females of the rare species may choose a male from a different species as this behaviour could be more likely to result in reproductive success rather than continuing the search for a conspecific male. The lack of discrimination for heterospecifics in the pairing behaviour of *C. pauper* females lead to a pattern of asymmetrical introgression, with gene flow skewed towards *C. parvulus* (Chapter 4). Together with the pairing observations and the analysis of song characteristics (Chapter 3), this provides an explanation for the formation of a hybrid swarm comprising *C. parvulus* and the hybrids. Analyses of genomic data and mitochondrial DNA would be useful to further investigate the asymmetrical introgression to test if particular alleles are being

preserved in this manner, and what functional genes are lost or gained in the hybrid birds.

While hybridisation is a common occurrence in at least ~ 16 % of bird species (Ottenburghs et al., 2015), fertility and fitness of hybrids varies greatly (Arnold and Hodges, 1995; Arnold and Martin, 2010; Burke and Arnold, 2001). My thesis presents the first evidence of hybrid fitness in relation to parasitism by larvae of the invasive fly *Philornis downsi* (Chapter 5). Hybrid nests contained fewer *P. downsi* than nests of their parental species *C. parvulus* and *C. pauper*. Particularly intriguing is (1) the fact that *P. downsi* infestation decreased with increasing genetic admixture, and (2) the lower nesting height of the hybrid compared to *C. pauper*.

This study, which uses genetically assigned birds, confirms findings from a previous study using morphologically assigned birds to species level and shows that nesting height correlated with *P. downsi* intensity. Additionally, my thesis is the first to examine the behavioural ecology of genetically assigned hybrid tree finches and suggests that the lower nesting height of the hybrids could be directly related to their lower infestation levels. *Camarhynchus pauper*, whose nests had the highest parasite loads, also had nesting and foraging heights in the forest canopy; the forest canopy generally also had fewer host nests.

Because of the encounter-dilution effect (Mooring and Hart, 1992), female *P. downsi* that have been shown to be abundant at ~ 7 m height (Kleindorfer et al., in press) have fewer host nests available, which may also explain more *P. downsi* per nest in canopy host nests. This idea remains to be tested on a larger scale, together with other ecological variables known to influence *P. downsi* infestation such as close nesting aggregations and the choice of nesting material. Furthermore, due to restrictions imposed by the low resolution of the genetic data, we could not identify the different

hybrid generations (F1, F2 and backcrosses). Future analyses using SNPs or genome sequencing could provide information on fitness differences between these generations. Since the host-parasite system comprising Darwin's finches and *P. downsi* clearly has high potential for coevolution (Chapter 4 & 5), genomic sequencing of *P. downsi* could elucidate coevolutionary dynamics such as the potential local adaptation to *G. fuliginosa* (Chapter 5).

When hybridisation is likely to increase the adaptive potential of species, it has been suggested that conservation management should consider the ecosystem as a whole rather than focussing on single species, as this will result in greater preservation of biodiversity (Becker et al., 2013). My thesis illustrates the value of this approach. In the case of the Darwin's finches on Floreana, hybrid tree finches likely serve as a reservoir for genetic variation of *C. pauper* (Chapter 4). Given *C. pauper*'s severe decline in the past decade (Chapter 3) and the high numbers of *P. downsi* found in its nests (Chapter 5), this species may not persist in the future. In this case, hybridisation will have preserved part of its genetic diversity that would have otherwise been lost. Therefore, hybridisation in this system contributes to the preservation of biodiversity.

The conclusions from my thesis are tempered by a few limitations, which are summarised here. The relatively small study site (constrained by site accessibility and restricted species range) and small population size limited sampling options for all species, but particularly for *C. pauper* (Chapters 2–5). These constraints resulted in small sample sizes that limit the confidence of certain analyses (Chapters 2–5). The relatively low number of nine microsatellite loci restrained the resolution of genetic data, which prevented more detailed analyses (Chapter 4 & 5). Population assessment would have been more representative if conducted over several days (Chapter 2). I

have acknowledged these limitations in each chapter and interpreted the findings with caution.

Implications for conservation

Nests of the critically endangered *C. pauper* had the highest *P. downsi* infestation levels of the four genetic groups (*C. parvulus*, *C. pauper*, hybrid and *G. fuliginosa*) and produced no successful fledglings in 2010, 2013 and 2014 (Chapter 5). This species also declined by 52 % from 2004–2013 (Chapter 3). The hybrids are serving as a reservoir harbouring genes from *C. pauper* (Chapter 4). While this retains genetic variation in the system, the population trends in *C. pauper* are highly alarming, and this species may possibly be heading towards extinction if *P. downsi* continues to infest nests at its current rate. Several bird species have already become locally extinct on Floreana, including the large tree finch *C. psittacula* (Kleindorfer et al., 2014a; Peters and Kleindorfer, in review), warbler finch *Certhidea olivacea*, (Grant et al., 2005b), Floreana mocking bird *Mimus trifasciatus* (Curry, 1986), and likely the vermillion flycatcher (Merlen, 2013b). While these species are still extant on other islands of the Archipelago, *C. pauper* is endemic to Floreana Island and its extinction here would mark the first complete extinction of an avian species of the Galápagos Islands since their discovery.

Population monitoring is an important part of species conservation management, and is particularly crucial for small and range restricted populations. Bioacoustical analysis of tree finch song showed that hybrids had similar song characteristics as *C. parvulus*, making these two species acoustically indistinguishable (Chapter 3). Contrastingly, *C. pauper* song differed from *C. parvulus* and hybrid song, and could henceforth be used for acoustically surveying *C. pauper* abundance. As acoustic surveys are the prime method used for monitoring Darwin's finches (Dvorak et al., 2012; Dvorak et al., 2004;

O'Connor et al., 2010c), my findings provide essential information for conservation management. Monitoring of the population development of the *Camarhynchus* group on Floreana should be conducted regularly given the low nesting success of all species and the critically endangered status of *C. pauper*.

Three important conservation issues for Floreana's tree finches are *P. downsi* parasitism, nest depredation, and habitat destruction and fragmentation. Floreana Island has been subject to habitat clearing for agricultural purposes, and while it still harbours *Scalesia* forest, areas are patchy and total habitat size does not exceed 4km² (O'Connor et al., 2010c). Analyses of foraging (Chapter 2) and nesting behaviour (Chapter 5) showed that Darwin's tree finches utilise the complete vertical range of their habitat. Conservation of the remaining habitat will be paramount to the survival of Darwin's tree finches on Floreana Island.

There have been ongoing efforts to develop management strategies for biological control towards both *P. downsi* and introduced rodents on the Archipelago, such as the production of the *Philornis* Action Plan (Causton et al., 2013) with the aim of mitigation and eradication of the fly, as well as bait dispersal in order to eradicate rodents from several islands. The *Philornis* Action Plan is a communal effort by a group of international researches collaborating with Galápagos National Park Authorities and the Charles Darwin Research station. It comprises the establishment of long-term strategies to eradicate the fly from the Archipelago, as well as short-term solutions to mitigate its impact on Galápagos' avifauna in the meantime. The identified research targets include identifying seasonal fly abundance, distribution and dispersal, host selection, reproductive behaviour, ecology of the fly and the establishment of methods for successful laboratory-based rearing of larvae. The *Philornis* Action Plan, of which my thesis forms a part, is a significant step towards the successful control and

possible future eradication of *P. downsi* to preserve the living legacy of Darwin's finches.

Appendix

Appendix 1 Inflection points of point-count survey data determined individually for different years and species following Reynolds et al. (1980). Only birds recorded within the distance of the inflection points were included in calculations of relative abundance and estimated population size

Species	2004	2008	2013	
			Observer 1 (KJP)	Observer 2 (SK)
Small Tree Finch *Camarhynchus parvulus* & hybrid group[1]	70	50	50	50
Medium tree finch *C. pauper*	70	50	40	40
Small ground finch *Geospiza fuliginosa*	60	50	30	40
Medium ground finch *G. fortis*	60	50	40	Not detected
Yellow warbler *Dendroica petechia*	30	20	30	30
Galápagos flycatcher *Myiarchus magnirostris*	Not detected	20	10	10
Smooth-billed ani *Crotophaga ani*	10	20	50	40
Dark-billed cuckoo *Coccyzus melacoryphus*	Not detected	50	80	70

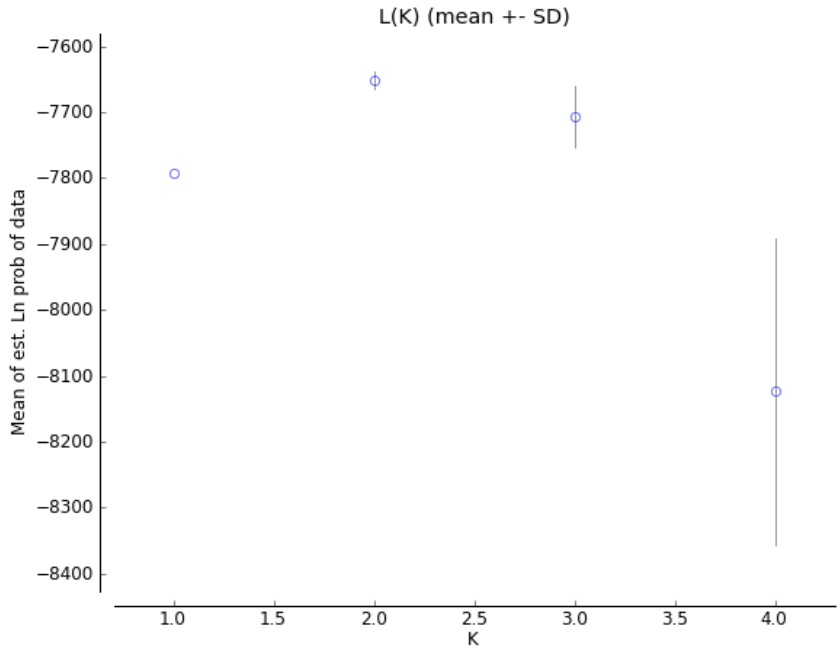
Appendix 2 Assessing the accuracy of three different threshold values of individual proportion of membership (qi). Error rates for genetic cluster assignment using 10 simulated datasets for each of the three different qi threshold values, data are shown as % error (SE). Error rates are calculated as the percentage of mismatches when assigning the simulated individuals to a genetic group based on the averaged probabilities derived from 10 runs in STRUCTURE 2.3.4 per dataset using the LOCPRIOR model. Based on these results, the inclusive threshold value of qi > 0.8 was chosen for the real data.

		Inclusive threshold for q_i		
		0.75	**0.80**	**0.85**
% Overall		16.6 (1.6)	8.7 (1.8)	23.2 (5.3)
% Per genetic group	***Camarhynchus parvulus* group**	38.4 (3.0)	17.4 (3.6)	63.6 (13.6)
	Hybrid	10.6 (1.3)	7.9 (1.4)	3.4 (1.5)
	***C. pauper* group**	0.7 (0.3)	0.8 (0.5)	2.4 (0.9)

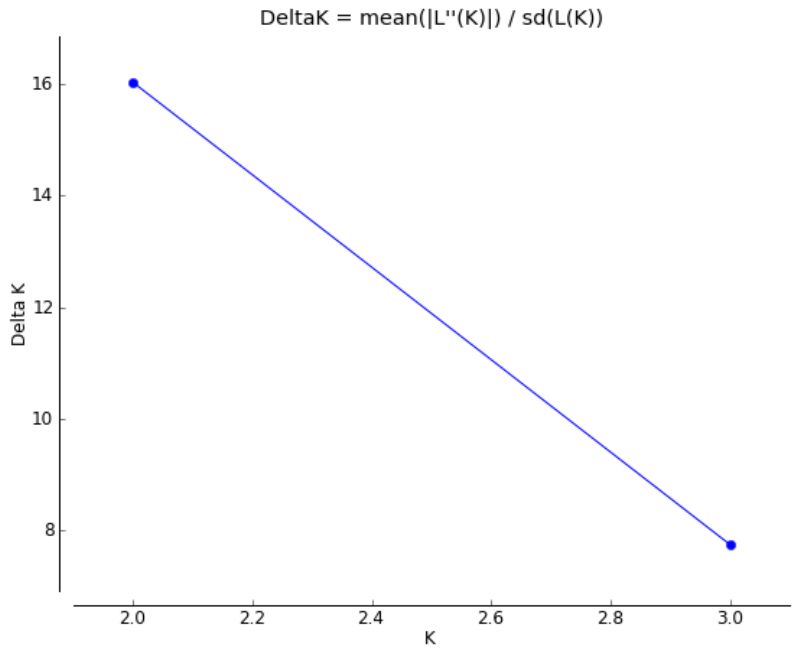
Appendix 3 Allelic variation for two putative populations at 9 microsatellite loci across 8 sampling periods over 10 years (2004, 2005, 2006, 2008, 2010, 2012, 2013, 2014). Loci that depart significantly from Hardy-Weinberg equilibrium are indicated in bold. N = sample size; Na = number of alleles; Ho = observed heterozygosity, He = expected heterozygosity; (GenAlEx 6.5; GenePop 4.2).

Putative population	Locus	N	Na	Ho	He
Population 1	**Gf1**	**226**	**19**	**0.84**	**0.89**
	Gf3	230	12	0.59	0.66
	Gf4	**248**	**4**	**0.11**	**0.16**
	Gf5	237	6	0.56	0.62
	Gf6	250	5	0.11	0.13
	Gf7	223	7	0.47	0.45
	Gf11	**230**	**12**	**0.50**	**0.57**
	Gf12	242	15	0.83	0.85
	Gf13	239	17	0.69	0.75
Population 2	Gf1	98	13	0.76	0.87
	Gf3	**96**	**10**	**0.54**	**0.72**
	Gf4	101	3	0.08	0.09
	Gf5	94	4	0.76	0.75
	Gf6	101	4	0.06	0.06
	Gf7	90	6	0.29	0.25
	Gf11	**80**	**8**	**0.41**	**0.53**
	Gf12	98	11	0.76	0.81
	Gf13	95	10	0.45	0.53

Appendix 4 Mean logarithm of probability of the data for K = 1–4, estimated using the LOCPRIOR model in STRUCTURE.



Appendix 5 Delta K for K = 1–4, calculated by transforming logarithm of probability of the data estimated using the LOCPRIOR model in STRUCTURE.



Appendix 6

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Divergent foraging behavior in a hybrid zone: Darwin's tree finches (*Camarhynchus* spp.) on Floreana Island

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Abstract Hybrid speciation is increasingly recognized as a mechanism for novel evolutionary trajectories. However, we know very little about the ecology of a contact zone that has arisen in sympatry. This study examines the foraging behavior and fitness of two species of Darwin's tree finches (*Camarhynchus parvulus*, *C. pauper*) and hybrid offspring on Floreana Island. Previous study showed that the percentage of hybrids in the tree finch population increased from 19% in 2005 to 41% in 2010, and their body and beak size increased by ~5% (parental phenotype did not change). In 2005–2006, all three tree finch groups (two parental species and hybrid birds) used the same foraging substrate, technique, and height. By 2010–2013, the small tree finch *C. parvulus* had changed its foraging technique and the medium tree finch *C. pauper* had changed its foraging height. Both parental species had higher body condition when foraging at (divergent) mean foraging heights per species but hybrid birds did not. We discuss the implications of conserving forest to facilitate vertical niche expansion and the role of hybridization for genetic persistence [*Current Zoology* 61 (1): 181–190, 2015].

Keywords Hybridization, Speciation, Adaptive radiation, Conservation, Scalesia, Ecological niche

The 14 species of Darwin's finches on the Galapagos Islands are a textbook example of adaptive radiation that have provided compelling evidence for both the magnitude and direction of selection in rapidly changing environments (Grant and Grant, 2014a; Grant and Grant, 2002; Grant and Grant, 2008). Other well-known examples of adaptive radiations that have produced morphologically distinct descendent species include the ~150 species of Anoles lizards in the Caribbean (Losos, 2009), ~250 species of cichlid fishes in Lake Tanganyika, Tanzania (Burress, 2014; Takahashi and Koblmüller, 2011), and ~52 species of Hawaiian Honeycreepers (Pratt, 2005). While these case studies have greatly increased our understanding of the processes underpinning divergence and speciation, the ecology at the time of the divergence has rarely been observed directly. Therefore, there is little direct information about the ecological context of hybridization events that occurred in the distant past.

