Conservation issues for Darwin's finches in the Galápagos Islands: invasive species and loss of genetic diversity

Jody Anne O'Connor

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School of Biological Sciences

Faculty of Science and Engineering

Flinders University

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Summary

This study examines the impacts of a novel host-parasite system for population dynamics in Darwin's finches on Floreana Island, Galápagos Archipelago. I focus in particular on the interaction between Darwin's finches and parasitic larvae of an introduced fly, *Philornis downsi*, which causes high nestling mortality. This is the first project to systematically study Darwin's finches on Floreana Island since the pioneering work of David Lack in the 1930s, and Robert Bowman in the 1960s. I provide the first descriptive study of the breeding biology of the locally endemic medium tree finch, *Camarhynchus pauper*, which – at the start of the study – was listed as "data deficient" on the IUCN RedList.

I begin with a study of the population status and population trends of finches on Floreana Island. The only population of Darwin's medium tree finches (*C. pauper*) had declined by 61% between 2004 and 2008 to ~1660 individuals. I also document evidence for lack of recruitment into the breeding population, given my finding that medium tree finches had an age-biased population, with few one year old or 5+ year old males. The survey reports on the lack of suitable habitat for highland birds. I devote several chapters to the study of the impacts of *P. downsi* on host mortality, and the potential for Darwin's finches to adapt to the negative impacts of this invasive parasite. *P. downsi* is unanimously considered the biggest threat to the survival of Galápagos landbirds, including Darwin's finches. The parasite caused 38-92% of nestling mortality across all five host species studied in this thesis. The impacts of this parasite are greatest for Darwin's tree finches (*Camarhynchus* spp) because parasite intensity is highest in their highland forest habitat. In particular, *P. downsi* is identified as the primary cause of nestling mortality in Darwin's medium tree finch. As a result of this research, the medium tree finch status

was reassessed from "vulnerable" to "critically endangered" on the IUCN RedList. Video surveillance of finch behaviour showed that parent and nestling finches now have a range of anti-parasite behaviours that can partially mitigate the impacts of the parasite, including preening and removal of larvae. Experimental studies using parasitised and parasite-free nests showed that finch parents increased food provisioning to parasitised nestlings, but did not compensate for the negative impacts of parasitism (*P. downsi* caused 92% of nestling mortality in 2010). Collectively, these findings indicate that *P. downsi* parasitism is a major conservation concern for the finches on Floreana Island. I also examine population genetic structure and gene flow between the three sympatric tree finch species on Floreana Island, and find evidence for the loss of genetic diversity in the sympatric tree finches. High levels of hybridisation were detected within the tree finch group, suggesting that the mechanism for loss of genetic diversity is via introgression with closely-related taxa, that is – "speciation in reverse".

This thesis represents a novel and multi-faceted approach to understanding the complex interactions of human impacts, introduced species, and endemic species decline in island birds. The results of this research will have immediate impacts on the development of *P. downsi* control programs, and highlight the need for focussed recovery plans for the medium tree finch.

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.

Jody Anne O'Connor

30th September 2011

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Data collection: S.K. (2004), J.O'C (2008)
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Manuscript writing: J.O'C, S.K., F.S.

Chapter 3
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Manuscript writing: J.O'C, S.K., F.S., J.R.

Chapter 4
Data collection: J.O'C, R.D., S.K.
Statistical analyses: J.O'C, S.K.
Manuscript writing: J.O'C, S.K., R.D.

Chapter 5
Data collection: J.O'C
Statistical analyses: J.O'C, S.K.
Manuscript writing: J.O'C, S.K., J.R.

Chapter 6
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Statistical analyses: J.O'C, S.K.
Manuscript writing: J.O'C, S.K., J.R.

Chapter 7
Data collection (field): J.O'C, S.K.
Laboratory analysis of DNA samples: J.O'C
Statistical analyses: J.O'C, S.M., R.D., S.K.
Manuscript writing: J.O'C, S.K., R.D., S.M., F.S.

1 General Introduction

1.1 Island birds and vulnerability to extinction

Understanding and managing the causes of species decline is fundamental to the conservation of biodiversity. This study aims to identify the processes and causal mechanisms behind bird population declines in the Galápagos Islands. Endemic island birds typically exist in small populations within restricted and specialised habitat (Simberloff, 1995), where they are 40 times more likely to go extinct than continental species (Johnson and Stattersfield, 1990). Since 1800, 90% of all bird extinctions have been island taxa (Banko and Banko, 2009), and very few island systems retain their full suite of avian inhabitants. For example, habitat degradation and the introduction of predators and pathogens following human settlement has caused the extinction of 90% of Hawaiian birds (Pimm et al., 1995, Banko and Banko, 2009) and 73% of land birds on Guam (Pregill and Steadman, 2009). One in eight species of birds are at risk of becoming extinct by the year 2100 (BirdLife, 2000), and most of these threatened species are tropical forest birds on islands (Johnson and Stattersfield, 1990, Banko and Banko, 2009). The ominous history and predicted continuation of island bird extinctions has provided the impetus for this study of Darwin's finches on the Galápagos Islands. So far, no species of bird on the Galápagos has become extinct, and the archipelago retains 95% of its original biodiversity. However, there is only a small window of opportunity to identify and mitigate the threats to Darwin's finches as we are already witnessing the first local extinctions (Grant et al., 2005) and population declines across the archipelago (Curry, 1986, Dvorak et al., 2004, Fessl et al., 2010, Dvorak et al., in press). This thesis is mainly focused on conservation issues for birds on Floreana Island (Figures 1.1 & 1.2), because the island has the longest history of human settlement, has the worst record for local bird

extirpations and is home to the only population of Darwin's medium tree finch. Current population sizes and recruitment processes for Floreana birds are unknown (Chapter 2).

1.2 Darwin's finches in the Galápagos Islands

The 14 species of Darwin's finches in the Galápagos Islands are textbook examples of evolution in action. They are internationally recognized as prime examples of natural selection and adaptive radiation in a unique natural laboratory (Grant, 1999, Schluter, 2001, Grant and Grant, 2008). The ancestors of Darwin's finches traversed over >1000km of ocean to colonise the volcanic islands about 2.3 million years ago (Sato et al., 2001), where they have since evolved into a diverse and endemic group in isolation from humans, pathogens, and with few predators. Charles Darwin himself noted that: 'The natural history of this archipelago is very remarkable: it seems to be a little world within itself" (Darwin, 1839). But since Darwin's famous voyage through the Galápagos in 1835, the islands have undergone intense and rapid degradation as a result of increasing human settlement and tourism (Steadman et al., 1991, Mauchamp, 1997). In fact the most pivotal Galápagos species for the development of Darwin's theory of evolution, the Floreana Mockingbird, is now critically endangered and only persists on two small islets after being extirpated from it's main habitat on Floreana (Curry, 1986, Deem et al., 2011). Darwin's finches are now under threat as their habitat is cleared for agriculture and introduced predators and parasites consume their nestlings (Curry, 1986, Steadman, 1986, Fessl and Tebbich, 2002, Grant et al., 2005, Chapters 2-5). In particular, the accidental introduction of the parasitic fly, *Philornis downsi*, to the Galápagos Islands is considered the most severe and imminent threat to the survival of endemic birds. *P. downsi* has been given the highest risk ranking for invasive organisms that threaten biodiversity in the Galápagos (Causton et al., 2006).

1.3 *Philornis downsi* parasitism causes endemic finch declines.

Parasites have such a strong impact on bird hosts they are 'likely to play a role in practically every aspect of (avian) evolutionary biology' (Price, 1991) Although Darwin's finches are famous for rapid evolution, they have certainly met their match in the new host-parasite relationship with *P. downsi*. Adult *P. downsi* flies are vegetarian but have three free-ranging parasitic larval instar stages, which feed on the blood and flesh of nestling birds (Fessl et al., 2006b)(Figure 1.3). Parasitism results in reduced haemoglobin concentration (Dudaniec et al., 2006), multiple body wounds and infections, substantial blood loss (18-55%) (Fessl et al., 2006a, Fessl et al., 2006b), reduced growth rates (Fessl et al., 2006a), grossly deformed nasal cavities of nestlings (Fessl et al., 2006b) and deformed beaks of fledglings that survive into adulthood (Galligan and Kleindorfer, 2009). Consequently, it is not surprising that *P. downsi* parasitism was found to cause 19-76% of Darwin's finch nestling mortality across years, when studied between 1998 and 2005 (Fessl et al., 2006b)(Figure 1.3).