Ecological speciation "involves the generation of reproductive isolation between populations as a result of ecologically based divergent selection pressures" (Price, 2008). When phenotypes adapt to local niches in heterogeneous environments, ecological divergence and speciation are favored (Schluter, 2000). The biological species concept defines species as populations with little or no gene flow as the result of reproductive barriers (Price, 2008). Hybridization occurs because the reproductive barrier between two species becomes porous or breaks down altogether (Mallet, 2005). Since hybridization may cause species to collapse into a single swarm, it can lead to reduced species richness and "reverse speciation" (Grant and Grant, 2014b; Seehausen, 2006; Seehausen et al., 2008; Taylor et al., 2006). On the other hand, hybridization may be a valuable source of genetic introgression that increases variance and gives rise to novel favorable genetic combinations (Barton, 2001; Burke and Arnold, 2001; Grant and Grant, 1992, 1994; Grant et al., 1996). Viewed in this light, hybridization can be the start of a new evolutionary lineage (Grant and Grant, 2014c).

Many extant species are the result of hybrid speciation that occurred thousands or millions of years ago (Johnston, 1969; Mallet, 2007; Price, 2008; Weir and Schluter, 2004). The ecology of previous hybridization events is largely unknown, but range expansion and contraction of parental and hybrid lineages are often invoked to explain current distribution patterns of species of hybrid origin. There are at least 200 known hybrid zones, but there is no compelling evidence for a contact zone that has arisen in sympatry (Price, 2008). It is not known if hybrid offspring use different resources

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compared with the parental species at the time of genesis, and whether the subsequent genetic and morphological divergence of the novel hybrid genotype can occur in situ. The study of Darwin's tree finches (*Camarhynchus* spp.) on Floreana Island offers a rare opportunity to witness contemporary hybridization and to test for ecological niche divergence in the contact zone in situ.

From 2005 to 2010, Darwin's tree finches on Floreana Island increased the proportion of hybrids from 19% to 41% (Kleindorfer et al., 2014a). During that same time period, hybrid body size increased by 5% while body size in both parental species remained similar (Kleindorfer et al., 2014a). Given the increase in relative abundance of hybrids and their body size, we ask if hybrid and parental tree finches occupy novel ecological space in sympatry. We compare foraging behavior (substrate, technique), foraging height, and fitness surrogates (body condition, time to successfully feed) to answer three questions. (1) Do hybrids and parental species occupy different foraging niches? (2) How have the foraging parameters changed over time comparing 2005–2006 and 2010–2013? (3) Is there evidence for fitness costs (lower body condition, longer time to success) related to foraging behavior in parental species versus hybrid individuals?

Darwin's finches feed on a variety of foods depending on the species; the finches are renowned for having morphological adaptations that enhance fitness for processing different dietary items (Grant, 1986). Darwin's tree finches are insectivorous, but their diet includes vegetable matter (Tebbich et al., 2004, Christensen and Kleindorfer, 2009). Diet composition is further affected by rainfall because this influences food availability (Tebbich et al., 2004; De Leon et al., 2014). In a comparative study of foraging behavior in arboreal Darwin's finches on Santa Cruz Island, Tebbich et al. (2004) found evidence for different foraging technique and substrate use among four Darwin's finch species including small tree finch *C. parvulus* and large tree finch *C. psittacula*. Therefore, we predict that small tree finch and medium tree finch *C. pauper* on Floreana Island will differ in foraging technique and substrate.

1 Materials and Methods

1.1 Study Location

This study was carried out on Floreana Island in 2005, 2006, 2010, 2012, and 2013. Different fieldwork activities were done during the months January to March, which coincides with the onset of peak breeding activity in Darwin's finches. Birds were mist-netted and observed in the *Scalesia* forest at the base of Cerro Pajas volcano (1°17′S, 90°27′W, elevation 250–350 m) (O'Connor et al., 2010a). The highland forest is dominated by the endemic trees *Scalesia pedunculata*, *Croton scouleri*, and *Zanthoxylum fagara*. Other main plant species are *Phoradendron henslowii* (Mistletoe), the shrub *Macraea laricifolia*, as well as several introduced fruit species (*Citrus limetta*, *Passiflora edulis*, *Psidium guajava*). Rainfall varies significantly across years on the Galapagos Islands (Grant and Boag, 1980), and is measured daily by the Galapagos National Park using rain gauges on Santa Cruz Island. The years 2005 and 2006 had lower rainfall from January to March (332 mm, 118 mm respectively) while 2010, 2012, and 2013 had higher annual rainfall (635mm, 672mm, 429mm respectively) (CDF Meteorological Database, http://www.darwinfoundation.org/datazone/climate/).

1.2 Study species across two study periods (2005–2006 and 2010–2013)

All guidebooks refer to three Darwin's tree finch species on Floreana Island: small tree finch, medium tree finch, and large tree finch (C. *parvulus*, *C. pauper* and *C. psittacula*, respectively). But a recent study by Kleindorfer et al. (2014a) found only two genetic groups and one hybrid cluster of tree finches on Floreana Island. The hybrid offspring were the result of pairings between females of the critically endangered medium tree finch and males of the common small tree finch (Kleindorfer et al., 2014a). At present we do not know whether hybridization extends beyond the F1 generation. Some level of introgression with the small tree finches is inferred due to ~15% higher genetic diversity compared to medium tree finches (Kleindorfer et al., 2014a). Unpublished data show that hybrid birds are fertile and offspring are viable, but more genetic analyses are required to gain insights into introgression patterns. The generation time for Darwin's finches is usually 1 year, but following extreme rainfall periods shorter generation times have been observed (Grant, 1986).

In this study we refer to the two parental populations (small tree finch, medium tree finch) and the hybrid birds. Hybrid birds increased in body size from 2005 to 2010 and were of intermediate body size between the parental species (Table 1) (Kleindorfer et al., 2014a). In the statistical analyses, the variable to denote the three groups is referred to as "genetic population".

1.3 Tree finch genetic assignment and morphology

We mist-netted, color-banded and measured 47 small

Table 1 Morphological trait values and body condition (mean ± *SE*) for males with genetic assignment to either parental species (small tree finch, medium tree finch) or hybrid birds on Floreana Island during the years 2005 and 2010

Trait	Small tree finch *C. parvulus*		Hybrid *Camarhynchus*		Medium tree finch *C. pauper*	
	2005 (n = 28)	2010 (n = 19)	2005 (n = 18)	2010 (n = 43)	2005 (n = 22)	2010 (n = 26)
Bill Length (mm)	7.4 ± 0.1	7.5 ± 0.1	7.5 ± 0.1	7.8 ± 0.1**	8.8 ± 0.1	8.8 ± 0.1
Bill Depth (mm)	7.1 ± 0.0	7.4 ± 0.1***	7.4 ± 0.1	7.7 ± 0.1**	8.4 ± 0.1	8.5 ± 0.1
Tarsus Length (mm)	20.3 ± 0.1	20.4 ± 0.2	20.5 ± 0.2	21.0 ± 0.2**	22.3 ± 0.2	22.4 ± 0.2
Wing Length (mm)	62.0 ± 0.2	61.7 ± 0.0	63.0 ± 0.0	64.0 ± 0.3*	67.0 ± 1.0	68.0 ± 0.5
Body Condition	-0.37 ± 0.3	-0.46 ± 0.3	-0.41 ± 0.5	-0.87 ± 0.2	1.22 ± 1.0	1.06 ± 0.3

The hybrid birds significantly increased in body size while the parental species had comparable body size across study periods, with the exception of bill depth in small tree finch. The P-values of independent *t*-tests are shown as **P*-value < 0.05; ** *P*-value<0.01; ***P*-value <0.001.

tree finches, 61 hybrid finches, and 48 medium tree finches (Table 1). We collected a blood sample for genetic analysis using microsatellite markers and assigned all birds to their respective genetic populations (for details see Kleindorfer et al., 2014a). Given the difficulty of assessing a Floreana tree finch using morphology, we only included color-banded birds with genetic assignment in this study. All foraging observations were done on color-banded and genetically assigned birds. Using calipers, we measured the following morphological traits per bird: (1) bill length nostril (mm), (2) bill depth (mm), (3) tarsus length (mm) and (4) wing length (mm). Body condition was calculated as the unstandardized residuals of mass against tarsus length (Brown 1996). Since the mass of eggs significantly alters the body mass of female finches, we only calculated body condition for male birds.

1.4 Foraging behavior, height, and time to foraging success

Foraging behavior was recorded for 156 color-banded birds observed during two weeks in February (2005, 2006, 2010, 2012, 2013). SK collected all data on foraging behavior and foraging height by walking a 1 km transect from 7–9 am and scoring every color-banded bird observed to forage. There were four different transects (one per study plot), each transect was traversed once per day across four days and repeated once (8 days sampling). Most observations were made at close range (< 8 m) due to the generally tame character of the finches. To avoid pseudo replication in the data, only the first foraging observation per bird was included for analysis (Kleindorfer et al., 2006; Myers et al., 2010). Table 2 lists the definitions for foraging substrate and technique used in this study. For analysis, we combined the foraging techniques "pick/glean" and "chip/pry". Additionally, SK visually estimated the height (m) at which the individual was foraging, and measured the time taken to successfully obtain a food item [time to success (s)] using a stop- watch from the moment a bird was first observed until it consumed a food item. The data collection was similar to methods described in Christensen and Kleindorfer (2009) for foraging *Camarhynchus* tree finches on Floreana Island during the years 2005 and 2006. For this paper, the data analysis differs from Christensen and Kleindorfer (2009) because we only use color-banded birds that have been assigned to a genetic population (Kleindorfer et al., 2014a); Christensen and Kleindorfer (2009) refer to the large tree finch *C. psittacula* while Kleindorfer et al. (2014a) make a case that the large tree finch is locally extinct on Floreana Island. Christensen and Kleindorfer (2009) refer to 2005 as a dry year (< 30 mm) but 2006 (~150 mm) as a wet year given reports for rainfall in the local study site near Cerro Pajas (pers. comm. E. Egas, Galapagos National Park Service). In this study, we refer to lower rainfall conditions during 2005 and 2006 compared with 2010, 2012 and 2013. Although we do

Table 2 Definitions used for foraging substrates and techniques

	Name	Definition
Technique	Glean	Removing prey from foliage surface
	Bite	Ingesting part of food item
	Probe	Inserting bill into substrate
	Pick	Removing prey from non-foliage surface
	Chip off	Downward thrust of bill
	Pry off	Using bill to lift substrate
Substrate	Foliage	Live and dead foliage
	Bark	Live and dead bark, branches
	Flower	Live or dead flower, flower bud
	Moss	Moss and lichen
	Fruit	Berries
	Other	Ground, seed heads

not explicitly analyze the effects of rainfall on foraging behavior, it is an important factor for resource abundance, especially in Darwin's finches. For this reason, we had a short timeframe for foraging data collection, to control for rainfall and resource distribution and to be able to compare foraging in the three genetic populations at the same time of year.

1.5 Statistical analysis

We used Chi-square tests to compare foraging technique and substrate use across genetic population and year. We used analysis of variance to test for differences in foraging height, time to success and body condition across genetic population and year, and multivariate analysis of variance to test if foraging height and foraging time to success differed in relation to genetic population and foraging technique. Once we had identified that foraging height differed across tree finch groups, we wanted to test if birds had lower body condition above and below their mean foraging height. Visual inspection of the pattern between foraging height and body condition showed a curvilinear relationship between the two variables. Therefore, we used quadratic regression within each genetic population to test if body condition was higher at a certain foraging height and lower for other foraging heights. All statistical analyses were performed using IBM® SPSS® Statistics Version 22.

2 Results

2.1 Morphological data

Table 1 presents the morphological data for male birds with known genetic assignment for the years 2005 and 2010. Hybrid birds became significantly larger within the 5 year study period, while small and medium tree finches did not change significantly in morphology.

2.2 Comparison of foraging between tree finch groups

Combining the data across all years, the three tree finch groups did not differ significantly in foraging technique ($\chi^2 = 7.279$, $df = 6$, $n = 157$, $P = 0.296$) (Table 3), foraging substrate ($\chi^2 = 9.461$, $df = 8$, $n = 157$, $P = 0.305$) (Table 3) or foraging "time to success" (ANOVA: $F_{2,153}=1.338$, $P = 0.265$) (Table 4). However, they differed significantly in foraging height (ANOVA: $F_{2,154}=4.705$, $P = 0.010$) (Table 4, Fig. 1).

Table 3 Foraging technique and foraging substrate in three genetic populations of Darwin's tree finches (*Camarhynchus* spp.) on Floreana Island (Galapagos) for two sampling periods (2005–2006, 2010–2013)

		Small tree finch *C. parvulus*			Hybrid *Camarhynchus*			Medium tree finch *C. pauper*		
		All years	05/06	10–13	All years	05/06	10–13	All years	05/06	10–13
Technique	Pick/ glean	59.5% (22)	33.3% (5)	77.3% (17)	46.5% (40)	40% (2)	46.9% (38)	45.5% (15)	0% (0)	53.6% (15)
	Bite	18.9% (7)	26.7% (4)	13.6% (3)	19.8% (17)	20% (1)	19.8% (16)	24.2% (8)	60% (3)	17.9% (5)
	Chip/ pry	2.7% (1)	0% (0)	4.5% (1)	19.8% (17)	20% (1)	19.8% (16)	12.1% (4)	20% (1)	10.7% (3)
	Probe	18.9% (7)	40.0% (6)	4.5% (1)	14.0% (12)	20% (1)	13.6% (11)	18.2% (6)	20% (1)	17.9% (5)
Substrate	Bark	15.8% (6)	6.7% (1)	21.7% (5)	31.8% (27)	20.0% (1)	32.5% (26)	21.9% (7)	20.0% (1)	22.2% (6)
	Foliage	65.8% (25)	73.3% (11)	60.9% (14)	40.0% (34)	80.0% (4)	37.5% (30)	50% (16)	20.0% (1)	55.6% (15)
	Flower	10.5% (4)	13.3% (2)	8.7% (2)	11.8% (10)	0% (0)	12.5% (10)	9.4% (3)	20.0% (1)	7.4% (2)
	Fruit	5.3% (2)	0% (0)	8.7% (2)	15.3% (13)	0% (0)	16.3% (13)	15.6% (5)	20.0% (1)	14.8% (4)
	Other	2.6% (1)	6.7% (1)	0% (0)	1.2% (1)	0% (0)	1.3% (1)	3.1% (1)	20.0% (1)	0% (0)

Data are shown as percentage of observations (N).

Table 4 Multivariate analysis of variance in foraging behavior of Darwin's tree finch groups on Floreana Island

	Dependent Variable	SS	*df*	MS	*F*	*P*
Genetic Population	Height	39.017	2	19.509	5.353	0.006
	Time to Success	21.969	2	10.985	0.245	0.783
Technique	Height	35.334	3	11.778	3.232	0.024
	Time to Success	1958.587	3	652.862	14.577	0.001
Genetic Population * Technique	Height	25.094	6	4.182	1.148	0.338
	Time to Success	165.369	6	27.561	0.615	0.718

The model tests the effects of genetic population (small tree finch, hybrid birds, medium tree finch) and feeding technique on foraging height and time to foraging success.

Given the increase in proportion of hybrids from 2005 to 2010 (Kleindorfer et al., 2014a), we compare changes in foraging behavior and height across these two time periods below.

2.3 Changes in foraging from 2005-2006 to 2010–2013

Foraging technique differed significantly across sampling period in the small tree finch (Likelihood Ratio: χ^2 = 11.08, *df* = 3, *n* = 38, *P* = 0.011) but not the medium tree finch (Likelihood Ratio: χ^2 = 7.581, *df* = 3, *n* = 33, *P* = 0.056) or hybrid birds (Likelihood Ratio: χ^2 = 0.174, *df* = 3, *n* = 86, *P* = 0.98), (Table 3). Comparing 2010-13 with 2005-06, small tree finches used the foraging technique "pick/glean" more often (Fisher's exact test < 0.010) and "probe" less often (Fisher's exact test < 0.011).

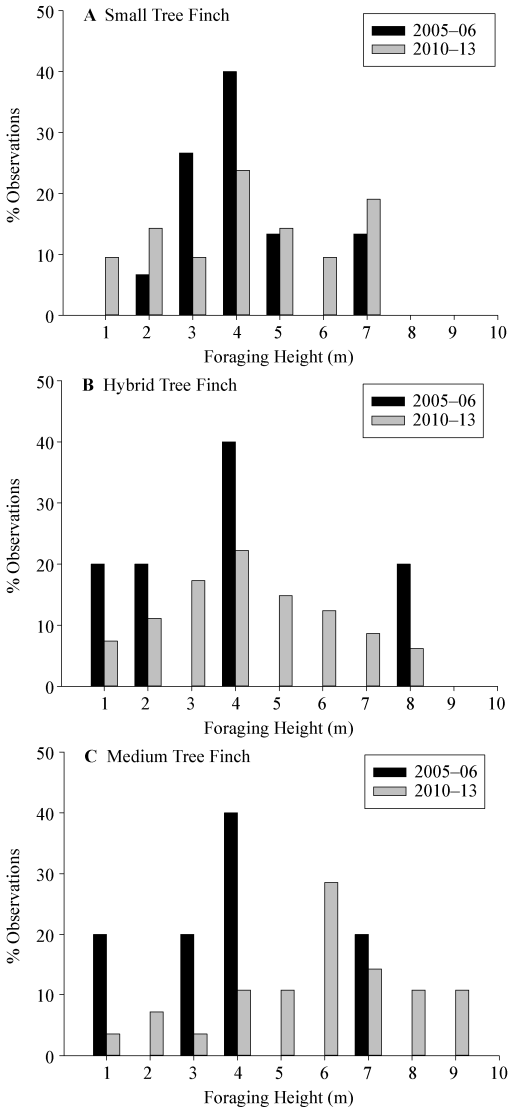

Fig. 1 The percentage of observations per foraging height (m) for three *Camarhynchus* tree finch groups (small tree finch, hybrid birds, medium tree finch) on Floreana Island across two study periods (2005–2006, 2010–2013)

Mean foraging heights (m) were: Small tree finch (2005–2006: 4.4± 0.4, 2010–2013: 4.3±0.4), Hybrid (2005–2006: 3.9±1.1, 2010-13: 4.4± 0.2), Medium tree finch (2005–2006: 3.6 ± 0.9, 2010–2013: 5.9±0.4).

Foraging time to success did not differ significantly across year or tree finch group (ANOVA, all *P* > 0.3). But time to success differed significantly in relation to foraging technique ($F_{3,154}$ = 54.447, *P* < 0.001) (see also Table 4). Therefore, to test if the three tree finch groups differed significantly in foraging efficiency, we compared time to success per foraging technique. Correcting for year, only "pick/glean" differed significantly across tree finch groups (ANOVA: $F_{2,76}$ = 3.583, *P* = 0.033), and was fastest in medium tree finch (2.9 ± 0.3 sec) compared with small tree finch (3.5 ± 0.6 sec) or hybrid birds (3.6 ± 0.5 sec). Foraging time to success was longest for birds using the foraging technique "chip/pry" (14–26 sec) (Table 5).

Substrate use per genetic population did not differ significantly across study periods. We found the same pattern of substrate use across the decade in small tree finches (Likelihood Ratio: χ^2 = 5.73, *df* = 4, *n* = 38, *P* = 0.220), hybrid birds (Likelihood Ratio: χ^2 = 4.85, *df* = 4, *n* = 86, *P* = 0.303), and medium tree finches (Likelihood Ratio: χ^2 = 5.69, *df* = 4, *n* = 33, *P* = 0.223) (Table 3).

Foraging height showed different patterns across study years comparing 2005–2006 and 2010–2013 (Fig. 1). There was no significant change in foraging height across years in small tree finch or hybrid birds (ANOVA: small tree finch: $F_{1,36}$=0.00, *P* = 0.993; hybrid: $F_{1,84}$= 0.273, *P* = 0.603) but the medium tree finch foraged higher in the canopy in 2010-13 compared with 2005–2006 ($F_{1,31}$=4.715, *P* = 0.038).

2.4 Body condition and foraging height

Body condition differed significantly between tree finch groups (ANOVA: $F_{2,207}$ = 7.377, *P* < 0.001) but not year ($F_{1,207}$ = 0.147, *P* = 0.702). The interaction term genetic population × year was not significant (*P* > 0.8). Medium tree finches had higher body condition than small tree finch or hybrid birds (Table 1).

We compared body condition and foraging height of individual birds observed to forage in 2010–2013 (sample size was too small for 2005–2006) (Fig. 2). The quadratic regression was significant for medium tree fin-

Table 5 Time to foraging success per foraging technique in three genetic populations of Darwin's tree finches (*Camarhynchus* spp.)

	Small tree finch *C. parvulus*	Hybrid *Camarhynchus*	Medium tree finch *C. pauper*	*F*	*P*-value
Pick/glean	3.5 ± 68 (22)	3.6 ± 0.5 (40)	2.9 ± 0.3 (15)	3.583	0.033
Bite/crush	5.9 ± 3.2 (7)	3.5 ± 1.1 (17)	3.8 ± 1.6 (8)	0.254	0.777
Chip/pry	14 (1)	23.1 ± 3.3 (17)	26.0 ± 11.4 (4)	0.384	0.687
Probe	6.5 ± 1.3 (7)	4.9 ± 1.6 (12)	3.7 ± 0.6 (6)	0.203	0.818

The data are shown as mean ± *SE* (*n*). The *F* and *P*-values are shown for ANOVA tests per foraging technique with time to success as the dependent variable and genetic population as the fixed factor.

ches ($R^2 = 0.490$, $P = 0.009$) and small tree finches ($R^2 = 0.411$, $P = 0.009$) but not hybrid birds ($R^2 = 0.027$, $P = 0.537$). The peak body condition occurred at the mean foraging height in each parental species, with no discernible pattern between body condition and foraging height in the hybrid birds (Fig. 2).

3 Discussion

Given the recent contemporary hybridization, Darwin's tree finches on Floreana Island provide a timely opportunity to test for changes in sympatric foraging niche in hybrid birds and parental species. The percentage of hybrid birds increased from 19% in 2005 to 41% in 2010 and they became ~5% larger in body size (Kleindorfer et al., 2014a, Table 1). In this study, we compared foraging behavior during 2005–2006 and 2010–2013. Across these study periods, both parental species changed their foraging niche while the hybrid birds did not. In 2005–2006, all three tree finch groups (two parental species and hybrid birds) used the same foraging substrate, technique, and height. By 2010–2013, the small tree finch had changed its foraging technique and the medium tree finch had changed its foraging height. Both parental species had higher body condition when foraging at (divergent) mean foraging heights per species but hybrid birds did not have higher body condition at any foraging height. Because of morphological overlap between the two parental species and hybrid birds, we restricted our analyses to birds with known population genetic assignment (Kleindorfer et al., 2014a). This decision limited our sample size and lowered the power of our analyses, but provided high confidence data. The rare opportunity to observe changes in ecological niche occupation at the time of increasing hybridization is the justification for our decision to proceed with data analysis using the smaller sample size. Aware of the limitations of small sample size, our findings suggest that different selection pressures could be operating along (possible) adaptive fitness peaks in a vertical niche distribution.

Variation in resource distribution is predicted to pro-

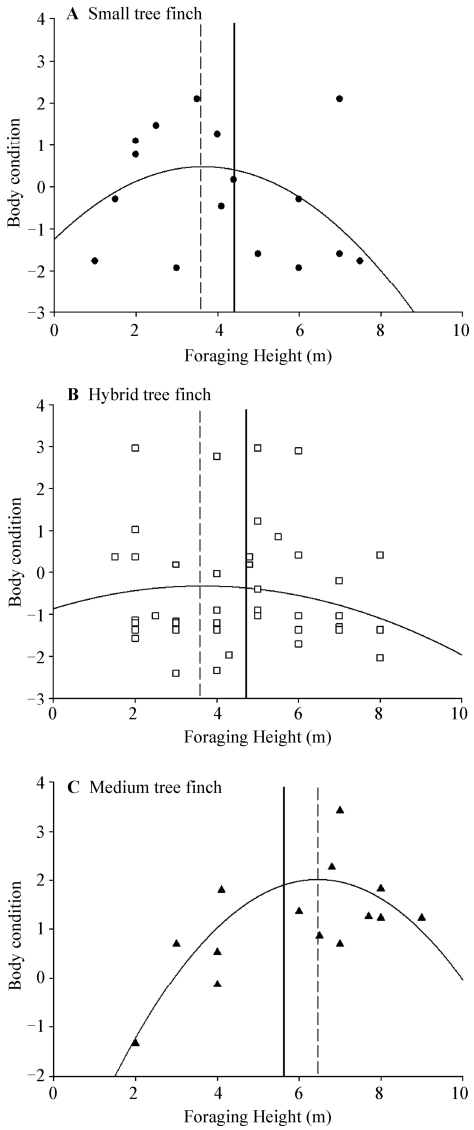

Fig. 2 Body condition plotted against foraging height for (A) small tree finch, (B) hybrid birds, and (C) medium tree finch
Dotted line represents the peak of the regression curve and solid line indicates the mean foraging height. There was a significant quadratic regression in small tree finch and medium tree finch but not hybrid birds (see results).

mote trait divergence across environments (Jeffries and Lawton, 1984; Schluter, 2000). In this way, natural selection acts on phenotypes. It is often difficult to identify if local phenotypes occur as the result of habitat-phenotype matching via dispersal, for example, or local selection (Galligan and Kleindorfer, 2010; Galligan et al., 2012; Sulloway and Kleindorfer, 2013). The advantage of this study is that the hybrid birds were born into the study area, and hence we can rule out habitat-phenotype matching as the mechanism for the intermediate hybrid phenotype. Once a given phenotype occurs in a particular environment, what is its fitness? There is much debate about relative hybrid fitness (e.g. Arnold and Martin, 2010), especially for ecological analyses of the novel genetic introgression. Here, we use body condition and time to foraging success as measures of fitness, which we compare for the different foraging domains of the sympatric tree finch groups. If local phenotypes are adapted to their current resource domain, we predict that they will extract resources with greater efficiency (shorter "time to success") and therefore have higher body condition. Animals with higher body condition are expected to have higher survival and more success rearing their young because they can more easily incur the high costs of reproduction (Golet and Irons, 1999; Reid, 1987). We found no evidence for higher hybrid fitness using these fitness surrogates. The medium tree finch had the fastest foraging time to success, and both parental species had higher body condition when foraging within their species-specific mean foraging height.

Adult males of the critically endangered medium tree finches, which are the largest birds in the Floreana tree finch group (see Table 1), had higher body condition than small tree finches and hybrids. Since large-bodied animals can competitively exclude smaller individuals from foraging in preferred resource patches (Rowland, 1989; Schoener, 1983), it is possible that the medium tree finches expanded their foraging into the upper forest because they encountered more insects and more inflorescences from *Scalesia* trees. Our findings of higher body condition and faster foraging success support this interpretation, but more work needs to be done to quantify the (possible) mechanism of competitive exclusion in sympatry (Abbott et al., 1977; Diamond, 1978; Grant and Grant, 1982). Data on vertical resource distribution within the study site are needed to more fully understand vertical patterns of resource use.

Hybrid tree finches did not have higher body condition and were not more efficient at extracting resources than their parental species. Perhaps we chose poor measures of fitness indicators (body condition and time to success), which is why we did not detect a pattern in hybrid birds. Other fitness variables could include clutch size, hatching and fledging success, parasite intensity, recruitment, annual survival and population abundance. From previous survey data and population estimates on Floreana Island, we know that the critically endangered medium tree finch population plummeted by 61% from 2004 to 2008 (O'Connor et al., 2010) but here we show that this species had the highest body condition. This finding seems counter-intuitive. Nesting success has been very low in medium tree finch, with 0% fledging success since 2012 (Kleindorfer et al., 2014b). In-nest chick mortality was caused by flesh-eating larvae of the introduced parasitic fly *Philornis downsi* (Kleindorfer et al., 2009, 2014b; O'Connor et al., 2010b; Dudaniec et al., 2010). Medium tree finch nests had more *P. downsi* larvae compared with most other Darwin's finch species (Dudaniec et al., 2007; Kleindorfer et al., 2014b). Although higher body condition is often linked with fewer parasites or higher survival under conditions of parasitism (Brown et al., 2000; Møller et al., 1998), this does not directly apply here because the parasite *P. downsi* consumes the blood of nestling birds and not adults (Dudaniec and Kleindorfer, 2006; Dudaniec et al., 2006; Fessl et al., 2006; Huber, 2008; O'Connor et al., 2014). Therefore, even if adult small and medium tree finches had better body condition and higher adult survival than the hybrid birds, their numbers could still be declining due to low nesting success. Likewise, even if adult hybrids have lower body condition, population numbers could still increase if they have higher nesting success due to lower parasite infestation. These ideas need to be tested with longitudinal data. Future research on host-parasite dynamics (including hybrid nesting success in relation to parasite infestation) and the ecology of host and *P. downsi* interactions are needed to better understand possible trade-offs between signaling environment, foraging environment, and fitness (Endler, 1993, 1992; Endler and Basolo, 1998).

Phenotypes that are close to the adaptive fitness peak should have higher fitness (Benkman, 2003; Price, 2008; Schluter and Grant, 1984). We found highest body condition per parental species aligned with their mean foraging height, whereas this was not the case for hybrid birds. Using the logic of local adaptedness to fitness peaks, these findings suggest the possible existence of only two adaptive peaks for foraging – each occupied

by one parental species. This reasoning could help to explain the local extinction of the large tree finch *C. psittacula* (Kleindorfer et al., 2014a) if its foraging niche was destroyed due to human impacts, for example. In the case of contemporary hybrid fitness when both parental species occupy the adaptive fitness peaks, selection should not favor the intermediate phenotype of the hybrid birds and consequently the intermediate-sized hybrids might not persist for long.

Rainfall has a significant influence on resource availability and distribution (Grant and Grant, 1980), which influences foraging behavior. A study on ground finches (*Geospiza* spp.) showed that during years with higher rainfall, the different ground finch species largely overlapped in diets, while during years with lower rainfall (when food was less abundant), each species had a more specific "private" diet (De León et al., 2014). In our study period, we classified the years 2005 and 2006 as 'dry years' and 2010, 2012 and 2013 as 'wet years'. Therefore, our findings of foraging differences across time periods could reflect the underlying effect of rainfall. However our findings were opposite to those of Grant and Grant (2014) and De León et al. (2014): we found foraging overlap during the low rainfall years (2005–2006) and foraging differences during the high rainfall years (2010–2013) in small tree finch and medium tree finch but did not observe changes in the hybrid birds.

The outcomes of this study are relevant to conservation biology for several reasons. First, there is widespread debate about the conservation value of hybrids if they dilute evolutionary significant units but generate biodiversity through genetic introgression and hybrid speciation (Allendorf et al., 2001; Barton, 2001; Fitzpatrick and Bradley Shaffer, 2007; Mallet, 2005; Soltis and Gitzendanner, 1999). Second, little is known about the ecology of hybridization events, which hampers conservation management of such processes. More is known about the ecology of extant hybrid zones (Price, 2008). Third, there is growing evidence that anthropogenic influences may increase hybridization rates through the introduction of competitor species, pathogens, and increasing habitat destruction and fragmentation (Allendorf et al., 2001; Seehausen et al., 2008). Therefore, understanding ecological resource use of hybrids in changing environments will generate insights into biological responses to a range of human-induced impacts. The forest on which Darwin's tree finches depend is highly threatened. On Santa Cruz Island, only 1% of the *Scalesia* forest remains; it has been virtually cleared from San Cristobal and Isabela Islands (Watson et al., 2010). Floreana Island harbors the last significant remnant of endemic *Scalesia* forest supporting the observed increase in tree finch hybridization over the past years. The remnant *Scalesia* is vulnerable to introduced plant and pest species (Watson et al., 2010). Besides habitat destruction and fragmentation, other significant threats for Darwin's tree finches include the spread of introduced species like *P. downsi* (Causton et al., 2006) and avian poxvirus (Kleindorfer and Dudaniec, 2006; Zylberberg et al., 2012). Our study shows that Darwin's tree finches use the entire vertical range of *Scalesia* forest (Kleindorfer, 2007; Kleindorfer and Dudaniec, 2009), and clearly, drastic efforts are needed to protect this vulnerable habitat.

The novelty of this study is the observation of sympatric habitat use in first generation hybrids and their two parental species. Despite a change in hybrid phenotype across the years, we did not find any evidence for different vertical habitat use by hybrid birds. We observed changes in the foraging behavior of both parental species as well as higher fitness in relation to their species-specific mean foraging height. These findings could support the idea of two adaptive fitness peaks each occupied by one parental species.

Even though we did not find evidence for contemporary hybrid fitness exceeding parental fitness (using our crude measures of body condition and time to foraging success), the emergence of the hybrid birds raises several conservation issues linked to fitness. Given the rapid population decline of the critically endangered medium tree finch, the hybrid birds could a) serve as a valuable genetic reservoir for the species, and b) replace the ecological role of the declining medium tree finch. Study is required to identify the risk of one or both of the parental species being swamped into a hybrid swarm.