The detrimental impacts of *P. downsi* on Darwin's finches are well documented, but because larval parasitism occurs within finch nests at night (Fessl et al., 2006a), we have very few observations of the host-parasite interaction in the wild. Developing effective control methods requires a more detailed understanding of within-nest activity such as the fly's reproductive characteristics, larval feeding strategies, and finch anti-parasite defences. This thesis represents the first study to use in-nest surveillance footage to examine real-time host-parasite interactions between Darwin's finches and *P. downsi* parasites (Chapters 5&6). Behavioural and nesting studies are used to examine the potential for Darwin's finches to adapt to parasitism, for example by showing anti-parasite behaviour and altering the expression of key life history variables such as clutch size and parental care (Chapters 3-6). I quantify fledging success and causes of brood

mortality across species, habitats, and islands to examine the vulnerability of Darwin's finch species to the effects of introduced parasites and rodent predators (Chapters 3-6).

1.4 Genetic diversity and hybridisation in Darwin's finches

The evolution of Darwin's finches has been greatly influenced by the isolation of the archipelago, extreme climatic variation, and limited opportunities for dispersal (Grant, 1999, Grant and Grant, 2008). These conditions created a unique environment, which promoted rapid adaptive radiation facilitated by niche competition and character divergence between species (Grant, 1999, Grant and Grant, 2008). But human-induced landscape changes such as the introduction of new food resources, predators and pathogens can alter the evolutionary trajectory of closely-related taxa, and should be considered when evaluating modern drivers of hybridisation, speciation and extinction (Hendry et al., 2006, Taylor et al., 2006, De León et al., 2011). To date, most field research about Darwin's finches has come from long term studies of the ground finches *(Geospizinae)*, while remarkably little is known about any aspect of the tree finches (*Camarhynchus* spp), including speciation scenarios, temporal and spatial patterns of hybridisation, and population genetic structure. This is the first study to systematically examine species boundaries, genetic diversity and divergence in three sympatric species of Darwin's tree finches on Floreana Island: the small, medium, and large tree finch (*C. parvulus, pauper* and *psittacula*)(Chapter 7). Notably, the medium tree finch is endemic to Floreana Island and at the time of commencing this research in 2006, the species was listed as "vulnerable" but "data deficient" on the IUCN redlist of threatened species. I studied gene flow between the three sympatric tree finch species on Floreana Island and contrasted patterns of gene flow between study years with low and high rainfall (Chapter 7). Previous research by Peter and Rosemary Grant has shown that Darwin's ground

finches are likely to hybridise during high rainfall years (Grant and Grant 1992). This finding is significant because hybridisation can result in extinction when declining taxa are genetically overwhelmed by introgression from closely-related species (Dabrowski and Fraser, 2005, Hendry et al., 2006, Taylor et al., 2006, De León et al., 2011), and this process is predicted to become increasingly prevalent as species' distributions are altered by human-induced changes (Dabrowski and Fraser, 2005, De León et al., 2011). Understanding patterns of hybridisation is therefore important for the effective conservation of sympatric populations of Darwin's tree finches that may be threatened by interspecific genetic interactions (Chapter 7).

1.5 Thesis scope and objectives

The chapters of this thesis examine the effects of bio-ecology of invasive species as well as an assessment of genetic processes that will affect the persistence of common and threatened Darwin's finch species. This information is integral to the development of control programs for invasive species and conservation management guidelines for threatened birds and habitats.

Specifically the aims of this study are to:

1. Survey population numbers of Darwin's finches in the degraded highland forest of Floreana island.
2. Identify the major cause of population decline in the critically endangered Darwin's medium tree finch.
3. Identify variation in *P. downsi* impacts and prevalence across species, years, habitats and islands.
4. Examine the impacts of nest predation and parasitism on the life history strategies of Darwin's small ground finch across two distinct habitats (arid lowlands and forest highlands).
5. Examine host-parasite interactions inside finch nests to increase our understanding of the life-cycle of *P. downsi* and the development of anti-parasite behavior in host birds.

6. Use experimental techniques to examine the role of parasitism on chick begging behavior and parental food compensation.
7. Use molecular techniques to test the idea that hybridisation will change in frequency across years that differ in rainfall, and will affect genetic diversity in Darwin's tree finches.

1.6 Organisation of this thesis

The thesis is presented as a series of manuscripts that are either published, submitted or in preparation for publication in peer-reviewed, scientific journals. The thesis is comprised of four published papers (Chapters 2-5), one paper that is submitted and "in review"(Chapter 6), and the final paper is in preparation (Chapter 7). A general discussion of the main findings of this research and suggestions for future work is included at the end of the research chapters.

Figure 1.1 Map of the Galápagos Islands (northern islands of Darwin and Wolf not pictured). The archipelago is located 1000km west of mainland Ecuador.

Figure 1.2 Floreana Island, Galápagos. Photo shows the island's largest volcano: Cerro Pajas (around the base of which lies the highland forest zone).