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Appendix 7

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Changes in *Philornis* infestation behavior threaten Darwin's finch survival

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Abstract The conservation behavior framework is useful to identify key linkages between behavior and conservation practice. We apply this framework to a novel host-parasite system on the Galapagos Islands and ask if there have been changes in parasite oviposition behavior and host mortality patterns across the first decade (2004-2013) of its known association. The Dipteran parasite *Philornis downsi* was first discovered in Darwin's finch nests in 1997 and is the biggest threat to the survival of Galapagos land birds. Host mortality has increased over the past decade. In Dipterans, pupation and pupae size are determined by access to host resources. Here, we test the hypothesis that *P. downsi* flies are laying eggs in finch nests earlier in the nestling phase to maximize larval feeding time and therefore chance of pupation success before host death. The results show fewer 1st instar larvae later in the host nesting cycle in support of earlier egg laying behavior by female flies. Between 2004 and 2013, parasite intensity increased from ~28 to ~48 parasites per nest, host mortality increased from ~50% to ~90%, and host age at death decreased from ~11 to ~5 days. The earlier age at host death was correlated with fewer pupae (from ~50% to ~20%) and smaller pupae size (~10% decrease). Changes in parasite behavior reveal new fitness costs to both the parasite and Darwin's finches. These findings underscore the need for urgent conservation action to save Darwin's finches from extinction due to a novel, lethal and introduced parasite [*Current Zoology* 60 (4): 542–550, 2014].

Keywords Host mortality, Parasite size, Darwin's finches, Ectoparasitism, Camarhynchus, Geospiza

The conservation behavior framework (Berger-Tal et al., 2011; Caro and Sherman, 2011) is useful to identify key linkages between animal behavior and conservation practice; it offers a hierarchical framework to bridge the gap between the two disciplines of ethology and conservation biology. Three basic themes that link the two disciplines are identified: (1) anthropogenic impacts on behavior that impact biodiversity, (2) behavior-based species management, and (3) behavioral indicators of other processes of conservation concern (Berger-Tal et al., 2011; Brearley et al., 2013; Civitello et al., 2013; Daly and Johnson, 2011). A range of questions follow on from this context. What anthropogenic changes have changed species behavior and fitness? What species-specific behavior needs to be managed to enhance fitness? What behaviors signal that a species is experiencing a threat to its persistence? Because parasites can affect host phenotype, foraging behavior, and ecological interactions (Britton, 2013; Crane et al., 2011; Goodman and Johnson, 2011; Houte et al., 2013), understanding the role of parasite behavior for host survival is key to managing biodiversity (Edeline et al., 2008; McCallum, 2008; Murray et al., 2013; Thompson et al., 2010).

We use the conservation behaviour framework to ask if there have been changes in parasite behaviour that impact host mortality in a novel host-parasite system on Floreana Island, Galapagos Archipelago. Specifically, we seek to identify temporal changes in reproductive behaviour that could threaten host species persistence or indicate altered parasite fitness since the onset of this parasite invasion. Larvae of *P. downsi* were first discovered in Darwin's finch nests on Santa Cruz Island in 1997 (Fessl and Tebbich, 2002) though the fly was present by 1964 (Causton et al., 2006). *P. downsi* is considered the biggest threat to the survival of all Galapagos land birds (Causton et al., 2011; Dvorak et al., 2011). The non-parasitic adult *P. downsi* fly lays its eggs in nests of land birds (O'Connor et al., 2014; O'Connor et al., 2010b). These eggs hatch into larvae that consume the blood and tissue of developing chicks (Fessl et al., 2006b; Fessl et al., 2006c; Koop et al., 2011; O'Connor et al., 2010a; O'Connor et al., 2010b), and in some instances the incubating female (Huber et al., 2010). In Darwin's finch chicks, the larvae cause up to 50% blood

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loss (Fessl et al., 2006a), naris deformation (Galligan and Kleindorfer, 2009), and between 17 and 100% mean annual mortality in chicks (Dudaniec et al., 2007; O'Connor et al., 2010b). From laboratory trials conducted on the Galapagos Islands, the minimum time for *P. downsi* to complete larval development is 4 days until pupation (Causton et al., 2011; O'Connor pers. observation). There has been a trend for increased host mortality across the decade (Dudaniec et al., 2007; Huber, 2008; Koop et al., 2011; O'Connor et al., 2010b; O'Connor et al., 2010c; O'Connor et al., 2010d).

Understanding parasite behavior is key to understanding sources of selection for host and parasite (Poulin, 2011; Schmid-Hempel, 2011). In Dipterans, the duration of larval development is dependent on resource availability provided by the host. If the larvae have not consumed sufficient resources to complete development and the host dies, the larvae will either perish or be forced to pupate sooner (Poulin, 2011; Schmid-Hempel, 2011). In addition to the time window for resource acquisition, host resource availability determines pupae size: pupae are generally smaller under conditions of low resource availability (Quiroga and Reboreda, 2013; Spalding et al., 2002; Teixeira, 1999). To increase resource availability, parasites may infest a host earlier in the nesting cycle. Recently, Quiroga and Reboreda (2013) showed that the size of larvae and pupae in *Philornis seguyi* was correlated with adult fly size. In insects, there is a wealth of data for the correlation between small adult size and lower fecundity. In a comparative study of 57 oviparous insects, the common slope of the fecundity/size relationship was close to one (Honěk, 1993). Therefore, *P. downsi* pupae size is a reliable proxy for adult size and fecundity.

This study examines possible changes in the timing of parasite infestation by *P. downsi* in Darwin's finch hosts on the Galapagos Islands between 2004 and 2013. If the parasite is infesting host nests earlier during the host nesting cycle, we predict fewer 1st instar larvae later in the nesting cycle, more pupae, and larger pupae size. Here we ask, is there evidence for (1) earlier *P. downsi* infestation during the nesting cycle of host nests, (2) increased pupae size as the result of longer larval development, and lastly (3) a correlation between earlier host mortality (i.e. nestling age), pupation success and pupae size.

1 Materials and Methods

1.1 Study site and host species

This study was conducted during the months February to April during the years 2004, 2006, 2008, 2010, 2012, 2013 on Floreana Island, Galapagos Archipelago (described in Kleindorfer et al., 2014). The avian focal species are the common small tree finch *Camarhynchus parvulus*, the critically endangered medium tree finch *C. pauper*, and the common small ground finch *Geospiza fuliginosa* (Grant, 1986; O'Connor et al., 2010b; O'Connor et al., 2010c; Sulloway and Kleindorfer, 2013). The highland study sites on Floreana Island were in *Scalesia* forest at the base of Cerro Pajas volcano, which is the stronghold of the tree finch population on Floreana Island (1°17′46″ S, 90°27′06″ W) (O'Connor et al., 2010a; O'Connor et al., 2010b; O'Connor et al., 2010c). Darwin's finches begin breeding with the onset of the rains that usually occur around January or February. Males build a display nest and sing to attract a female (Kleindorfer, 2007b). Females visit the singing male and inspect the nest. If accepted, a female will subsequently lay a clutch size of 2–5 eggs per nest (Kleindorfer, 2007a); some nests had 6 eggs in 2008 and 2010. In all three focal species, the female is the sole incubator and both parents provide food to chicks. The incubation and feeding phase are ~14 days each.

The three focal species build domed shaped nests with a thick nest base that provides the nesting substrate for larvae of the introduced parasite *P. downsi* (Kleindorfer and Dudaniec, 2009). On Floreana Island, the study sites were characterized by low annual rainfall (~500 mm) in 2004 and 2006, high annual rainfall (~1,500 mm) in 2008 and 2010, and moderate annual rainfall (~800 mm) in 2012 and 2013 (Kleindorfer et al., 2014; Charles Darwin Foundation Meteorological Database: http://datazone.darwinfoundation.org/climate/).

1.2 Sample size

We monitored nesting outcome at 561 active Darwin's finch nests between 2004 and 2013 on Floreana Island. The sample size per focal species was 139 small tree finch, 196 medium tree finch, and 226 small ground finch nests. We analyzed the following subsets of data for this study: nests with *P. downsi* (238 nests), nests with data on percentage of 1st, 2nd, 3rd instar (88 nests), the percentage of *P. downsi* pupae in relation to larvae (191 nests), in-nest chick mortality (222 nests), chick age at death (150 nests), pupae size (66 nests), pupae size and chick age at death (39 nests).

1.3 Parasite species: Background information

Philornis downsi is a Dipteran parasitic fly that has a two-stage life-cycle, which is unusual for the genus: first instar larvae feed internally on the nasal and body cavities of its avian nestling hosts, and 2nd and 3rd instar

larvae feed externally on the chicks (Fessl et al., 2006c). The genus *Philornis* has a Neotropical distribution comprised of ~50 species (reviewed in Dudaniec and Kleindorfer, 2006; Quiroga et al., 2012). Possible sources of introduction of *P. downsi* to the Galapagos Islands are via known mainland hosts, such as smooth-billed ani *Crotophaga ani* that was first recorded in the Galapagos in 1962, as well as the rock pigeon *Columbia livia* that was introduced to the Galapagos in 1972. Because adult *P. downsi* feed on fruit, the fly could have been introduced to the Galapagos Islands via cargo boats laden with fruits and vegetables.

Philornis downsi is the only ectoparasite that causes measurable fitness costs in Darwin's finches; blood parasites have not been detected and intestinal protozoan parasites are rare (reviewed in Dudaniec et al., 2005; Dudaniec et al., 2006). From our previous genetic analysis of maternity, paternity, and offspring genetic structure of *P. downsi* within Darwin's finch nests, we found that each female fly mates with an average of ~2 males (range 1–5 males per female) and 1 to 6 females each contribute an average of five larvae per Darwin's finch nest (range = 1–24 eggs; Dudaniec et al., 2010). Female *P. downsi* flies generally carry ~60 eggs, therefore the female appears to only oviposit a portion of the available clutch per host nest (Causton et al., 2011; Dudaniec et al., 2010).

From in-nest video recordings, there is evidence that *P. downsi* flies enter active finch nests that contain eggs or chicks and lay eggs on the inner nest surface when parents are absent (O'Connor et al., 2010a; O'Connor et al., 2014). After host eggs hatch, the fly eggs hatch within ~6 hours (P. Lincango and C. Causton, unpublished data) and the *P. downsi* larvae crawl into the naris of the chick. The 1st and early 2nd instar larvae feed within the nares and body cavities of chicks (Fessl et al., 2006c). Late instar larvae (2nd and 3rd instar) move from inside the chick's naris and body to feed externally on the chick; the late instar larvae reside in the nest base during the day and emerge at night to feed on the chicks (O'Connor et al., 2014). Larvae pupate in the nest base after feeding on chicks for 4–7 days (O'Connor and Kleindorfer, unpublished data) and emerge as flies after 7–18 days (P. Lincango and C. Causton, unpublished data).

1.4 Host mortality

Host nesting status was determined from repeated 20-minute observations (every two days) of parental activity at each nest, as well as by nest inspection using a ladder (2004–2006) or mirror/camera on an extendable 6m pole (2008–2013). Chick age was determined by the date of hatching, and chick age at death in the nest was determined from nest inspections every two days per active nest. Darwin's finches fledge at ~14 days (O'Connor et al., 2010d) and in-nest mortality was calculated from the percentage of chicks per clutch size that died in the nest. For this study, we analyze chick age at death in relation to parasite intensity, percentage of larvae and pupae, and pupae size.

1.5 *P. downsi* instar distribution and pupae size

All Darwin's finch nests with chicks in this study had *P. downsi* parasites (100% prevalence). We collected all parasite samples per nest 1–2 days after the death or fledging of the last chick. The nesting material was dismantled and all *P. downsi* larvae, pupae and pupae cases were counted to calculate the total number of parasites (parasite intensity) per nest. The larvae were assigned to instar using a microscope in a laboratory. Chicks that had recently died were immersed in alcohol so that larvae within the body would float out and could be counted. We stored the pupae and larvae in ethanol within 24 hours of collection from the host nest.

To estimate the timing of *P. downsi* laying behavior in the host nest, we compare the percentage of larval instar phases in relation to host age at death. If *P. downsi* is laying earlier in host nests across the decade, over time we predict (1) a higher proportion of 1st instar larvae for younger hosts, (2) fewer 1st instar larvae in older hosts, and (3) more pupae in younger hosts. Because we were unable to measure 1st instar larvae during the first days of nesting when the host was still alive, we test for fewer 1st instar larvae and more pupae when hosts died by 4–6 days after hatching. We compare the proportion of larvae and pupae for different chick ages at death across the decade, which we use as a measure of pupation success. We assume higher pupation success when there are more pupae than larvae.

To test for changes in pupae size across the decade, we measured *P. downsi* pupae size from 66 Darwin's finch nests. In a controlled laboratory environment, we measured the pupae per nest to the nearest 0.1 mm using callipers. The nest sample size to calculate *P. downsi* pupae size is as follows: small tree finch (n = 22), medium tree finch (n = 18), and small ground finch (n = 26). For 40/66 nests we had information on chick age at death, which we analyzed in relation to pupae size.

1.6 Statistical analysis

Data were analyzed with SPSS 20 for Windows (SPSS Inc., Chicago, USA). We used linear regression analyses to test for changes across years in parasite in-

tensity, in-nest mortality, chick age at death (days post-hatching), proportion of pupae and larvae, and mean pupae size (mm) per nest; we also compared pupae size and chick age at death per species. We used ANOVA to test for an effect of Year and Species on parasite intensity, and MANOVA to test for an effect of Year and Species on the proportion of 1st, 2nd, and 3rd instar flies.

2 Results

2.1 Ectoparasite intensity

Total *P. downsi* intensity in Darwin's finch nests on Floreana Island increased significantly across years, from 27.5 ± 4.6 in 2004 to 48.4 ± 6.5 in 2013 (r = 0.261, n = 238, P < 0.001). To test for effects of Year and Species on parasite intensity in a single model, we used ANOVA. Parasite intensity differed significantly between years and focal species (ANOVA: Year: $F_{6,237}$ = 6.01, P < 0.001, partial eta^2 = 0.141; Species: $F_{2,237}$ = 22.87, P < 0.001 partial eta^2 = 0.173). The interaction term was significant (Species × Year: $F_{11,237}$ = 2.96, P = 0.001, partial eta^2 = 0.129). The critically endangered medium tree finch had significantly higher parasite intensity (54.7 ± 5.4) compared with the common small tree finch (28.7 ± 2.4) and common small ground finch (31.0 ± 2.1) (Tukey's post-hoc: P < 0.001), but there was no significant difference in parasite intensity between small tree finch and small ground finch (P = 0.819). Parasite intensity increased significantly across the decade in small tree finch (r = 0.252, n = 60, P = 0.050), medium tree finch (r = 0.478, n = 54, P < 0.001), and small ground finch (r = 0.178, n = 122, P = 0.049).

2.2 Host age at death

Nests with many *P. downsi* had earlier chick death (r = -0.370, n = 105, P < 0.001). Across the decade, Darwin's finch chicks died at a younger age in nests infested with *P. downsi* parasites (r = -0.65, P < 0.001, n = 150). We found the same pattern in small tree finch (r = -0.69, P < 0.001, n = 44), medium tree finch (r = -0.74, P < 0.001, n = 40), and small ground finch (r = -0.58, P < 0.001, n = 66). For all species combined, chick age at death was 10.6 ± 0.5 days in 2004 and 5.4 ± 0.3 days in 2013.

2.3 Ectoparasite oviposition behavior and pupation

To indirectly test if *P. downsi* oviposition behavior changed across the decade and occurred earlier during the host nesting cycle, we compared the percentage of *P. downsi* instar stages for the minimum period of rapid pupation (4 days) between 2004–2008 (n = 8 nests at which chicks died 4–6 days after hatching) and 2010–2013 (n = 26 nests at which chicks died 4–6 days after hatching). If the parasite is laying eggs earlier in the host nesting cycle, we should find fewer 1st instar larvae later during the nesting cycle. We found that *P. downsi* infested host nests significantly earlier in the nestling period in recent years (Table 1, Table 2). There were 6%–16% fewer 1st instar larvae in chicks that died 4–6 days after hatching comparing 2010–2013 versus 2004–2008 (Table 1, Fig. 1). There were 1%–20% fewer 1st instar larvae in chicks that died 8–10 days after hatching comparing 2010–2013 versus 2004–2008 (Table 1), but this change was not significantly different, which is likely due to small sample size as a result of high early mortality (all P>0.2) (Table 1).

Table 1 The percentage (mean ± *SE*) of *P. downsi* instar and pupae in Darwin's finch nests

(A) Early resource termination (host chicks die 4-6 days after hatching)

	Small tree finch *C. parvulus*		Medium tree finch *C. pauper*		Small ground finch *G. fuliginosa*	
P. downsi (%)	2004–2008 (n=2)	2010–2013 (n=10)	2004–2008 (n=3)	2010–2013 (n=10)	2004–2008 (n=3)	2010–2013 (n=6)
1st instar	18.8±7.5	2.6±2.0	13.7±2.0	1.3±1.3	16.1±3.1	10.1±9.1
2nd instar	16.2±4.8	37.7±9.9	14.1±3.4	22.3±8.9	12.7±1.5	37.7±5.4
3rd instar	40.0±12.7	56.7±10.6	38.5±6.6	65.8±9.5	51.0±4.5	46.1±8.4
Pupae	25.0±25.0	19.8±7.3	45.1±13.1	8.2±4.0	39.4±20.6	34.3±15.0

(B) Late resource termination (host chicks die 8-10 days after hatching)

	Small tree finch *C. parvulus*		Medium tree finch *C. pauper*		Small ground finch *G. fuliginosa*	
P. downsi (%)	2004–2008 (n=4)	2010–2013 (n=4)	2004–2008 (n=3)	2010–2013 (n=2)	2004–2008(n=10)	2010–2013 (n=3)
1st instar	3.8±3.8	2.6±2.6	21.4±6.7	0±0	11.9±19.9	0±0
2nd instar	10.5±4.3	27.0±13.1	15.2±2.4	3.5±3.5	10.9±5.8	1.0±1.0
3rd instar	41.8±6.9	45.4±11.9	43.7±3.6	67.6±22.0	36.2±11.5	44.8±29.3
Pupae	49.4±8.7	30.4±10.1	53.1±12.3	38.2±14.1	58.4±8.4	72.5±19.3

Table 2 MANOVA results for effects of Year and Species on the percentage of *P. downsi* instar in Darwin's finch nests across sampling periods (2004–2008, 2010–2013) on Floreana Island

		F value	*P* value	Partial Eta^2
Year	1st instar (%)	6.910	0.014	0.198
	2nd instar (%)	3.218	0.084	0.103
	3rd instar (%)	1.328	0.259	0.045
Species	1st instar (%)	0.606	0.553	0.041
	2nd instar (%)	0.293	0.748	0.021
	3rd instar (%)	0.052	0.949	0.004
Year × Species	1st instar (%)	0.442	0.647	0.031
	2nd instar (%)	0.276	0.761	0.019
	3rd instar (%)	0.760	0.477	0.051

Data are shown for early resource termination (host chicks die 4-6 days after hatching) in three Darwin's finch host species: small tree finch *Camarhynchus parvulus* (*n*=12), medium tree finch *C. pauper* (*n*=13), and small ground finch *Geospiza fuliginosa* (*n*=9). Only the percentage 1st instar changed significantly across the decade.

Pupation success was higher when hosts survived for longer. Using multiple regression analysis, there were more pupae when chicks survived for longer (r_{part} = 0.516, P = 0.003) and more larvae when chicks died younger (r_{part} = -0.540, P = 0.001). The percentage of pupae decreased significantly across the decade in small tree finch (r = -0.470, P < 0.001, n = 57) and medium tree finch (r = -0.423, P = 0.002, n = 49), but not small ground finch (r = 0.135, P = 0.217, n = 85). Because we stored the pupae and larvae in ethanol within 24 hours of collection from the host nest, we cannot assess rates of larval pupation after nest collection. Here we report on the finding of significantly fewer 1st instar larvae, fewer pupae, and smaller pupae size when hosts died younger – which became increasingly evident as the decade progressed.

2.4 Ectoparasite pupae size

Mean *P. downsi* pupae size decreased significantly across the decade (r=-0.54, P<0.001, n=66). Pupae size was 9.8±0.8 mm in 2006 compared to 8.6±0.2 mm in 2013. The pattern was comparable in small tree finch (r=-0.86, P<0.001, n=22), medium tree finch (r=-0.57, P=0.011, n=18), and small ground finch (r=-0.33, P=0.090, n=26) (Fig. 2). Larvae pupated at larger size when chicks survived for longer (r=0.34, P=0.031, n=39). Thus, *P. downsi* showed a change in behavior to earlier cessation of parasitism (i.e., via pupation) during the host life cycle that was associated with earlier host mortality.

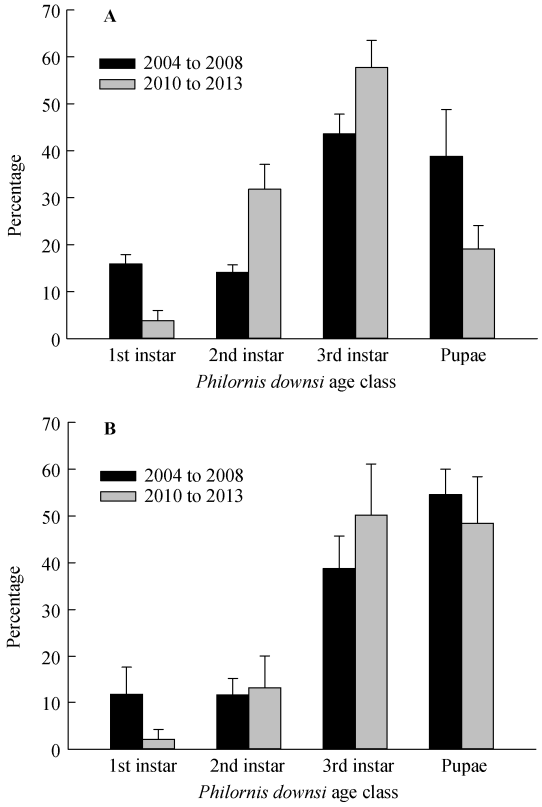

Fig. 1 The percentage (mean ± *SE*) of *P. downsi* instar and pupae in Darwin's finch nests across two sampling periods (2004–2008, 2010–2013) on Floreana Island
A. Early resource termination (host chicks die 4–6 days after hatching). **B.** Late resource termination (host chicks die 8–10 days after hatching). The minimum feeding time for *P. downsi* larvae to pupate is four days. From 2010 onwards, there were fewer 1st instar larvae as the nesting cycle progressed.

3 Discussion

The introduced fly *P. downsi* is considered the biggest threat to the survival of Galapagos land birds. The results of this study on Floreana Island support this view. Across the decade from 2004 to 2013, *P. downsi* parasite intensity nearly doubled (~28 to ~48 parasites per nest), in-nest mortality nearly doubled (~50% to ~90% in-nest mortality), and chicks died in half the time (~11 to ~5 days after hatching). The earlier age at host death predicted smaller pupae size. Also, across the decade, pupae size got 10% smaller (~10 mm to ~9 mm). In Dipterans, pupa size and adult fecundity co-vary nearly 1:1; therefore a 10% reduction in pupae size equates to a 10% decrease in parasite fecundity. The earlier death of the chicks and the smaller pupae size could be the result of a change in parasite behavior to infest the nest

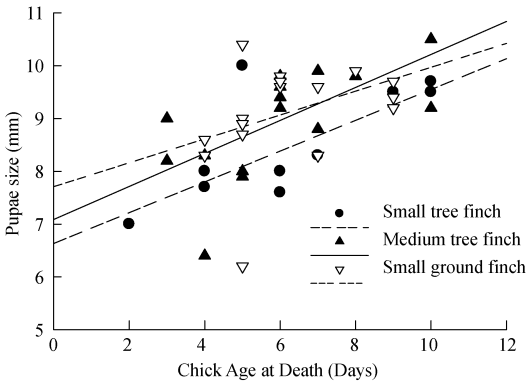

Fig. 2 The positive relationship between chick age at death and *P. downsi* pupae size for common small tree finch *Camarhynchus parvulus* (*n*=22 nests), critically endangered medium tree finch *C. pauper* (*n*=18 nests), and common small ground finch *Geospiza fuliginosa* (*n*=26 nests) on Floreana Island. Later host death predicted larger *P. downsi* pupae size (*r*=0.34, *P*=0.031, *n*=39)

earlier during the nestling phase. If *P. downsi* were infesting Darwin's finch nests earlier, one would predict fewer 1st instar larvae as the nesting cycle progressed, which was supported by our data. The combination of higher *P. downsi* intensity and a more synchronous age class of parasites means that Darwin's finch hosts were exposed to older (and hence larger) parasites consuming their blood and tissue from an earlier age, when nestlings are more vulnerable. This could explain why we found increased mortality across the decade in Darwin's finch hosts, as well as earlier age of death.

Parasites are predicted to exploit their hosts prudently to ensure maintenance of their resource base – without killing them too soon (Frank, 1996; Hanken and Wake, 1993). But this is clearly not the case with *P. downsi*. Since it was first discovered in Darwin's finch nests in 1997, the introduced parasite has been killing Darwin's finches at an ever-earlier age. Generalist parasites like *P. downsi* infest a range of host species, and have been found in Darwin's finch nests across habitats on Santa Cruz and Floreana Island (Dudaniec et al., 2007; Fessl et al., 2010; O'Connor et al., 2010a; O'Connor et al., 2014). Maladaptive virulence (whereby parasite behavior results in reduced parasite fitness and ability to reinfect) is predicted to be more common in generalist parasites (Leggett et al., 2013) because the generalist parasite is more likely to infest a novel host without shared co-evolutionary history. This study finds preliminary evidence for variable maladaptive virulence in *P. downsi*. Parasite pupation success (percentage of pupae) decreased across the decade in the two tree finch species but not in the small ground finch – despite the fact that parasite intensity across the decade increased significantly in all three focal species, as did host mortality. To summarize: host mortality patterns showed a similar pattern but parasite pupation showed a different pattern between host species. What attributes in the small ground finch compared with tree finches lead to higher pupation success in the novel parasite? This question should be the subject of future enquiry.

The Galapagos Islands are a natural laboratory to study host-parasite impacts and offer a unique window to study the importance of pathogens as selective agents in a relatively simple and pristine ecological context. Previous work on Santa Cruz Island strongly suggests that *P. downsi* intensity increases with high rainfall (Dudaniec et al., 2007), but this finding is not consistent (Koop et al., 2013). Rainfall on the Galapagos Islands is unpredictable within and across years (Grant and Grant, 2014). Future study should examine the role of rainfall and other ecological predictor variables for *P. downsi* intensity on Floreana Island. The aim of this study was to identify changes in parasite behavior and host mortality. Clearly much work remains to be done to more fully understand the ecological context of host-parasite associations (Auld et al., 2013; Duffy et al., 2012), including the data presented here.

In the current study, we document a potential trade-off between the parasite life-cycle (i.e. size at pupation) and host mortality, such that adult parasite fecundity (inferred from smaller pupae size) becomes reduced with earlier chick death. This observation suggests that a co-evolutionary arms race between maximizing parasite fecundity and keeping host resources available is occurring in this system. This brings about further consequences for reproductive investment by *P. downsi* female flies, which may co-infest nests with up to six additional females with each depositing up to approximately 24 eggs per nest (Dudaniec et al., 2010). With earlier parasite oviposition in host nests, earlier host death, and increased fecundity costs for parasites, it is feasible that *P. downsi* may be under selection to oviposit fewer eggs per nest, perhaps with fewer co-infesting females, in order to maximize fitness through reduced larval competition under a narrowing, temporary resource. In turn, this could have consequences for Darwin's finch hosts that must balance the benefits of the parasite-dilution effect observed for larger clutch size (Dudaniec et al., 2006) with a more synchronous parasite life-cycle that is

evolving under increasing levels of virulence. Rapid evolution of parasite life history traits has been observed in other systems (Duffy and Sivars-Becker, 2007; Jones et al. 2008; Kelehear et al., 2012) and requires further study in this system.

Theory predicts that parasites should become locally adapted – that is, have a fitness advantage in sympatric hosts over allopatric hosts that cannot be invaded by other non-adapted parasites (Kaltz and Shykoff, 1998). There is growing experimental evidence for local parasite maladaptation, indicating specificity for parasite attack and host defense in sympatric versus allopatric populations (Adiba et al., 2010; Lemoine et al., 2012). In the novel *P. downsi* and Darwin's finch association on Floreana Island, we found evidence for local parasite maladaptation across the decade given fewer pupae in tree finch nests, and evidence for local adaptation given more pupae in small ground finch nests. These findings would be complemented by further sampling, as well as replicated allopatric associations (Blanquart et al., 2013), which is possible within this naturally replicated island system.

The critically endangered medium tree finch, a species endemic to Floreana Island, warrants special concern as it had the highest mean *P. downsi* intensity of any Darwin's finch species studied to date (O'Connor et al., 2010d). Alarmingly, this species had 100% in-nest mortality (no fledging success) since 2012 (Kleindorfer, unpublished data). Even on the same island and in the same forest, the medium tree finch had more *P. downsi* compared with two sympatric Darwin's finch species (small tree finch, small ground finch), which raises further questions to be answered regarding host-specific virulence.

The central question posed at the beginning of this study was about changes in parasite behavior that may signal elevated extinction risk in naïve Darwin's finch hosts. The timing of *P. downsi* infestation behavior became earlier in the nesting cycle over the first decade of this host-parasite association, as inferred from the percentage of 1st instar larvae. The number of *P. downsi* per host nest also increased in all three host species across the decade. We suspect that these two factors explain why we found elevated host mortality and earlier age at death as the decade progressed. Our study therefore reveals changes in parasite behavior that pose additional challenges for Darwin's finch survival. These challenges should be considered as we develop conservation management strategies for this invasive parasite.

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Appendix 8

Flight behaviour of an introduced parasite affects its Galápagos Island hosts: *Philornis downsi* and Darwin's finches

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Abstract

Island populations have high extinction risk from introduced diseases that can spread quickly among geographically clustered naïve individuals. To conserve endemic island species, we need a suite of tools to analyse current threats posed by introduced species. Darwin's finches of the Galápagos Islands are being decimated by parasitic larvae of the introduced fly *Philornis downsi*, which was first discovered in Darwin's finch nests in 1997. We use the conservation behaviour framework to identify species-specific behaviours of the host and parasite in order to discover possible means of mitigating the impact of *P. downsi* on endemic Galápagos land birds. We measured Darwin's finch nesting height and number of *P. downsi* larvae per nest, and we placed McPhail traps to collect *P. downsi* adults and to determine their abundance in relation to fly trap height. Data were collected for three Darwin's finch species on Floreana Island during 2004-2014: small tree finch (*Camarhynchus parvulus*), Medium Tree finch (*C. pauper*), and small ground finch (*Geospiza fuliginosa*). Nesting height of Darwin's finches was positively associated with *P. downsi* intensity: higher finch nests had more *P. downsi* larvae. Fly traps higher in the canopy (7m) caught significantly more female *P. downsi*, while fly traps at 4-5m height caught more male *P. downsi*. The critically endangered medium tree finch nested at a mean height of 6.8m, and this species had the highest *P. downsi* intensity – perhaps because it was most likely to be encountered by female *P. downsi* given the typical nest height of this species. The findings can be used to inform conservation management strategies (for example, by placing fly traps at 6-8m height in order to increase the probability of removing egg-laying female flies). In conclusion, study of the vertical nesting behaviour of naïve avian hosts and vertical flight behaviour of introduced parasites can be applied to conserve endemic island birds.

Introduction

Disease and parasite outbreaks have become more frequent and more rapid as the consequence of increasing global interconnectedness, trade, and travel (Kilpatrick, 2011). Ecological theory predicts that a parasite epidemic will end when the hosts have died or evolved defences against the pathogen (Duffy and Sivars-Becker, 2007). Given that island populations are especially prone to extinction from introduced disease, islands warrant particular conservation scrutiny (Wikelski et al., 2004). There is growing information about the molecular ecology of virulence, which is useful in predicting the likelihood of acquired defences such as immunity (Alizon and Van Baalen, 2008, Kovaliski et al., 2014, McCallum, 2008). Populations not only evolve, but they are also composed of individuals with diverse behaviours. Because behaviour can shape evolutionary trajectories, understanding the behaviour of novel pathogens and naïve hosts can inform insights into evolutionary scenarios that may usefully be applied to conservation and epidemiological modelling (Nelson, 2014).

Linking insights from animal behaviour with conservation policy seems logical, but has been slow to take off in practice (Nelson, 2014, Caro, 1999). It is easy to imagine how an understanding of a species' dispersal, diet, and mating system would usefully inform conservation practice. In addition to these life history behaviours, there are many untapped approaches to integrating behaviour with conservation science that have not been sufficiently promoted by ethologists. This lack of integration has motivated some researchers to champion the "conservation behaviour framework" (Berger-Tal et al., 2011; Caro and Sherman, 2011), which identifies three major linkages between animal behaviour and conservation practice: (1) anthropogenic impacts on behaviour that impact biodiversity, (2) behaviour-based species management, and (3) behavioural indicators of other processes of conservation concern (Berger-Tal et al., 2011, Brearley et al., 2013, Daly and Johnson, 2011, Palestis, 2014, Kleindorfer et al., 2014a, Caro and Riggio, 2014). The proposed linkages between animal behaviour and conservation practice can be used to identify relevant thematically structured frameworks to generate research approaches that test ideas about the role of behaviour for species persistence. As Nelson (2014) summarises, the challenge is for behavioural biologists to "demonstrate how behavioural knowledge can make a difference to conservation management problems in light of the far more dominating effects of anthropogenic threats" (Caro and Riggio, 2014).