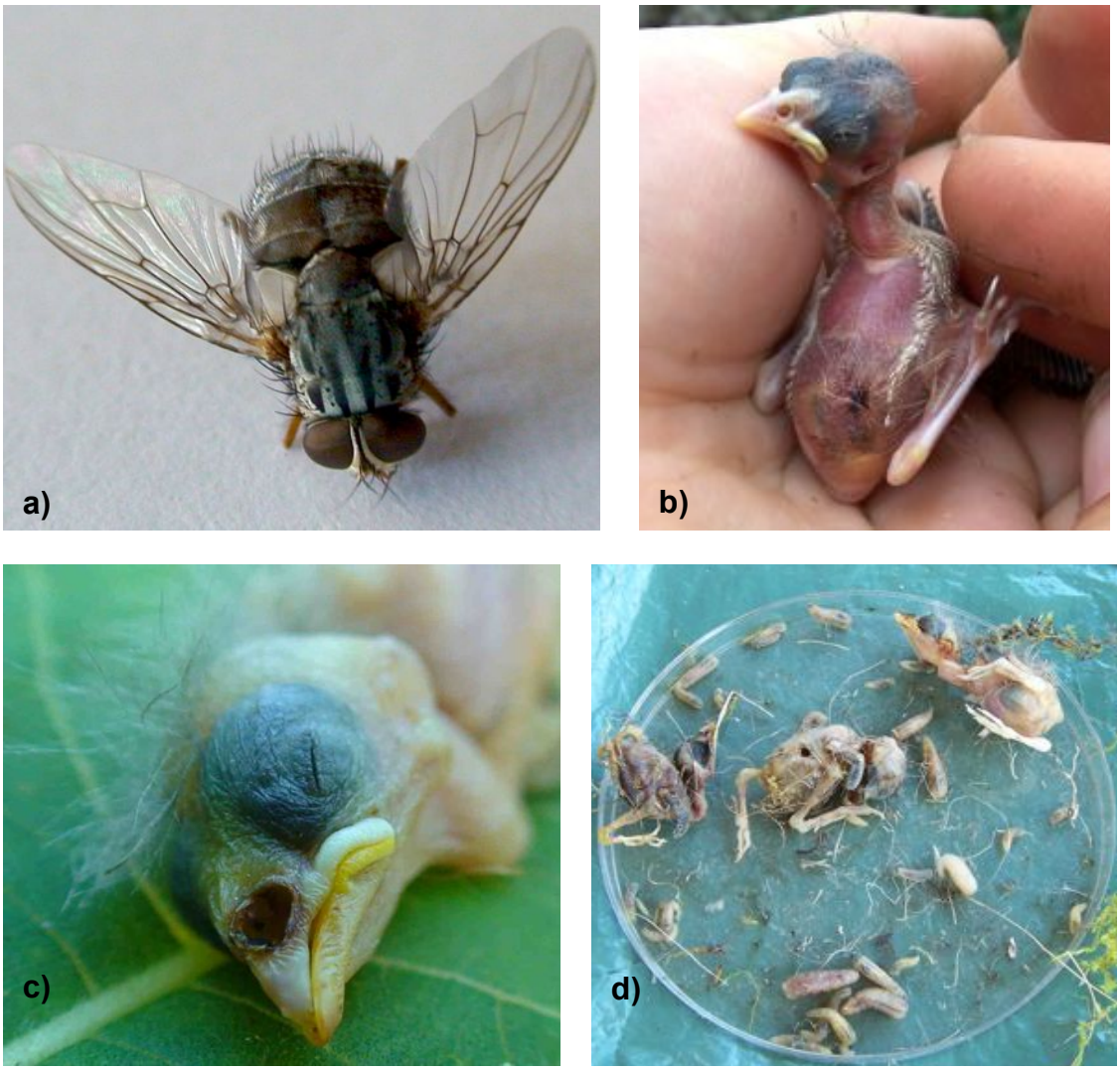

Figure 1.3 a) Adult *Philornis downsi* fly, b) small ground finch (*Geospiza fuliginosa*) nestling with signs of *P. downsi* larval feeding (body wounds and enlarged nares), c) dead medium tree finch (*Camarhynchus pauper*) nestling, d) dead medium tree finch nestlings with signs of parasitism along with 2nd and 3rd instar *P. downsi* larvae collected from the same nest

2 Avian Population Survey in the Floreana Highlands: Is the medium tree finch declining in remnant patches of *Scalesia* forest?

Jody A. O'Connor, Frank J. Sulloway, and Sonia Kleindorfer.

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2.1 Abstract

Island species typically exist in pathogen and predator sparse environments before human settlement, and are particularly vulnerable to the impacts of invasive species. In this study, we used the variable circular-plot method to estimate the density of birds in the highlands of Floreana Island, Galápagos Archipelago, where introduced parasites, predators, and habitat degradation are a known threat to endemic species. We recorded the number of birds seen and heard at 15 locations near Cerro Pajas Volcano in 2004 and 2008, an area that harbours the largest expanse of highland *Scalesia* forest on Floreana Island. We estimated the change in population density for 9 bird species, including 5 species of Darwin's finches. We specifically address changes in population density for the locally endemic medium tree finch, *Camarhynchus pauper*, which only occurs on Floreana Island and has a small population size. Comparing 2004 and 2008, our study found lower population density in the medium tree finch, but stable population density in Small and large tree finches. Based on data from three additional highland sites surveyed in 2008, we estimate that the maximum size of the medium tree finch population is 1,620 individuals. In addition to the survey data, we observed breeding males in 2006 and 2008. We found: (1) low nesting success (6 out of 63 nests produced fledglings) and high *Philornis downsi* parasite intensity, and (2) a biased age structure of the breeding population. No breeding males were one year old in 2006, and no males were five years old in either study year, indicating low reproductive success as well as limited lifespan. This research has contributed to the recent re-evalution by the International Union for Conservation of Nature, which has changed the Red List status of the medium tree finch from "vulnerable" to "critically endangered".

2.2 Introduction

Endemic island birds typically exist in small populations within restricted and specialised habitat (Simberloff, 1995) where they are 40 times more likely to go extinct than continental species (Johnson and Stattersfield, 1990). Birds that are highly adapted to restricted, elevated forest habitats on islands are under a particularly high risk of extinction when their habitat is fragmented via land clearance and/or invaded by introduced species (Savidge, 1987, Johnson and Stattersfield, 1990, Steadman, 1995). In Hawaii, the cooler high-elevation areas provide refuge for rare endemic honeycreepers (Benning et al., 2002). In contrast, elevated habitats in the Galápagos Islands have high prevalence and infestations of introduced fly larvae parasites (*Philornis downsi*) (see (Dudaniec et al., 2007, Wiedenfeld et al., 2007), and these highland areas have been extensively cleared for agriculture, leaving only fragmented and invaded habitats for mid-to-high elevation birds.

The *P. downsi* invasion is one of the most recent, yet most significant threats to all landbirds in the Galápagos Islands (Fessl and Tebbich, 2002, Causton et al., 2006), including the iconic group of Darwin's finches. Adult *P. downsi* flies lay eggs in bird's nests that hatch into larvae and proceed to feed on the blood and body tissues of developing nestlings by external attachment or by entering through the nasal cavity to feed internally (Fessl et al., 2006b, O'Connor et al., 2010b). Impacts of *P. downsi* parasitism in Darwin's finch nestlings include up to 55% blood loss, multiple body wounds and infections, increased mortality (Fessl et al., 2006b), reduced fledging success (shown experimentally in Fessl et al., 2006a), and beak deformation of fledglings that survive into adulthood (Galligan and Kleindorfer, 2009). The parasite is more prevalent on the three elevated islands with human settlements (Wiedenfeld et al., 2007), such as Floreana Island (which was visited in 1835 by Charles Darwin). With noticeable human

impacts even in Darwin's time, Floreana Island now has the longest history of human settlement and avian extinctions in the entire Galápagos archipelago. Native habitat on Floreana Island has been disturbed via agricultural clearance (Lack, 1947, Sulloway, 1982, Steadman, 1986), and invaded by introduced plants (Mauchamp, 1997), predators (Curry, 1986, Baskin, 2002, Grant et al., 2005), and avian parasites and infectious diseases (Fessl and Tebbich, 2002, Dudaniec et al., 2006, personal communication, Sharon Deem), To date, three Darwin's finch species have become extinct on Floreana Island including both the large ground finch, *Geospiza magnirostris*, and sharp beaked finch, *G. nebulosa*, by about 1870 (reviewed in Sulloway, 1982, Steadman, 1986), and the reported loss of the warbler finch, *Certhidia fusca*, by 2004 (Grant et al., 2005). Once common, the Floreana mockingbird, *Nesomimus trifasciatus*, disappeared from Floreana by 1895 and only survives today on two small islets: Champion and Gardener-by-Floreana (Curry 1986). Floreana Island also supports the only population of the medium tree finch (Lack, 1947, Grant, 1999), which is restricted to fragmented forest patches in the humid highland area. A recent study has found that, across years, medium tree finch nests have one of the highest *P. downsi* intensity documented in any Darwin finch species, and that *P. downsi* parasitism was responsible for mortality in 41% of nestlings (O'Connor et al., 2010d).

The preferred nesting tree of the medium tree finch, *Scalesia pedunculata*, is endangered (Boada, 2005) and only remains in small patches within the Floreana highlands. The size of the medium tree finch population is unknown, yet evidence suggests it may be declining due to lack of suitable habitat and high nestling mortality. Adult age structure within the population is also unknown, yet is an essential component for predicting adult survival. Another closely related species, the large tree finch, is also suspected to be declining on Floreana Island (Grant et al., 2005).

In this study we conducted surveys of all birds in the highlands across four sites, three of which contain the largest remnant patches of *Scalesia* forest, to estimate the maximum population size of several highland bird species. Our objectives were to (1) survey densities of bird species in the highlands of Floreana Island across years, with special reference to Darwin's finches, flycatchers, and yellow warblers; and (2) assess male age structure of nesting medium tree finches across years.

2.3 Methods

Study site: Cerro Pajas Volcano (2004 and 2008)

We surveyed bird abundance at the base of the Cerro Pajas Volcano (ca 300-400m), which is the highest volcano (max elevation 550m) on Floreana Island (173km^2, 1°28S, 90°48W) (Figure 2.1). The survey was conducted across seven days in mid-February of 2004 and 2008. The Cerro Pajas study site covers an area of approximately 2.4km^2 and is characterised by humid forest habitat, dominated by the tallest endemic *Scalesia pedunculata* (up to 15m high) found on the island (Table 2.1).