The Galápagos Islands offer a timely case study to understand the behaviour of a novel host-parasite system. Endemic land birds including naïve Darwin's finch hosts (Passeriformes: Emberizidae) are experiencing extensive malformation and/or mortality from the introduced fly *Philornis downsi* (Diptera: Muscidae) (Causton et al., 2011). Larvae of *P. downsi* were first discovered in Darwin's finch nests on Santa Cruz Island in 1997 (Fessl et al., 2001), although the fly was present on the island by 1964 (Causton et al., 2006). The abundance of *P. downsi* in nests differs across Galápagos Islands (Wiedenfeld et al., 2007), and gene flow is largely unrestricted across three of the major islands (Dudaniec et al., 2008). The adult fly is a vegetarian, but its larvae feed on the blood and tissue of developing chicks (Kleindorfer et al., 2014a, Koop et al.,

2011, Huber, 2008, Fessl et al., 2006a). The intensity of *P. downsi* ("intensity" refers to the number of parasites per nest) has generally been lower during years with lower rainfall (Dudaniec et al., 2007, but see Koop et al., 2013). Since 2008, under conditions of higher rainfall, studies on Darwin's finch nesting success have reported more than 90% annual chick mortality due to *P. downsi* (O'Connor et al., 2010d, Kleindorfer et al., 2014a, Koop et al., 2011, O'Connor et al., 2010b). The few chicks that survive may persist as adults with malformed beaks (Galligan and Kleindorfer, 2009). *Philornis downsi* parasites are considered the biggest threat to all Galápagos land birds (Causton et al., 2006), and understanding the behaviour of the fly and Galápagos land birds is a top management and research priority (Causton et al., 2011).

Since the initial discovery of *P. downsi* in Darwin's finch nests in 1997, *P. downsi* intensity in finch nests has doubled on both Santa Cruz and Floreana Islands. Between 2000-2002 on Santa Cruz Island, small tree finch (*Camarhynchus parvulus*) nests with chicks had ~15 larvae per nest (Kleindorfer unpublished data), but between 2010-2012 they had ~30 larvae per nest (Cimadom et al., 2014). On Floreana Island between 2004-2013, *P. downsi* intensity nearly doubled (~28 to ~48 parasites per nest), in-nest mortality nearly doubled (~50% to ~90%), and chicks died in half the time (~11 versus only ~5 days after hatching) (Kleindorfer et al., 2014a). On both islands, finch populations have been declining (O'Connor et al., 2010d, O'Connor et al., 2010c, Dvorak et al., 2012). Kleindorfer et al. (2014a) applied the conservation behaviour framework to understand how change in *P. downsi* oviposition behaviour was driving Darwin's finch mortality patterns on Floreana Island. Kleindorfer et al. (2014a) showed that, across the decade, female *P. downsi* laid eggs earlier in the Darwin's finch nesting cycle and that the size and age of larvae in host nests was more synchronous. The combination of higher *P. downsi* abundance per nest, earlier egg-laying, and a more synchronous age class of parasites has meant that Darwin's finch hosts were exposed to older (and hence larger) parasites, which were consuming their blood and tissue from an earlier age. Within the conservation behaviour framework, the findings from Kleindorfer et al. (2014a) provided evidence for behavioural indicators (early fly oviposition behaviour, synchronous larval cohorts) that signal processes of conservation concern (early and elevated host death).

The evolutionary theory of ecological specialisation predicts that parasites should become locally adapted (Kawecki and Ebert, 2004). Parasites that are locally adapted will have a fitness advantage in sympatric hosts as opposed to allopatric hosts, because locally adapted parasites cannot be invaded by nonadapted parasites from different populations (Kaltz and Shykoff, 1998). Parasite and host behaviour may contribute to the process of local parasite adaptedness. Given slight initial differences in host populations (including nesting behaviour), the selection pressures exerted by novel parasites (including search behaviour) should lead to different evolutionary trajectories across populations. In the case of *P. downsi* and Darwin's finches, Kleindorfer et al. (2014a) found preliminary evidence for local parasite adaptation: there were more live *P. downsi* pupae in the nests of small ground finch (*Geospiza fuliginosa*) than in the nests of small tree finch (*C. parvulus*) or medium tree finch (*C. pauper*) – a difference that needs further study. From the hosts' perspective, some species consistently had

more *P. downsi* per nest than others. In particular, the critically endangered medium tree finch consistently had more *P. downsi* larvae per nest compared with small tree finch, small ground finch, or hybrid tree finches on Floreana Island (Kleindorfer et al., 2014b). High *P. downsi* intensity is considered the primary cause of decline in the medium tree finch (O'Connor et al., 2010d) and is considered a possible causal factor in the recent local extinction of the large tree finch (*C. psittacula*) and Warbler finch (*Certhidea fusca)* on Floreana Island (Kleindorfer et al., 2014b, Grant et al., 2005). Thus there are urgent conservation imperatives to explain why *P. downsi* intensity, Darwin's finch host mortality, and *P. downsi* pupation success differ among host species that inhabit the same patch of forest and breed at the same time (Kleindorfer et al., 2014a,b). In particular, we need to understand what attributes make certain hosts more attractive to *P. downsi*. Viewed from the perspective of the parasite, do some aspects of *P. downsi* behaviour make this parasite more likely to encounter particular hosts?

This study examines vertical flight behaviour in *P. downsi* and nesting height in Darwin's finch hosts on Floreana Island. We use an observational approach to compare *P. downsi* intensity in relation to nesting height in three Darwin's finch host species, and we measure *P. downsi* abundance and sex ratio at different forest heights. If we find significant differences in fly abundance according to forest height, we predict that Darwin's finch nests at the height at which *P. downsi* are most commonly encountered will have the highest *P. downsi* intensity. In this case, data would support the view that parasite intensity is shaped by the probability of encountering a host nest. The conservation management implication of this work is to place fly traps at the height preferred by *P. downsi* flies (to remove the greatest number of parasites from the study site), and to place artificial nest boxes below mean fly height (to manage host reproductive success in threatened populations). If neither fly abundance nor host nesting height show a consistent pattern of association, then the data would support the view that host attributes (and not nest attributes) predict parasite intensity. The conservation management suggestion would be to place fly traps at different forest heights (by varying trap height one would remove more flies from the study site), and to apply pyrethrum to nests of threatened populations to manage host reproductive success until the fly population is reduced (Knutie et al., 2014). This chapter is a case study demonstrating how insights from host and parasite behaviour can be harnessed to inform conservation management.

Methods

Study site

This study was conducted during February (2004, 2005) and during February to April (2006, 2008, 2010, 2012, 2013, 2014) on Floreana Island, Galápagos Archipelago (described in Kleindorfer et al., 2014a,b). The study site was in *Scalesia* forest at the base of Cerro Pajas volcano (1° 17' 46S, 90° 27' 06W) at an elevation of 300-400m; the area is the stronghold of the tree finch population on Floreana Island (O'Connor et al., 2010c). We sampled *P. downsi* flies and Darwin's finch nesting outcome from four 100m x 200m study plots, as previously described (Kleindorfer et al., 2014b, O'Connor

et al., 2010c). The preferred habitat of Darwin's tree finches is dominated by endemic *Scalesia pedunculata* trees, which are endangered on Floreana Island and only remain in fragmented patches totalling less than 3km^2; the highland *Scalesia* forest overlaps with agricultural land (O'Connor et al., 2010c).

Host species

We examined nesting height and parasite intensity in three Darwin's finch species: the common small tree finch (*Camarhynchus parvulus*), the critically endangered medium tree finch (*C. pauper*), and the common small ground finch (*Geospiza fuliginosa*) (Grant and Grant, 2008, O'Connor et al., 2010d, Sulloway and Kleindorfer, 2013). Based on surveys in 2008, the estimated maximum highland population size on Floreana Island was 3,700 small tree finches, 1,620 medium tree finches, and 4,680 small ground finches (O'Connor et al., 2010c).

Nesting in Darwin's finches begins with the onset of the rains that usually occur in January or February. Males build a display nest and sing to attract females (Kleindorfer, 2007a). Females visit the singing male and inspect the nest. If accepted, a female will subsequently lay a clutch size of 2-5 eggs per nest (Kleindorfer, 2007b); some nests contained 6 eggs in 2008 and 2010. In all three species, the female is the sole incubator and both parents provide food to chicks. The incubation and feeding phase are ~14 days each. In the lowlands, small ground finch males typically nest in *Opuntia cacti* or *Acacia* trees; but in the highlands, they generally nest in Cat's Claw (*Zanthoxylum fagara*) or *S. pedunculata* trees (O'Connor et al., 2010a, Kleindorfer, 2007b). Highland tree finches mostly build nests in *S. pedunculata* and occasionally in *Z. fagara.*

Parasite species

Philornis downsi is a parasitic Dipteran that has two temporally distinct feeding modes: first instar larvae feed internally on the nasal and body cavities of its avian nestling hosts, and 2nd and 3rd instar larvae feed externally on the chicks (Fessl et al., 2006b, O'Connor et al., 2010b, O'Connor et al., 2014). Adult female *P. downsi* generally carry ~60 eggs; each female fly mates with an average of ~2 males (range of 1-5 males per female), and 1 to 6 females each contribute an average of five larvae per Darwin's finch nest (range = 1-24 eggs) (Dudaniec et al., 2010). Larvae pupate in the nest base after feeding on chicks for 4-7 days, and they emerge as flies after 7-18 days (P. Lincango and C. Causton, unpublished data; Kleindorfer et al., 2014a). The instars can be identified based on the size and shape of the posterior spiracles: first instars are the smallest in body length and have no discernible spiracles; 2nd instars have two light brown spiracles and vary in body length from 4-7mm; 3rd instars have two large black spiracles and vary in body length from 6-12mm.

The genus *Philornis* has a Neotropical distribution comprised of ~50 species (Dudaniec and Kleindorfer, 2006, Quiroga et al., 2012). It is not known how *P. downsi* arrived in the Galápagos Islands, but there are two likely scenarios: (1) introduction via known mainland hosts such as Smooth-billed Ani (*Crotophaga ani*) and/or Rock Pigeon (*Columbia livia*), which were both introduced to the Galápagos between 1962-1972

(Wikelski et al., 2004, Thiel et al., 2005, Santiago-Alarcon et al., 2006); http://www.issg.org/database/species/ecology); and (2) given that adult *P. downsi* feeds on fruit, the fly may have arrived via cargo boats from mainland Ecuador carrying produce (Causton et al., 2011, Dudaniec and Kleindorfer, 2006). The latter possibility is rendered more plausible given the recent discovery of *P. downsi* in bird nests on mainland Ecuador (Bulgarella et al., 2015).

Philornis downsi is the only parasite that causes measurable fitness costs in Darwin's finches. Avian poxvirus (*Poxvirus avium*) has existed on the Galápagos Islands since the 1890s (Parker et al., 2011), and has increased sharply from 2000-2009 (Zylberberg et al., 2012, Gottdenker et al., 2008, Kleindorfer and Dudaniec, 2006). Both paramyxovirus and adenovirus have been found in Darwin's finches on Floreana Island (Deem et al., 2011). Blood parasites have not been detected in Darwin's finches, and intestinal protozoan parasites are rare (Morales, 2013, Dudaniec et al., 2005, Dudaniec et al., 2006). Eight genera of feather mites have been found on Darwin's finches, and mite abundance increases with host body mass (Villa et al., 2013).

Host nesting height and nest contents

Nesting height (m) was visually estimated, and the estimate was calibrated using a 6m extendable video scope inserted into nests to check nest contents during routine nest status inspections. We used two methods to assess nesting status: 20-minute observations every two days to quantify parental activity at each nest, as well as nest inspection using a ladder (2004-2006) or mirror/camera on an extendable 6m pole (2008-2014) (Kleindorfer et al., 2014a). Within two days after the death or fledging of the last chick, we collected the nest and counted the number of *P. downsi*. The nesting material was dismantled and all *P. downsi* larvae, pupae and pupae cases were counted to calculate parasite intensity per nest. Chicks that had recently died were immersed in alcohol so that larvae within the body would float out and could be counted. We stored the pupae and larvae in ethanol within 24 hours of collection from the host nest. All Darwin's finch nests with chicks in this study had *P. downsi* parasites.

We monitored nesting outcome at 582 active Darwin's finch nests between 2004 and 2014 on Floreana Island. The sample size per species was 150 small tree finch, 198 medium tree finch, and 234 small ground finch nests. We analysed the following subsets of data for this study: nests with information about *P. downsi* intensity (N = 254), brood size (N = 253), percentage chick mortality (N = 225), nesting height (N = 466), and nests with information on both nesting height and *P. downsi* intensity (N = 206). The sample sizes per species for nests with information on both nesting height and *P. downsi* intensity were 40 small tree finch, 48 medium tree finch, and 118 small ground finch.

Vertical distribution of P. downsi in fly traps

During 15 March to 15 April 2014 we collected 365 *P. downsi* flies from 28 McPhail traps sampled four times each (N = 112 trapping events; mean = 3.3 ± 0.3 flies per trap, maximum = 19). McPhail traps are ball-shaped plastic traps with a yellow bottom and a clear top with narrowing entrance in the middle; the traps are designed

to hang in trees. The bottom contained a liquid lure (see details below) whose odour, together with the yellow colour, attracts insects to the trap; the insects subsequently drown in the fluid or fail to exit the trap. We placed 7 fly traps every 15m along each of 4 x 90m transects in the *Scalesia* forest, for a total of 28 traps. The four transects (A-D) along which fly traps were placed were located within study plots used to monitor Darwin's finch nesting biology; the study plots are referred to as plot 1 (W090°27'05.1", S01°17'50.5"), plot 2 (W090°27'09.8", S01°18'02.9"), plot 3 (W090°27'09.3", S01°18'05.9") and plot 4 (W090°27'02.0", S01°17'54.0"). The onset of each fly trap transect was separated by 100m. Traps were allocated different heights that remained the same across the four-week sampling period, and sequential traps never had the same height. Table 1 shows the height of each fly trap within each transect. The number of replicates per trap height was 6 traps at 2m, 3 traps at 3m, 7 traps at 4m, 4 traps at 5m, 6 traps at 6m, and 2 traps at 7m.

To lure the flies to the trap, we filled them with 140 ml "bait juice" made from one Hawaiian papaya (blended), four litres of tap water, and six table spoons of white sugar (pers. comm. P. Lincango and C. Causton). Each fly trap was placed on a metal hook at a specific height on a *Scalesia* tree. The height of fly traps was either 2, 3, 4, 5, 6 or 7m; the height was chosen randomly and allocated to each trap and transect before going to the field, but some heights needed to be changed in the field to suit the height of available trees. Following the trapping protocol of P. Lincango and C. Causton, insects were collected twice per week and stored in ethanol for later sorting at the field station. The bait lure was changed every 7 days. We repeated this procedure per trap across four weeks.

Identification of P. downsi from fly traps

The wing of *P. downsi* is very distinctive compared to other Galápagos Muscidae species. The R4+5 and M1 veins are sinuous or wavy and the distance separating them at the wing margin is greater than other Galápagos muscids (pers. comm. B. Sinclair). *Philornis downsi* have dark abdomen and thorax; body length ~8mm; wing length ~9mm in females and ~10mm in males. The flies collected from the McPhail traps were sexed at camp using a magnifying glass. Males have longer, pale yellowish legs, and the eyes are positioned differently compared to females. Male eyes are closely approximated and are dorsally separated by the width of the ocellar triangle (~0.2mm); male eyes appear to be almost touching when viewed from above (Figure 1a). Females have shorter, darker legs, and female eyes are dorsally separated by ~0.5mm; female eyes appear more parallel (Figure 1b).

Statistical analysis

Data were analysed with SPSS 22 for Windows (SPSS Inc., Chicago, USA) and SAS 9.4 for Windows (Cary, North Carolina, USA). Before conducting statistical analyses, we examined the data to determine if they conformed to assumptions of normality and homogeneity of variance. For statistical analyses, nesting height data were log transformed and *Philornis downsi* data were square root transformed to satisfy requirements of normality. We found significant heterogeneity of variance for nesting

height as assessed by Levene's test for equality of variances ($P = .034$). Therefore we used a Welch ANOVA to test for differences in nesting height across host species. To test if higher nests had more *P. downsi* parasites, we used multiple regression analysis with *P. downsi* intensity (square root transformed) as the dependent variable and nesting height (log transformed) and year as independent variables. In a separate analysis, we examined the effects of host brood size, percentage chick mortality in nests, and chick age at death in relation to nesting height, again using multiple regression analysis.