Study site: Other highland forest areas (2008 only)

To gain a better understanding of the entire highland forest bird community, we surveyed three additional highland sites in 2008 (Figure 2.1). These sites were situated around the base of mountains and volcanoes and are considered to be the last patches of native highland forest containing *Scalesia* (personal communication, Walter Cruz, Walter Simbaña). The characteristics of each site, including total patch size (km^2), *Scalesia* patch size (km^2), and dominant plant species are shown in Table 2.1. Notably, Peor es Nada supports only a small 100mx100m patch of *Scalesia* on its north-western slope. Cerro

Ventanas does not contain *Scalesia*, but it does contain native highland shrubs (not trees). Our study has essentially surveyed finch populations in prime habitat in the peak breeding season when song and foraging activity is very high. Birds--especially tree finches--do not generally nest in the agricultural area due to lack of suitable nesting substrate, and would only be temporary visitors there to forage on crops.

Survey methods

We followed protocols of the variable circular plot method to obtain our survey data (see (Martin et al., 1997). At Cerro Pajas we sampled from 15 point counts along the trail leading to the inner crater. Fourteen point counts were made at Cerro Ventanas, 11 point counts at Asilo de la Paz, and 12 at Peor es Nada. All point counts were conducted a minimum of 150m apart. At each survey point we recorded: (1) GPS co-ordinates, (2) species identity, (3) estimated radial distance of each bird from the observer (in ten-metre intervals), and (4) detection method (sight or sound). Birds were recorded up to a distance of 200m away, but we only analysed data from within a 20-70m radius due to a decline in rates of detection, which varied by species. Surveys were conducted for 5 minutes at each point, which was sufficient time to identify all birds actively using the area (this was trialled in a pilot study on Santa Cruz Island in 2000 and repeated in 2008). At each point, the observer surveyed the area facing 0°, 90°, 180° and 270° to eliminate visual or audio bias while facing only one direction. Counts were taken during peak bird activity which was between 06:00h and 12:00h.

Analysis of population density

Population density estimates (individual birds per square kilometre) and species detectability estimates were initially tested using the software program DISTANCE version 5.0 (Thomas et al., 2006), but our data did not meet the model assumptions/criteria. We also did not obtain the minimum 60-100 detections per species recommended for calculating accurate density estimates with DISTANCE (Somershoe et al., 2006) because of restrictions imposed by habitat patch sizes/accessibility.

We instead calculated population density using the inflection-point-per-species method (Reynolds et al., 1980). We plotted the number of detections of each species within 10-metre concentric bands and determined the distance from the observer at which its rate of detection begins to decline (the inflection point). The detectability of each species is affected by: (1) the ability of its song to be heard through thick vegetation, and (2) its visibility to the observer (due to differences in foraging behaviour). Habitat density analyses only include birds observed at a distance within the inflection point. For the tree finches and ground finches this point was 70m, but it differed for the remaining species (see Table 2.2). The number of birds/km^2 was determined by calculating the number of birds from each species within each 10m concentric circle, dividing the number by the total area within each circle, and dividing the density estimate by the number of survey points.

Male age structure in medium tree finches

We are particularly interested in estimating changes in population density in the medium tree finch, because (1) this species is locally restricted, (2) has high *P. downsi* parasite intensity (O'Connor et al., 2010d), and (3) only six of the 63 monitored nests produced fledglings (O'Connor et al., 2010d). We examined the age structure of medium tree

finches nesting in *Scalesia* forest at the base of Cerro Pajas during the peak finch-breeding period (February-April) of 2006 and 2008. Male tree finches increase the proportion of black on their chins and crowns with each year of annual moult until attaining a fully black head by five years of age (Lack, 1947, Grant, 1999, Kleindorfer, 2007b). Therefore, male age is considered to covary with male plumage coloration. Each male found singing at a display nest was assigned with a unique colour category based on the length of black on the chin (cm) and the extent of black on the crown (see Kleindorfer, 2007b). Black 0 males are yearling males and Black 5 males are five years and older. Females of all species of Darwin's finches remain brown throughout their lives; therefore female plumage coloration gives no indication of age.

Our analysis of male age structure was restricted to males that sang at nests to attract females. We located nests by systematically searching four 100m x 200m study plots at elevations of 300-400m within the Cerro Pajas site and locating either (1) singing males with display nests, (2) males building new nests or (3) active nests with a female present. We found 27 medium tree finch males with display nests in 2006, and 36 in 2008. We noted the nesting tree and height for each nest, and GPS co-ordinates were recorded with a hand held Garmin GCX12. Nesting activity was monitored for every unpaired singing male using 20-minute continuous focal sampling at least every second day to determine the status of the nest. At this time, we recorded male colour category.

2.4 Results

Avian population trends in 2004 and 2008 at Cerro Pajas

A total of 344 individuals of 9 bird species was counted between years at the Cerro Pajas site within 70-metre point counts and was included in the population density analysis (Table 2.2). The population density (number of birds/km^2) of some species differed

across years, while it remained stable in others (Table 2.2). Notably, we found that the medium tree finch population declined from 154 birds/km^2 in 2004 to 60 birds/km^2 in 2008 at the Cerro Pajas site, a reduction of 61%. In 2008, we observed substantially higher densities of 6 of the other 8 species, and collectively these 8 species increased their numbers by an average of 280%.

Avian population size across four highland sites in 2008

In 2008, the density of most species was similar across sites (Table 2.2) with the exception of large tree finches, which were not seen or heard at Cerro Ventanas. Avian species composition was most different at Peor es Nada where we observed (1) fewer small ground finches and Galápagos flycatchers, and (2) more medium ground finches and yellow warblers compared with other sites.

Calculating population estimates for the medium and large tree finch according to habitat suitability

The highlands area of Floreana covers an area of approximately 25km^2. It contains an inner region of approximately 2.5 km^2 that has been cleared for agriculture. The Floreana highlands thus contain a 22.5 km^2 uncleared area that may contain suitable forest habitat for medium tree finches. *Scalesia* dominated forest is estimated at 3.71km^2 (see Table 2.1).

A mean of 72 medium tree finches/km^2 was observed in surveyed forest habitat (calculated as an average over the 4 survey sites in 2008). Using 22.5km^2 as the maximum habitat size, we estimate that the entire medium tree finch population on Floreana Island consists of up to 1,620 individuals (Table 2.2). Using the same reasoning,

the large tree finch population has a mean of 22 finches/km^2 with up to 490 individuals on Floreana Island in 2008. However, large tree finches were not detected at Cerro Ventanas. If we exclude this area from our calculation, then the maximum number of large tree finches on Floreana Island in 2008 was 450 (Table 2.2).

Male age structure in medium tree finches

We recorded data from 63 males that were observed singing at 77 display nests to attract a female. Some males built multiple display nests. Most nest (83%) were built in *S. pedunculata* (n=63), 14% in *Z. fagara* (n=11), 2% in *C. scouleri* (n=2), and 1% in guava (n=1). Mean nest height was 6.15 metres (± s.e. 0.22), though nest height ranged from 3-12 metres high. Most nesting males were young (88% of nesting males were Black category 0-3). Only 12% of males were in category Black 4, and no Black 5 males were seen or mist-netted. There was a significant difference in the age structure of nesting males between 2006 and 2008: no Black 0 males were found in 2006 and more Black 1 males were found in 2008 (Likelihood ratio=21.79, df=4, P<0.001) (Figure 2.2). This finding suggests that breeding did not occur in 2005, which was a drought year in the archipelago.

2.5 Discussion

Population trends in tree finches

This is the first study to estimate the population size/density and range restrictions of any highland bird community in the Galápagos Islands. Of the 3 tree finch (*Camarhynchus*) species observed in the Floreana survey, small tree finches were the most common (Table 2.2). Medium and large tree finches were observed at lower densities relative to small

tree finches using both point count sampling and non-targeted mist netting (Christensen and Kleindorfer unpublished data). Large tree finches were always the least common tree finch species in both the point count and mist netting surveys. This species warrants close monitoring on Floreana Island because it exhibits three hallmark features of a species that could be on a trajectory to extinction: (1) small population size: <500 individuals, (2) large body size (which is associated with higher *P. downsi* parasitism levels), and (3) more specialist foraging behaviour (Bennett and Owens, 1997, Bennett and Owens, 2002, Christensen and Kleindorfer, 2009). In addition, we know that only one large tree finch nest that we monitored over two years produced fledglings, and this nest had high parasite intensity (50 *P. downsi* larvae in the nest).

Although the small and large tree finch populations appear to be stable, the intermediately-sized form of these two species – the medium tree finch – has declined in numbers. By 2008, the medium tree finch population at Cerro Pajas had dropped to 39% of its size in 2004, which is equivalent to a decline of ~15% per year. Natural populations of birds are known to fluctuate in numbers across years (Holmes and Sherry 2001), and declining populations can recover from major losses (Roth and Johnson, 1993, Holmes and Sherry, 2001, Hale and Briskie, 2009). However, in the case of the medium tree finch, the probable causes for its decline (introduced parasites and predators, and habitat loss) are not likely to subside without concentrated effort. Even in the very wet year of 2008 (when resources were abundant, bird nesting activity was very high, and females laid larger clutch sizes), high levels of nestling depredation and parasitism reduced the number of nests with any fledging success to lower than that in the significantly drier year of 2006 (O'Connor et al., 2010d). The entire medium tree finch population currently consists of a maximum 1620 individuals, which is significantly less common than it was 50 to 100 years ago (Kleindorfer and Sulloway, in preparation). This finding may be

explained by a combination of low nesting success and low adult survival. In a recent comparison of *P. downsi* parasite intensity in three Floreana Island tree finch species, (O'Connor et al., 2010d) found that the larger bodied medium and large tree finches had higher parasite intensity than the small tree finch. The study also found that medium tree finches have a higher *P. downsi* parasite intensity than would be expected based on body mass alone and that parasitism was responsible for 41% of nestling mortality. The medium tree finch has since been uplisted from 'vulnerable' to 'critically endangered' on the 2009 Red List of the International Union for Conservation of Nature (Birdlife International 2009).

Tree finch habitat on Floreana Island

We estimated maximum population sizes of each species according to their densities/km^2 across sites in 2008, multiplied by the area of native forest area. However not all of the maximum uncleared highland forest area used to calculate population range/size may be suitable tree finch habitat, as non-*Scalesia* forest is of considerably lower height (eg., Cerro Ventanas, maximum 4m) (Table 2.1) and lower vegetation density. For example, large tree finches were only observed at sites with tall trees, and where *Scalesia* is present (Table 2.2), and medium tree finches nested at a mean height of 6.15m, and 83% of their nests were in *Scalesia* trees (at Cerro Pajas). Displacement of native *Scalesia* forest by exotic fruit trees was identified as a conservation problem on Floreana as early as 1957 (Eibl-Eibesfeldt, 1959), and the species now remains only in small patches because of agricultural clearance, competition from invasive plants, and destruction by introduced mammals.

Male age structure in medium tree finches

Interestingly, of the 63 medium tree finch males observed singing at nests to attract females, most were less than four years old (see Figure 2.2), as indicated by the extent of black coloration in crown and chin plumage. Recent studies have found that older male small tree finches (*C. parvulus*) have higher pairing success compared with young males of the same species (Kleindorfer 2007, Kleindorfer *et al*. 2009). Kleindorfer (2007) also found that older males built more concealed nests that were less likely to be depredated and experienced higher fledging success. Thus, the scarcity of older medium tree finch males in the population may negatively influence nesting outcome if male age predicts nesting success.

Our finding of no young (Black 0) males in 2006 suggests unsuccessful breeding in the dry year of 2005 (see Dudaniec et al., 2007 for rainfall data). Many two-year-old (Black 1) males were found in 2008 and may represent a cohort that fledged in 2006. Few Black 4 and no Black 5 males were observed, which suggests that adults are not surviving past 4-5 years of age. The overall young age structure of the medium tree finch population signifies that adult males are not generally surviving to full maturity (5 years or more).

Population trends in other bird species

Significant changes in Galápagos finch population sizes have been reported from small, low-elevation islands such as Daphne Major where selection pressures are high and extremely variable across years (Grant and Grant 1999). The six elevated Galápagos islands with forest highland regions receive higher and more consistent annual rainfall (Wiedenfeld *et al*. 2007), which should support more stable bird population sizes. Here, we show that some bird species experienced rapid and dramatic population fluctuations in

as little as four years on the elevated island of Floreana. Between 2004 and 2008 at the Cerro Pajas site, the medium tree finch declined in numbers and the density of all other species either increased or remained relatively stable (Table 2.2). The ratio of change in densities of all other species in Table 2.2, compared to the medium tree finch, is 7.2 to 1. Thus the medium tree finch population, as of 2008, represented about 14% of what would be expected based on the more favourable ecological conditions that year, as reflected in the population sizes of other species. Warblers and flycatchers are insectivorous and may have been more commonly observed in 2008 because of an increase in insect abundance due to heavier rainfall. Small ground finches have the widest foraging breadth among Darwin's finches, which enables them to exploit modified habitats from which more specialised finches could be excluded from (see also Kleindorfer et al., 2006, Kleindorfer and Mitchell, 2009; Sulloway and Kleindorfer, in preparation for a discussion of habitat use and range expansion on Santa Cruz Island). The increase in small ground finch abundance may be due to their expansion into the highlands during a period of drought, where they can use the available food resources given their generalist foraging behaviour and diet.

The vegetarian finch (*Platyspiza crassirostris*) was not detected in surveys, though one female was observed at the Cerro Pajas site by J.O'C in 2008. The vermilion flycatcher (*Pyrocephalus rubinus*), once considered relatively common in the highlands of Floreana Island (Edwin Egas and Walter Cruz, personal communication), was not detected in our bird surveys, although two vermilion flycatchers were observed by S.K. in 2004 at the Cerro Pajas site while conducting other fieldwork. Finally, although the warbler finch (*Certhidea fusca*) was considered locally extinct by 2004 (Grant et al., 2005), we heard a male singing in 2008 (approximately 20m high in a *Cedrela odorata* tree at Asilo de la Paz).

2.6 Conclusion

Here we show that the sole population of Darwin's medium tree finch is small, declining, and at risk of extinction. Three other species (vermillion flycatchers, vegetarian finches, and warbler finches), once common in the Floreana highlands, have also become extremely rare. The Galápagos National Park has recently implemented programs to control and eradicate invasive plants and feral goats within the Floreana highlands. To help prevent another local avian extinction on the island, there is a need for effective *P. downsi* parasite and rodent predator control, as well as regeneration and expansion of the endemic *Scalesia* forest.

2.7 Acknowledgements

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Table 2.1 Description of vegetation found at each of the four survey sites. The total area with native forest is regarded as any forested area dominated by native tree species. We have noted the occurrence of seven of the most common dominant highland plant species across sites in the following descending order of abundance: (1) Dominant, (2) Common, (3) Patch (common, but only within a specified area in km2), (4) Present, (5) Absent. Site sizes were calculated using Google Earth ProTM.

† Native species
* Used as nesting substrate by tree finches (see results)

	Survey site and characteristics			
	Cerro Pajas	**Cerro Ventanas**	**Asilo de la Paz**	**Peor es Nada**
Max elevation above sea level	550m	420m	450m	370m
Total area with native forest	2.4 km^2	2 km^2	1.3 km^2	1 km^2
Size of *Scalesia* patch	2.4 km^2	0 km^2	1.3 km^2	.01 km^2
Height of tallest vegetation	15m	4m	10m	15m
Scalesia pedunculata†*	Dominant	Absent	Dominant	Patch
Zanthoxylum fagara†*	Common	Present	Common	Present
Croton scouleri†*	Common	Dominant	Common	Dominant
Macraea laricifolia†	Present	Dominant	Present	Dominant
Cinchona pubescens	Present	Present	Present	Common
Cedrela odorata	Absent	Absent	Present	Common
*Psidium guajava**	Common	Absent	Present	Common

Table 2.2 Population density estimates for bird species surveyed at Floreana Island highland forest sites in 2006 & 2008. Methods for calculating density are detailed in the methods section. The maximum population size for 2008 was calculated using the mean density (birds/km2) across the four sites divided by the total area of native forest (22.5 km2). For the large tree finch, a range in population size was calculated (see results, Cerro Ventanas was excluded from the lower estimate). The inflection point (distance from the observer at which the density of each species declined) was 70m for each of the five species of Darwin's finches, 50m for the smooth-billed ani and dark-billed cuckoo, 40m for the yellow warbler, and 30m for the Galápagos flycatcher.