We found significant heterogeneity of variance for male trap counts by height, for female trap counts by week, and for percent male and female flies trapped by week. We also found excessive kurtosis (> 3) in the data for male trap counts by height and by week. We therefore applied natural log transformations to normalise the data, which resolved these problems. The data by trapping events (N = 112) are not statistically independent. For this reason we analysed these data using multilevel modelling in SAS (PROC MIXED). Our statistical design nested weekly capture data within collecting sites, and collecting sites within transects. Examination of the intraclass correlations between levels revealed that multilevel modelling was justified. Fifty percent of the variation was found between transects and collection sites, and the remaining 50% of the variation occurred within individual traps.

Results

Philornis downsi *intensity and nesting height in Darwin's finch host species*

Nesting height differed significantly between the three species (Table 2). Nesting height was lowest in small ground finch (3.5 ± 0.1, N = 204), intermediate in small tree finch (4.8 ± 0.2, N = 90), and highest in medium tree finch (6.8 ± 0.2, N = 158) (Welch's ANOVA $F_{2,254.2} = 101.49$, $P < .0005$). We examined changes in nesting height per year from 2004-2014 in each species. Nesting height did not change significantly in small tree finch or medium tree finch (linear and quadratic regression analysis; all $P > .5$), but nesting height increased significantly across the decade in small ground finch (r = .22, N = 206, $P = .002$) (Table 2).

Host nesting height was positively correlated with the number of *P. downsi* parasites across Darwin's finch species (nesting height: rpartial = .55, $P < .001$; year: rpartial = .24, $P = .001$, N = 208): higher nests had more *P. downsi* parasites, and *P. downsi* intensity increased across the decade (Table 2). Within each species, the pattern between nesting height and *P. downsi* intensity was similar: higher nests had significantly more *P. downsi* in small tree finch (nesting height: rpartial = .37, $P = .018$; year: rpartial = .22, $P = .184$, N = 40) and small ground finch (nesting height: rpartial = .62, $P < .001$; year: rpartial = .23, $P = .01$, N = 118), and a marginally significant trend for more *P. downsi* in higher nests of medium tree finch (nesting height: rpartial = .28, $P = .056$; year: rpartial = .45, $P = .001$, N = 48) (Figure 2).

We used multiple regression analysis to test if other factors covaried with nesting height. In a single model, we tested host nesting height as the dependent variable against host brood size, percentage chick mortality in nests, and chick age at death. In

small tree finch and medium tree finch, none of the variables was significantly associated with nesting height (small tree finch brood size: rpartial = -.37, $P = 0.15$; percentage chick mortality: rpartial = .10, $P = 0.71$; chick age at death: rpartial = .19, $P = .46$, N = 26; medium tree finch brood size: rpartial = -.39, $P = .34$; percentage chick mortality: rpartial = 0.64, $P = .09$; chick age at death: rpartial = 0.19, $P = .66$, N = 27). In small ground finch, higher nests had larger brood size (brood size: rpartial = .39, $P = .015$; percentage chick mortality: rpartial = -.06, $P = 0.73$; chick age at death: rpartial = -.20, $P = .22$, N = 40).

P. downsi abundance at different heights

Table 3 and Figure 3 show the average number and percentage of male and female *P. downsi* flies caught in traps. To test specific hypotheses about fly behaviour we first computed linear and quadratic contrasts for trap height. Controlling the nonsignificant linear trend in height among males, we found that traps at an intermediate height caught a substantially greater number of male flies than traps placed at the very lowest and the very highest elevations (for the linear trend, rpartial = -0.03, $t_{1,96.3} = 0.28$, $P = 0.79$; for the quadratic trend, rpartial = 0.25, $t_{1,105} = 2.67$, $P = 0.009$). In our analysis of female flies we found a significant linear trend by height and, controlled for this trend, a nonsignificant trend for females to be trapped at the very lowest and the very highest elevations (for the linear trend, rpartial = .24, $t_{1,94.8} = 2.36$, $P = .02$; for the quadratic trend, rpartial = -.15, $t_{1,105} = -1.50$, $P = .14$, N = 112).

Analysing the data for both sexes collectively and controlling the nonsignificant linear and quadratic trends, we found a significant interaction effect between sex and the linear trend, as well as a significant interaction between sex and the quadratic trend (for the linear trend, rpartial = 0.21, $t_{1,112} = 2.35$, $P = 0.02$; for the quadratic trend interaction, rpartial = -0.34, $t_{1,112} = -3.84$, $P = 0.0002$). These findings show that males tended to be found at lower elevations than females, and especially at intermediate heights; whereas females tended to occur at the lowest and especially the highest elevations.

Because of the relative small number of male flies captured at 7 meters, and because only 2 traps were placed at this height (versus an average of 5 traps placed at other heights), we repeated this last test by combining the data for flies trapped at 6 and 7 meters and adjusting the model contrasts accordingly. The significant interaction between sex and height as a linear trend was no longer significant, being replaced by a significant linear trend for the two sexes as a whole (rpartial = .22, $t_{1,94.3} = 2.16$, $P = .03$); however, the interaction between sex and the quadratic trend for height remained significant, confirming the previous finding that females are the least common where males are most common, namely, at intermediate heights (rpartial = -.25, $t_{1,112} = -2.74$, $P = .007$).

Discussion

This study applied the conservation behaviour framework to a newly evolving host-parasite system on the Galápagos Islands, with the aim of identifying species-specific

behaviour that could be used to alleviate the extremely high levels of virulence caused by *P. downsi* for its naïve Darwin's finch hosts. The study identified two behavioural traits that suggest new ways of thinking about *P. downsi* and Darwin's finch hosts. First, nesting height of Darwin's finches on Floreana Island is positively associated with *P. downsi* intensity: higher finch nests had more parasitic larvae. We found this pattern within and between host species using a substantial sample size (206 host nests sampled between 2004 and 2014). Second, fly traps placed higher in the canopy (7m) caught significantly more female *P. downsi*, while fly traps placed at 4-5m height caught more male *P. downsi*. Despite any limitations of the fly trap data, which pertain to a single year (2014), the findings are noteworthy for two reasons. First, they provide a plausible explanation for why we have consistently documented higher parasite intensity in the critically endangered medium tree finch (O'Connor et al., 2010d, Kleindorfer et al., 2014a,b). The average nesting height in medium tree finches is 6.8m – and therefore the nests of this species appear to be more susceptible to being located by female *P. downsi* flies given that females are more common at 7m. Second, these findings can be used to generate useful conservation management strategies to control *P. downsi* abundance. One obvious recommendation is to place fly traps at 6-8m height to remove egg-laying female flies, and at 4-5m height to remove male flies, from the habitats of critically endangered species such as medium tree finch on Floreana Island (O'Connor et al., 2010d) and Mangrove finch (*C. heliobates*) on Isabela Island (Fessl et al., 2010). The results of this study suggest that *P. downsi* flight behaviour makes this parasite more likely to encounter particular hosts, rather than hosts having specific attributes that make them particularly attractive to the parasite.

Different research groups have been monitoring the impact of *P. downsi* intensity for Darwin's finch chick mortality since 1998 (Cimadom et al., 2014, Dudaniec et al., 2007, Fessl et al., 2006a, Huber et al., 2010, Kleindorfer et al., 2014a, Knutie et al., 2013, Koop et al., 2011, O'Connor et al., 2010a-d). One finding that has intrigued researchers for over a decade is that some Darwin's finch species have higher *P. downsi* intensity than others. On Santa Cruz Island between 1998 and 2006, the highest *P. downsi* intensity (57 ± 4) has been found in the large-bodied (22g) Woodpecker finch (*Camarhynchus pallidus*); and the second highest *P. downsi* intensity (41 ± 6) occurs in the small-bodied (9g) Warbler finch (*C. olivacea*) (Kleindorfer and Dudaniec 2009). Both species have had the strongest population declines from 2000 to 2010 (Dvorak et al., 2012). In contrast, the medium-sized (13g) small tree finch (*C. parvulus*) generally has fewer *P. downsi* (23 ± 3) (Dudaniec et al., 2007), and its population on Santa Cruz Island has remained stable from 2000 to 2010 (Dvorak et al., 2012). Suggestive hints that body size could be important for parasite intensity come from the finding that larger-bodied Darwin's finches build larger nests, and larger nests have more *P. downsi* (Kleindorfer and Dudaniec, 2009). Despite the appeal of host body size as a predictor of parasite intensity, the evidence has so far been inconclusive for *P. downsi* and Darwin's finches (Cimadom et al., 2014). Rather, evidence is growing that nest attributes (nest size and location) predict parasite intensity. In a previous study, Kleindorfer and Dudaniec (2009) showed that nests that were larger in size and nests with many close neighbours had higher *P. downsi* intensity. It remains to be tested if host nesting density on Santa Cruz Island has changed across the past decade, and to

what degree the height or abundance of *Scalesia* trees used for nesting may be associated with host nesting height. The conservation implications of managing vertical and horizontal forest attributes are important for biodiversity (e.g. DeVries et al., 1997) but are only beginning to be explored for host-parasite systems (Peters and Kleindorfer, 2015). If the vertical forest is associated with particular patterns of parasite community, then forest height can become a target of tailored conservation management approaches.

As on Santa Cruz Island, there were different interspecific patterns of *P. downsi* intensity in Darwin's finch hosts on Floreana. The critically endangered medium tree finch has higher parasite intensity (55 ± 5) than the common small tree finch (31 ± 2) and small ground finch (31 ± 2) (Table 2). Since 2012, all medium tree finch chicks have died in the nest due to *P. downsi* parasites – a rather alarming finding (Kleindorfer et al., 2014a). In addition to low nesting success and declining populations, tree finches on Floreana Island are hybridising. In particular, female medium tree finches have increasingly paired with male small tree finches and have produced hybrid offspring. As a result, the proportion of hybrid tree finches has increased from 19% in 2005 to 41% in 2010 (Kleindorfer et al., 2014b). Intriguingly, the parental tree finch species had 60-500% more *P. downsi* per nest compared with hybrid birds (Kleindorfer et al., 2014b). Therefore, the initial evidence suggests that hybrid offspring are being favoured by selection, perhaps because they are less likely to be parasitised by *P. downsi*. From another study, we know that hybrid tree finches forage at ~4m in the *Scalesia* canopy while medium tree finches forage at ~6m (Peters and Kleindorfer, 2015), evidence that supports the findings presented here: birds that foraged lower in the canopy also had fewer *P. downsi* parasites. Nesting height remains to be tested in hybrid finches, and we await a larger sample size to draw any firm conclusions. The increase in mean *P. downsi* intensity on Floreana Island coincides with species collapse via hybridisation in the iconic Darwin's finches, the bird group that is known as a classic textbook example of speciation (Grant and Grant 2014). Because *P. downsi* was likely introduced as the result of human activity, there are urgent conservation imperatives to explain why *P. downsi* intensity and Darwin's finch chick mortality differ between host species, and to do so before the populations collapse into a single hybrid swarm or become extinct (Kleindorfer et al., 2014b).

This study suggests that the flight behaviour of *P. downsi* could be a key factor that predicts its frequency of occurrence in Darwin's finch nests. One major difference in *P. downsi* flight height is sex: we found significantly more female flies at 2m and 7m, and male flies at 4-5m. This pattern suggests that gravid females may be avoiding males. Although we acknowledge the limits of our study design and its small sample size, we note that our findings are consistent with evidence from other study systems of female behavioural tactics designed to reduce the cost of mating and to avoid males once a female is gravid. *Drosophila melanogaster* is a model system to study the costs of reproduction because the mating frequency that maximises male reproductive success is higher than that which maximises female reproductive success (Bateman, 1948). Female *D. melanogaster* that mate frequently have a shorter lifespan and lower reproductive success (Fowler and Partridge, 1989). Wigby and Chapman (2005)

identified a sex peptide in male *D. melanogaster* ejaculate that stimulates female egg production but also lowers female reproductive success. Clearly, sexual selection should favour females that avoid costly matings. In damselflies (*Ischnura elegans*) males will attempt to mate multiply with females, leading to male harassment and selection on the frequency of cryptic or male-type colour polymorphisms among females (Van Gossum et al., 2001). Svensson and colleagues have shown that male species recognition in banded demoiselle (*Calopteryx splendens*) is fixed at emergence, but females learn to recognise heterospecifics to reduce costly hybrid matings (Svensson et al., 2014). Male harassment has been shown to reduce reproductive success in damselflies (*I. senegalensis*), and females accordingly avoid oviposition sites having many males (Takahashi and Watanabe, 2010). Thus, there is evidence across taxonomic groups for behavioural tactics to avoid the high costs of multiple mating in female insects. From molecular data on family groups of *P. downsi* in nests of Darwin's finches, we know that female *P. downsi* remate between 1 and 5 times (an average of 1.91 ± 0.08 times) (Dudaniec et al., 2010); previous studies have not tested for potential fitness costs of multiple mating by *P. downsi* females – a topic of potentially fruitful future research.

Little is known about the reproductive behaviour of *P. downsi* in the wild. We believe the morphology of *P. downsi* male and female eyes provides indirect evidence concerning reproductive behaviour as it relates to flight height in this system. Although this matter was not an explicit focus of this study, we used eye morphology (and other morphological traits) as a means of sexing the adult flies caught in traps. In *P. downsi*, male eyes are close together, whereas female eyes are wider apart (see Figure 1). From previous study of other flies, mate search behaviour can be predicted from eye morphology and vision. For example, Zeil (1986) recorded the flight paths of male house flies (*Fannia canicularis*) patrolling the airspace below indoor landmarks, such as lampshades. Male house flies approached these landmarks from below and defended the airspace immediately below the landmark. If a patrol area is occupied, the next arriving male occupies an area below that of the first male. Zeil (1986) hypothesised that female flies might approach landmarks from the side, and not from below as the males do, and that female flies would therefore pass through the dorsal visual field of the males. The sexual dimorphism in compound eye organisation in *P. downsi* may provide indirect evidence that male vision and female flight behaviour are related. As is the case in many fly species (e.g. Collett and Land, 1975, Zeil, 1983, Land and Eckert, 1985), the eyes of male *P. downsi* extend more medially in the frontal and dorsal visual field, compared with female eyes, which is an indication of a fronto-dorsal acute zone involved in detecting and chasing females. We therefore predict that male *P. downsi* at lower heights than females are better able to detect a female flying above – a hypothesis that remains to be tested.

Studies of differences in flight height in Diptera have found significantly more female flies higher in the canopy compared with males (Aluja et al., 1989, Herczeg et al., 2014), and more females closer to the ground compared with males (Birtele and Hardersen, 2012, Gersabeck and Merritt, 1983). In addition to avoiding costly reproduction with males, gravid female flies move to foraging areas to increase their

foraging efficiency for egg production (Irvin et al., 1999, Maguire et al., 2014, Mavoungou et al., 2013). These findings show that the vertical distribution of flies must be considered in relation to reproductive behaviour. Adult *P. downsi* feed on fruit (such as papaya) and decaying organic matter (Fessl et al. 2001), and gravid *P. downsi* females in search for food may therefore fly at both lower and higher elevations, as we found in this study. Clearly, many different factors could influence the vertical distribution of flies, including meteorological conditions, vegetation type and cover, host location, and oviposition habits (Van Hennekeler et al., 2011, Birtele and Hardersen, 2012, Maguire et al., 2014, Mavoungou et al., 2013, Roberts, 1985, Swanson et al., 2012). One limitation of this study is that fly abundance per trap height was sampled within a single year and across a single month, and therefore that the findings could reflect other unexamined factors, such as micro-climate and seasonality. Ideally, fly trapping should be carried out across the year and under varied environmental conditions.

In conclusion, this study has found consistent evidence from the systematic examination of vertical nesting and flight behaviour in host and parasite that can usefully be used to manage the extreme virulence caused by *P. downsi* on its naïve Darwin's finch hosts. The conservation behaviour framework advocates for synergistic discourse between ethology and conservation. Accordingly, a fruitful approach to manage the impacts of invasive species is to identify behavioural traits that can be targeted for maximal efficacy of limited human and financial resources. Here we showed that both host nesting height and parasite flight height could be useful targets of conservation intervention. Maintaining a broad range of vertical forest is likely to improve host survival by creating stratified nesting areas for the avoidance of airborne parasites that preferentially occur at particular heights. The findings can also be applied to nest-box studies: if nesting height is associated with high parasite intensity then researchers should alter nest-box height. Finally, when attempting to remove airborne parasites from the population, trap heights that target female flies would remove more eggs and hence would maximise conservation dollars for trapping efforts. In these ways, one can imagine how linking insights from ethology and conservation management should generate more biologically relevant and cost effective approaches to managing threatened species.