	Number of birds/km^2: Estimated (Observed)					**Estimated maximum highlands population size, 2008**
	2006	**2008**				
	Cerro Pajas	**Cerro Pajas**	**Cerro Ventanas**	**Asilo de la Paz**	**Peor es nada**	
Small Tree Finch *Camarhynchus parvulus*	162 (43)	184 (57)	129 (32)	195 (38)	151 (32)	3,700
Medium tree finch *C. pauper*	154 (41)	60 (19)	61 (15)	97 (19)	71 (15)	1,620
Large Tree Finch *C. psittacula*	49 (13)	47 (1)	0	21 (4)	19 (4)	450-490
Small Ground Finch *Geospiza fuliginosa*	132 (36)	234 (47)	246 (61)	206 (40)	146 (31)	4,680
Medium Ground Finch *G. fortis*	26 (6)	18 (4)	8 (2)	5 (1)	38 (8)	390
Yellow Warbler *Dendroica petechia*	225 (18)	690 (56)	696 (49)	741 (41)	812 (49)	16,530
Galápagos Flycatcher *Myiarchus magnirostris*	142 (8)	401 (18)	429 (17)	289 (9)	88 (3)	6,790
Smooth-billed Ani *Crotophaga ani*	30 (1)	91 (3)	18 (2)	35 (3)	85 (8)	1,290
Dark-billed Cuckoo *Coccyzus melacoryphus*	0	34 (4)	18 (2)	35 (8)	18 (2)	590

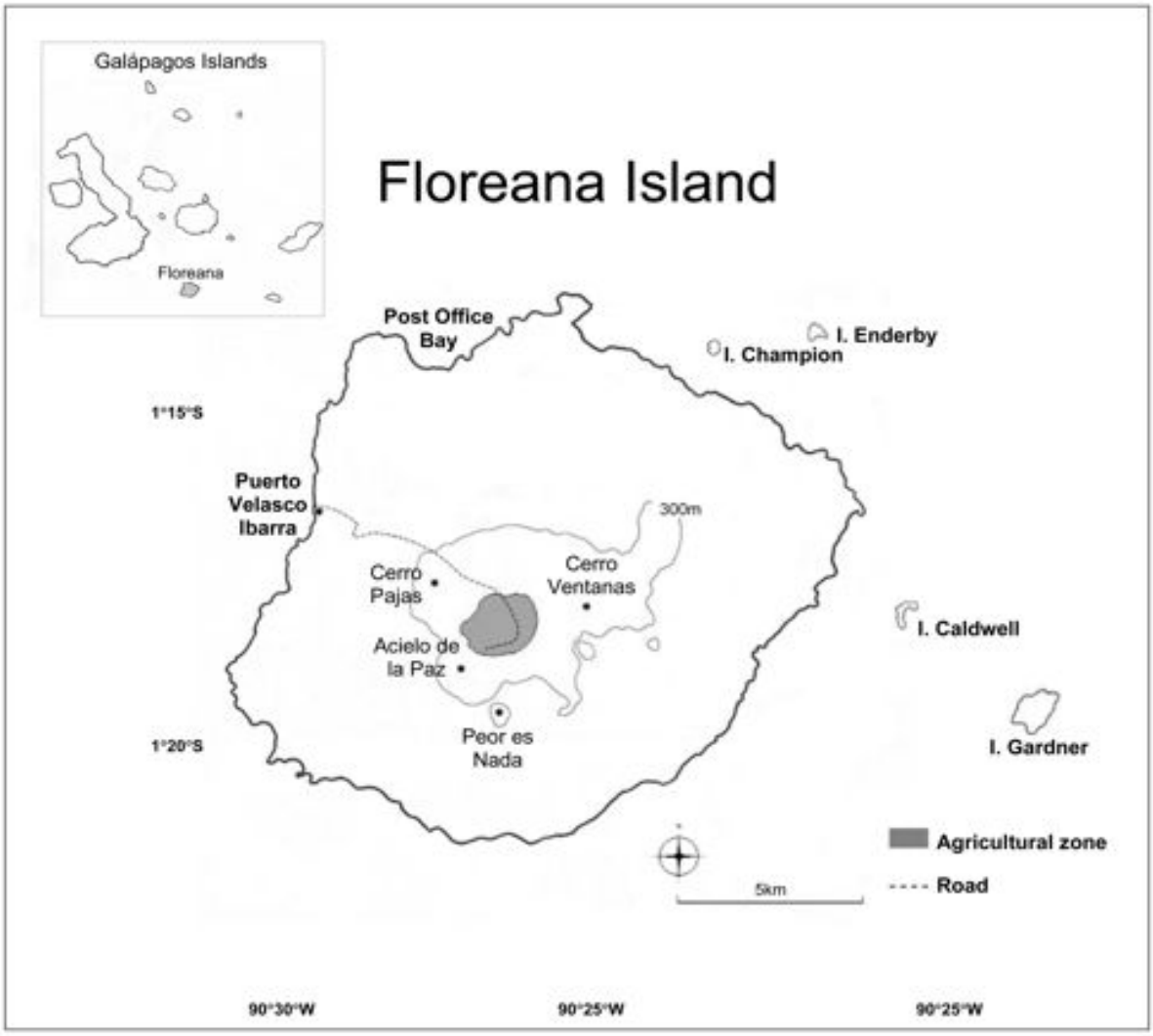

Figure 2.1 Map of Floreana Island, Galápagos Archipelago, Ecuador. The highlands zone includes all areas within the 300m contour line. Site locations and contour lines were established using GPS coordinates and Google Earth ProTM.

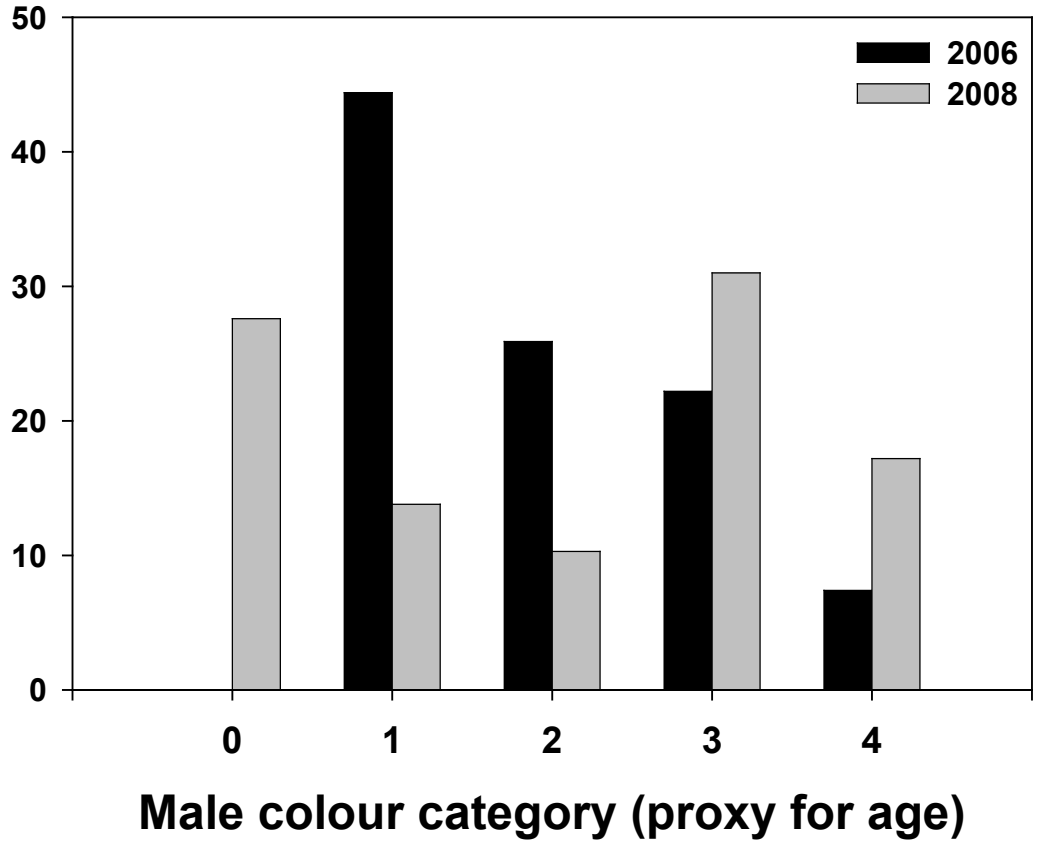

Figure 2.2 Change in the percentage of male colour categories at active medium tree finch nests in the Floreana highlands between 2006 and 2008. Note that there were no Black 0 (young) males in 2006, which suggests unsuccessful breeding in the previous year(s). No Black 5 (old) males were observed in either study year.

3 *P. downsi* parasitism is the primary cause of nestling mortality in the critically endangered Darwin's medium tree finch (*Camarhynchus pauper*).

Jody A. O'Connor, Frank J. Sulloway, Jeremy Robertson, and Sonia Kleindorfer.

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3.1 Abstract

Darwin's medium tree finch (*Camarhynchus pauper*) meets the 2009 International Union for Conservation of Nature Red List criteria for a critically endangered species because it has "a very small range on a single island" and is "declining rapidly owing to the effects of the parasite *Philornis downsi*", habitat degradation, and introduced predators. The medium tree finch is only found in patches of remnant highland forest on Floreana Island, where it co-exists with breeding populations of small and large tree finches (*C. parvulus* and *C. psittacula*). Here, we examine the intensity of *P. downsi* in nests of small, medium, and large tree finches on Floreana. We expected that parasite intensity would increase with finch body size, and with greater rainfall, and would also correlate with increased nestling mortality. We found a trend in the expected direction for parasite intensity and rainfall. Combined meta-analytically with data from a previous study, the overall trend for the two studies was significant. We also found a significant linear trend in parasite intensity with finch body size. In addition, the medium tree finch exhibited a somewhat higher parasite intensity than would be expected based on body mass alone. Of 63 active medium tree finch nests, 17 nests had nestlings: all of which were infested with *P. downsi*. Only 25% of medium tree finch nestlings fledged, 28% were depredated, 41% died due to *P. downsi* parasitism, and 6% died for other reasons.

Keywords: bird, body size, fledging success, Galápagos Islands, introduced species, larvae, mortality, parasite.

3.2 Introduction

The Galápagos Islands are a natural laboratory to examine oscillating evolutionary dynamics of natural populations in response to shifts in natural selection pressures (Grant, 1999, Grant and Grant, 2008). The strength and type of natural selection affecting endemic Galápagos organisms has changed remarkably over the past century because of the sharp rise in the number of introduced species to the archipelago and increased human impacts (see Wikelski et al., 2004, Causton et al., 2006). Currently, 29% of insects, 32% of vertebrates, and 57% of vascular plants in the archipelago have been introduced (reviewed in Durham, 2008). Collectively, these invasive alien species have been identified as the prime threat to terrestrial life in the Galápagos (Baskin, 2002, Causton et al., 2006), and are implicated in the current and future population declines of some of the iconic Darwin's finches (see Grant and Grant, 1997b, Dvorak et al., 2004, Grant et al., 2005).

The introduction and distribution of invasive species in the Galápagos archipelago follows patterns of human settlement and mobility. The first human settlement was established on Floreana Island in 1832, just three years before Charles Darwin's historic visit. Within approximately 50 years following settlement, six local vertebrate species became extinct on the island, including the Floreana tortoise (*Geochelone nigra*), Galápagos snake (*Alsophis biserialis*), Galápagos barn owl (*Tyto punctatissima*), Floreana mockingbird (*Mimus trifasciatus*), and two species of Darwin's finch: the large ground finch (*Geospiza magnirostris*), and sharp-beaked ground finch (*G. nebulosa*) (Sulloway, 1982, Steadman, 1986, Estes et al., 2000). Floreana Island has the longest history of local vertebrate extinctions following human settlement, but compared to the larger and most central island in the archipelago, Santa Cruz, its avifauna are relatively

understudied. More recently (by 2004), yet another Darwin's finch species--the warbler finch (*Certhidea fusca*)--was reported as extinct on Floreana Island (Grant et al., 2005), indicating ongoing extinction pressures for the island's bird populations. The medium tree finch (*Camarhynchus pauper*) is one of five tree finch species inhabiting the Galápagos Islands. In contrast to other Darwin finch species, most of which occur on more than one island, the medium tree finch is restricted to the highland area of Floreana Island (Lack, 1947, Grant, 1999). To date, there has been no systematic study of nesting success in the medium tree finch, or indeed any tree finch on Floreana Island.

The introduced parasitic fly *Philornis downsi* (Diptera, Muscidae) is one of the greatest current threats to the survival of Darwin's finches and to many other land birds on the Galápagos Islands (Causton et al., 2006). *P. downsi* was discovered in nests of Darwin's finches in 1997 (Fessl et al., 2001, Fessl and Tebbich, 2002, Dudaniec and Kleindorfer, 2006, Fessl et al., 2006b). Adult *P. downsi* flies feed mainly on fruit, but oviposit in birds nests where their eggs hatch into parasitic larvae (Fessl et al., 2006b). 1st instar larvae feed within the nasal cavities of nestlings, whereas 2nd and 3rd instar larvae reside in the nest-base and emerge at night to feed both internally (entry via the nares) and externally (by attachment to host integument) on nestlings (Dudaniec and Kleindorfer, 2006). *P. downsi* larvae consume up to 55 % of the blood volume of nestlings (Fessl et al., 2006a) and hence cause significant mortality, which may be 100% in some years, but varies between 16 % and 95 % in all finch nests (Fessl and Tebbich, 2002, Dudaniec and Kleindorfer, 2006, Fessl et al., 2006a, Huber, 2008). Larvae were found in 98% of active finch nests on Santa Cruz Island (Fessl and Tebbich, 2002). A survey of the fly's distribution across the Galápagos Islands found the highest prevalence of *P. downsi* across nests, and highest intensity per nest, on Santa Cruz (Wiedenfeld et al., 2007). Parasite intensity was not found to vary across lowland or highland habitats on Santa

Cruz (Dudaniec et al., 2007), but more fly larvae were found per nest on other elevated islands with a humid highland forest (Wiedenfeld et al., 2007). The forest highlands are always moist and provide the fruit and abundant decaying organic matter on which the adult flies feed, hence providing a refuge for the adult flies during unfavourable climatic conditions. This ecological characteristic may allow adult flies to colonise other habitats from highland refuges during more favourable conditions (Wiedenfeld et al., 2007). As the moist highland forests are the preferred habitat of Darwin's tree finches across the archipelago, the tree finches appear to be at elevated risk of parasitism from *P. downsi*. Darwin's finch nests are usually infested with a mean of 30-50 *P. downsi* larvae (Fessl and Tebbich, 2002, Dudaniec et al., 2007), but up to 182 parasites have been found in one nest (Fessl and Tebbich, 2002). The number of parasites feeding on each nestling may be diluted in nests with larger broods (Dudaniec et al., 2006), but tree finches generally have small clutch sizes (2-3 eggs) (Kleindorfer, 2007b), which may increase the intensity of larvae per nestling and intensify larval feeding on fewer nestlings. More *P. downsi* parasites are found in nests of larger-bodied Darwin's finch species that build larger nests, possibly because they provide greater resources for developing larvae (Dudaniec et al., 2007). A larger host may be able to sustain a greater number of *P. downsi* larvae, while larger nests may offer greater space for resting and pupating larvae (see Kleindorfer and Dudaniec 2009).

The aim of this study is to identify the extent to which the medium tree finch is experiencing low reproductive success, in comparison to other tree finches in the same habitat on Floreana Island. We focus on nesting outcome in relation to *P. downsi* intensity (the number of parasites per nest) in three tree finch species on Floreana Island. From previous research on Santa Cruz Island, we know that *P. downsi* intensity was highest in tree finches with large body mass and in years with increased rainfall

(Dudaniec et al., 2007, Kleindorfer and Dudaniec, 2009). Therefore, we examine *P. downsi* intensity in the small tree finch, medium tree finch, and large tree finch (*C. parvulus, C. pauper, C. psittacula*) on Floreana Island, and across two years with different rainfall. We expected: (1) *P. downsi* intensity would covary with host body size, such that intensity is highest in the large tree finch, intermediate in the medium tree finch, and lowest in the small tree finch; (2) *P. downsi* intensity would be higher in years with high rainfall; and (3) within the medium tree finch, high *P. downsi* intensity will correlate with high nestling mortality.