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Table 1. The height (m) of 28 McPhail traps to capture *Philornis downsi*. Each fly trap was placed at a distance of 15m from the preceding fly trap along one of four straight-line transects (A-D) spanning 90m in the highlands of Floreana Island. The number of replicates per trap height was 6 traps at 2m, 3 traps at 3m, 7 traps at 4m, 4 traps at 5m, 6 traps at 6m, and 2 traps at 7m.

	Transect A (Plot 1)	**Transect B (Plot 2)**	**Transect C (Plot 3)**	**Transect D (Plot 4)**
Trap 1	2m	5m	6m	2m
Trap 2	6m	3m	4m	6m
Trap 3	4m	6m	3m	2m
Trap 4	7m	2m	4m	6m
Trap 5	5m	4m	6m	4m
Trap 6	2m	6m	5m	7m
Trap 7	3m	4m	2m	5m

Table 2. *Philornis downsi* intensity and nesting height (m) per species per year; data are shown as mean ± SE with sample size (N = number of nests). Nesting height differed significantly between species but not across years: lowest nesting height in small ground finch (3.5 ± 0.1), intermediate in small tree finch (4.8 ± 0.2), and highest in medium tree finch (6.8 ± 0.2). The intensity of *P. downsi* differed significantly between species: lower in small ground finch (31.7 ± 2.0) and small tree finch (31.1 ± 2.4), and higher in medium tree finch (55.2 ± 5.2) (ANOVA: species $F_{2, 254} = 17.32$, $P < .001$).

	Small Tree finch (*Camarhynchus parvulus*)		**Medium Tree finch (*C. pauper*)**		**Small Ground finch (*Geospiza fuliginosa*)**	
	P. downsi Intensity (N)	Nesting Height (m)	*P. downsi* Intensity (N)	Nesting Height (m)	*P. downsi* Intensity (N)	Nesting Height (m)
2004	30.9 ± 8.3 (13)	5.4 ± 0.5	65.5 ± 10.5 (5)	7.5 ± 0.5	19.9 ± 4.5 (14)	3.2 ± 0.4
2005	12.5 ± 0.5 (2)	4.0 ± 0.7	36.9 ± 3.4 (7)	4.7 ± 0.5	27.1 ± 5.7 (7)	3.3 ± 0.4
2006	20.4 ± 3.9 (10)	4.5 ± 0.3	36.1 ± 5.6 (35)	7.4 ± 0.4	28.2 ± 3.9 (68)	3.0 ± 0.2
2008	20.4 ± 4.2 (9)	4.9 ± 0.6	40.4 ± 7.6 (46)	5.6 ± 0.3	39.5 ± 4.1 (57)	3.6 ± 0.2
2010	31.5 ± 4.9 (42)	4.7 ± 0.3	64.3 ± 11.3 (53)	5.8 ± 0.3	21.3 ± 4.3 (58)	3.6 ± 0.3
2012	32.3 ± 3.8 (31)	4.2 ± 0.3	89.7 ± 31.4 (36)	6.8 ± 0.3		
2013	36.3 ± 5.2 (32)	4.7 ± 0.2	103.8 ± 28.7 (12)	6.8 ± 0.4	41.8 ± 5.2 (22)	4.4 ± 0.5
2014	51.9 ± 8.6 (11)	5.4 ± 0.5	68.0 ± 3.0 (3)	6.8 ± 0.6	43.0 ± 8.2 (8)	4.1 ± 0.6

Table 3. The percentage and number of male and female *Philornis downsi* per McPhail trap in relation to trap height (m) and the number of *P. downsi* per nest in relation to nesting height (m) in Darwin's finch species on Floreana Island. Data are shown as mean ± SE. Higher fly traps caught more female *P. downsi* and higher finch nests contained more *P. downsi* (statistical analyses in results).

	Male *Philornis downsi*	**Female *P. downsi***	**Small Tree finch (*Camarhynchus parvulus*)**	**Medium Tree finch (*C. pauper*)**	**Small Ground finch (*Geospiza fuliginosa*)**
Height (m)	%*P. downsi* in fly traps (number of flies)	%*P. downsi* in fly traps (number of flies)	#*P. downsi* per nesting height (number of nests)	#*P. downsi* per nesting height (number of nests)	#*P. downsi* per nesting height (number of nests)
2 m	30.0 ± 9.1 (1.1 ± 0.3)	28.3 ± 1.9 (1.0 ± 0.3)	27.0 ± 9.0 (5)	25.0 ± 0.0 (1)	17.7 ± 3.3 (76)
3 m	43.1 ± 3.7 (1.3 ± 0.1)	15.2 ± 5.0 (0.7 ± 0.2)	41.2 ± 8.2 (13)	27.0 ± 0.0 (1)	31.1 ± 4.3 (36)
4 m	50.5 ± 7.4 (2.1 ± 0.6)	35.3 ± 5.9 (1.6 ± 0.3)	21.0 ± 3.6 (23)	48.4 ± 10.7 (21)	29.5 ± 3.4 (38)
5 m	70.3 ± 8.1 (3.4 ± 1.3)	11.0 ± 3.9 (1.4 ± 0.5)	28.3 ± 6.3 (20)	38.0 ± 11.3 (21)	45.3 ± 4.0 (23)
6 m	36.3 ± 7.2 (1.8 ± 0.4)	38.8 ± 6.6 (1.5 ± 0.3)	37.2 ± 6.7 (12)	72.7 ± 21.5 (37)	59.2 ± 6.3 (21)
7 m	16.0 ± 7.8 (0.9 ± 0.7)	83.9 ± 7.9 (3.3 ± 0.5)	65.7 ± 9.1 (7)	52.0 ± 7.5 (27)	69.0 ± 5.1 (7)
8 m			63.0 ± 0.0 (1)	67.2 ± 13.1 (22)	None
9 m			None	87.8 ± 19.5 (15)	None

(a) Male *(b) Female*

Figure 1. The frontal view of male (left) and female (right) *Philornis downsi* from Santa Cruz Island, Galápagos Archipelago. Male eyes are closely approximated and are dorsally separated by ~0.2mm; female eyes appear more parallel and are dorsally separated by ~0.5mm. Images provided by Bradley Sinclair.

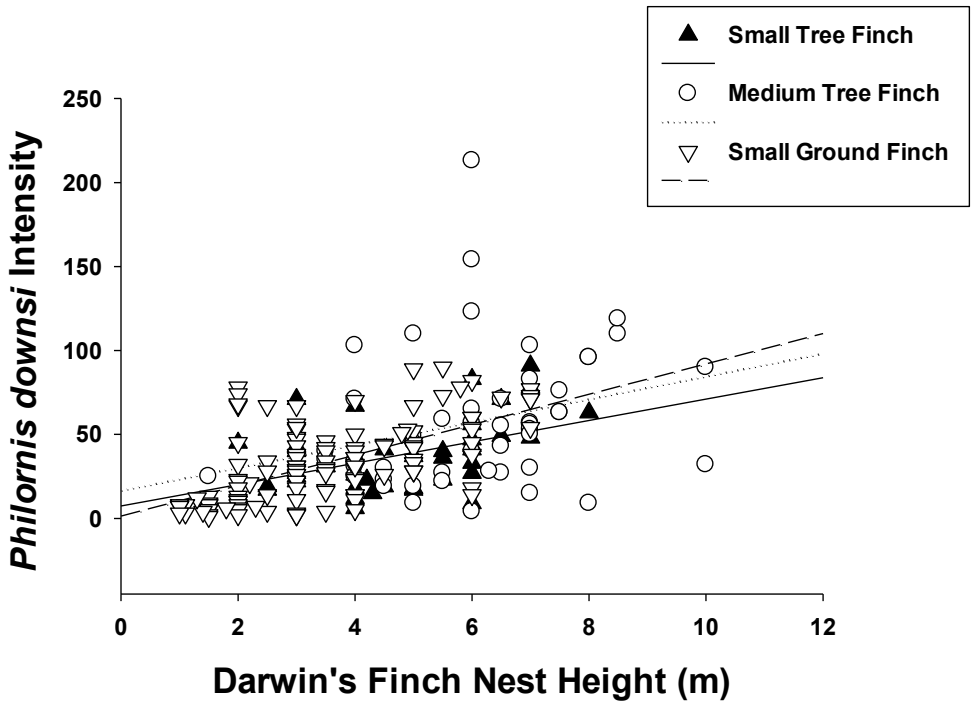

Figure 2. The significant linear association between host nesting height (m) and number of *Philornis downsi* parasites per nest on Floreana Island for data collected during the years 2004-2014. The sample size per species was 40 small tree finch nests (*Camarhynchus parvulus*), 48 medium tree finch nests (*C. pauper*), and 118 small ground finch nests (*Geospiza fuliginosa*).

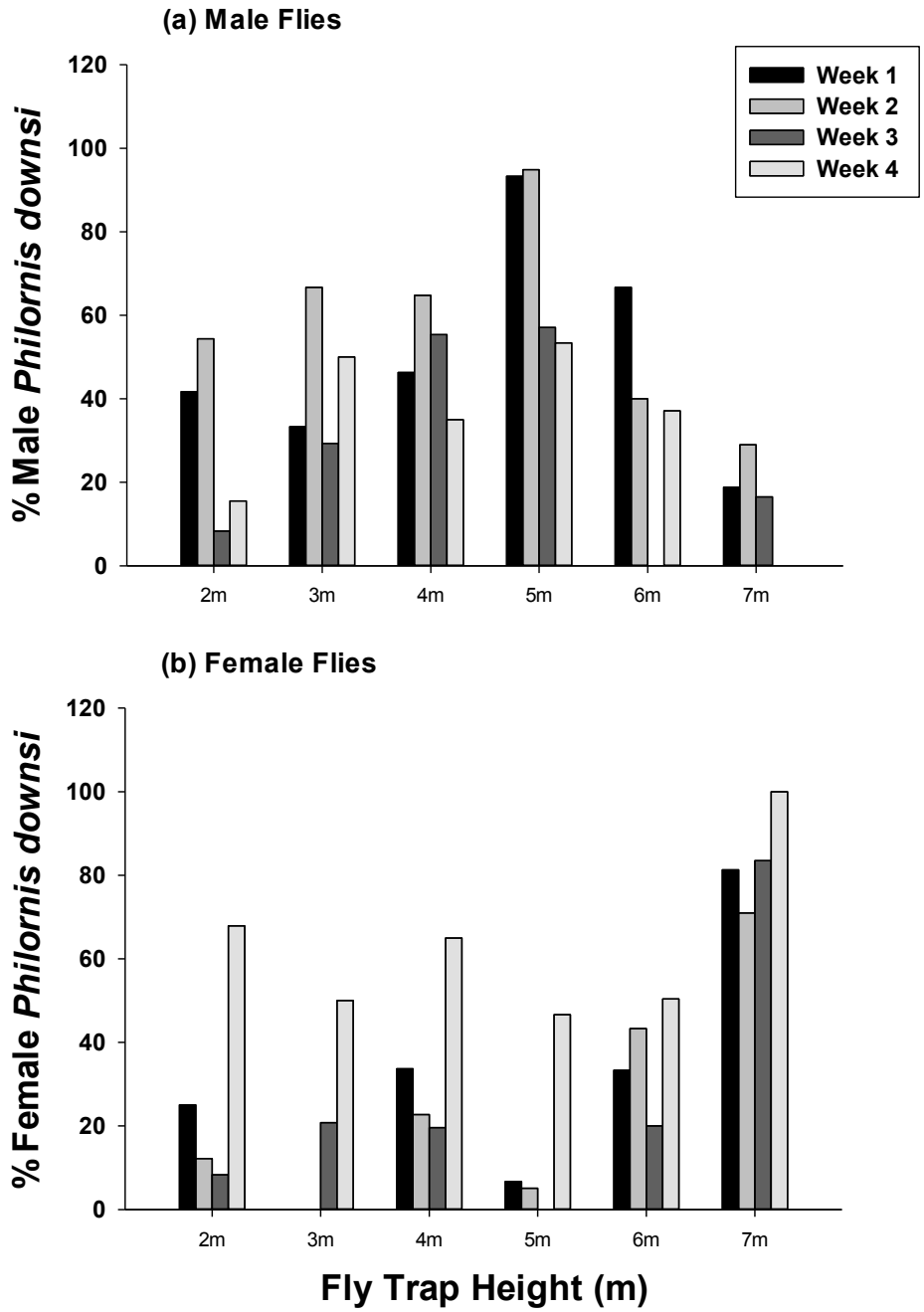

Figure 3. The percentage of male and female *Philornis downsi* caught in relation to the height (m) of McPhail traps on Floreana Island. The data are shown as means per week from 15 March to 15 April 2014 for each trap height. The sample size was 365 *P. downsi* caught in 112 trapping events using 28 McPhail traps.

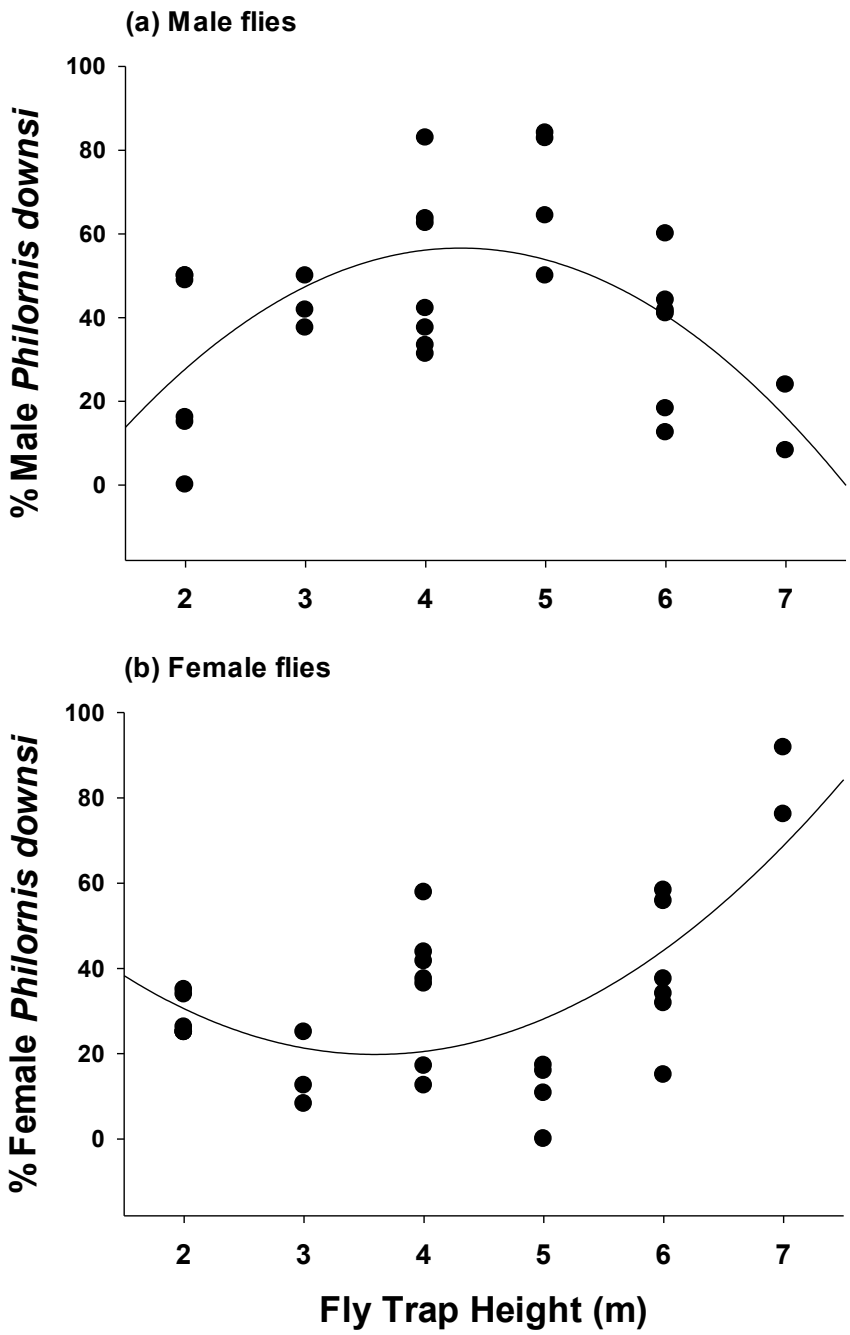

Figure 4. The quadratic association between fly trap height (m) and percentage of male and female *Philornis downsi* per trap. Data are shown for the mean percentage of flies per trap (N = 28) across four weeks of sampling. Females exhibited a significant linear trend, and a near-significant quadratic trend. The two quadratic trends were significantly different from each other: traps at a height of 4-5m caught more male *P. downsi*, and traps at the lowest and highest elevations caught more female *P. downsi* (see results).

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