3.3 Methods

Study species and site

This study examines *P. downsi* intensity in the small tree finch (*C. parvulus*), medium tree finch (*C. pauper*), and large tree finch (*C. psittacula*) in the highlands of Floreana Island (01°17 S, 090°26W), Galápagos Archipelago (Figure 3.1). As the names suggest, the three species generally differ in body and beak size and shape (Grant, 1999) as well as diet and foraging habit (Christensen and Kleindorfer, 2009). Here, we provide a summary of the mean wing length (mm) and tarsus length (mm), shown as mean±se, for the small tree finch (*n*=125), medium tree finch (*n*=37), and large tree finch (*n*=14) sampled between 2004 and 2006 in the Floreana highlands near Cerro Pajas (Christensen and Kleindorfer, unpublished data): 61.5±2.0 (wing length), 20.1±0.7 (tarsus length); 66.5±2.9 (wing length), 21.7±0.9 (tarsus length); 68.5±2.2 (wing length), 22.7±0.5 (tarsus length), respectively.

P. downsi intensity and nesting outcome were recorded between 1 March and 8 April 2006 and between 26 February and 7 April 2008 on Floreana Island. The island has an area of 173 km^2 (described in Snell et al., 1996) and a maximum elevation of 550 m (Figure 3.1). The study site consisted of four 100m x 200m plots at the base of a volcanic cone, Cerro Pajas, at elevations of 300-400m. Approximately 95% of Floreana Island is protected by National Park (personal communication Edwin Edgas, Galápagos National Parks). The entire highland region covers an area of approximately 25km^2, and contains an inner, cleared agricultural area (2.5km^2). Tree finches prefer to nest in endemic *Scalesia pedunculata* trees, which are endangered on the island and only remain in fragmented patches (totalling <4km^2) that overlap with agricultural land. Geographic co-ordinates for the southeast corners of study plots are available on request.

Parasite intensity

The intensity of *P. downsi* per nest was examined using methods established by Fessl and Tebbich (2002) and Dudaniec et al. (2006). Total intensity refers to the number of *P. downsi* per nest, whereas mean intensity refers to the number of *P. downsi* per nestling (Bush et al., 1997, Dudaniec et al., 2007). *P. downsi* intensity was only analysed from nests in which nestlings survived to at least six days to minimise the effects of nestling age on the accumulation of parasite numbers within the nest (Fessl and Tebbich, 2002). If nests were known to have become inactive (by either checking nest contents for dead or absent nestlings, or by 30 minutes of continuous focal observation with no nest activity), nests were removed from the nesting tree, sealed in plastic bags, and carefully dismantled after returning from the field. All larvae, pupae, and pupae cases were counted to obtain total *P. downsi* nest intensity and were preserved in 95% ethanol. We collected *P. downsi*

data from the following number of nests of each species across years: (1) small tree finch (2006, *n*=10; 2008, *n*=5), (2) medium tree finch, (2006, *n*=8; 2008, *n*=5), (3) large tree finch, (2008, *n*=1). In both years, finches began breeding in late February, and the majority of nesting activity had ceased by early April. Nests were collected over a four-week period spanning from early March to early April, during which we did not detect any significant intra-seasonal changes in parasite intensity. No finches that built nests and formed pairs were observed with active pox lesions.

Nest Monitoring

We provide data on *P. downsi* intensity in nests of small, medium, and large tree finches from Floreana Island and in doing so, we present the first data on nesting outcome for our focal species, the medium tree finch. Nesting outcome was not monitored with equal effort in the small and large tree finches; however, we present comparable data on *P. downsi* intensity in these species (all data is from Floreana Island). Dudaniec et al. (2007), however, have already reported the effects of *P. downsi* parasitism on fledging success of small and large tree finches on Santa Cruz Island, where both species are common.

To monitor nesting outcome in the medium tree finch, we located nests by systematically searching our 4 study plots and locating either (1) singing males with display nests, (2) males building new nests, or (3) active nests with a female present. We found 27 medium tree finch males with display nests in 2006, and 36 in 2008. The GPS co-ordinates of each nest were recorded with a hand held Garmin GCX12. Nesting activity was monitored for every unpaired singing male using 20-minute continuous focal sampling at

least every second day to determine the status of the nest. Active nests were checked every day. Clutch size was determined between days 7-10 of the incubation period. If the date was unknown for the commencement of egg-laying, nests were rechecked after 2 days to confirm completion of egg-laying. Nesting outcome was categorised at the end of the nesting into 5 possible outcomes: (1) abandoned, (2) fledged, (3) depredated, (4) dead nestlings (partial or total brood loss), or (5) unknown outcome. Nests were collected and inspected for the presence of abandoned eggs, egg shell remains, or dead nestlings. We also counted the total number of *P. downsi* larvae, pupae, and pupae cases in nests to examine the relationship between *P. downsi* intensity and partial or total brood loss. To cover possible cases of premature fledging, fledging was inferred when nests were empty and chicks had reached the 9^{th} day from hatching (the nestling phase is usually 13-16 days; Grant, 1999), or when the nest was empty and fledglings were seen around the nest. Predation was assumed for empty nests that had previously contained eggs or nestlings (≤8 day old). Suspected fledging, predation, parasitism, or abandonment, was confirmed by 30 minutes of inactivity at the nest.

Measuring Rainfall

Within our highland study site, we positioned a rain gauge at an elevation of 343m (S01°17’48.4, W 90° 27’07.0) on a 1.5m post in a 4m radius clearing (to prevent tree canopy from blocking rainfall). The gauge was checked for rainfall every day between 7am and 8am when the temperature was still cool (to avoid water loss due to evaporation). Rainfall data was collected between 1 March – 8 April 2006 and 26 February – 7 April 2008.

Statistical Methods

Data for total parasite intensity did not differ significantly from a normal distribution (Kolmogorov Normality test P=0.35), and the same was true for mean parasite intensity (P=0.81). For consistency, data for both total and mean *P. downsi* parasite intensity were transformed using natural logs to give more acceptable scores for skewness and kurtosis respectively in both variables (total parasite intensity= -0.63, -0.44, mean parasite intensity= -0.39, -0.21). In similar studies, data on *P. downsi* intensity has typically shown a non-normal distribution and has been transformed via square root or natural log for subsequent analyses (Fessl and Tebbich, 2002, Dudaniec et al., 2007). We calculated effect sizes using the point-biserial correlation coefficient (r_{pb}).

We obtained data on parasite intensity from only one large tree finch nest (2008), which has been included in regression analyses (involving parasite intensity and its relationship to species body size and to rainfall), but has been omitted from ANOVAs.

Ethical Note

All procedures followed the Guidelines for the Use of Animals in Research (Flinders University, Charles Darwin Research Station, Galápagos National Parks), the legal requirements of Ecuador, and were approved by the Animal Welfare Committee of Flinders University (permit E189).

3.4 Results

Philornis downsi intensity across the three tree finch species

As predicted, total *P. downsi* intensity (mean±s.e.) was significantly different between the two smaller tree finch species, but did not differ significantly across years (for species, $F_{1,24}$=7.01, r_{pb}=0.48, P=0.01; for year: $F_{1,24}$=0.03, r_{pb}=0.04, P=0.86; for the interaction effect, which was significant, $F_{1,24}$=4.57, r_{pb}=0.40, P=0.04) (Figure 3.2). *P. downsi* intensity was lowest in the small tree finch, which had the smallest body size (Lack, 1947, Christensen and Kleindorfer, in prep)) . The medium tree finch had the highest *P. downsi* intensity (51.77± s.e. 7.77, n=13), which was significantly higher than that for the small tree finch (29.93± s.e. 5.29)(t_{26}=2.38, r_{pb}=0.42, P=0.03). In the large tree finch, nestlings survived to six days post hatching in only 1 of 4 active nests, where *P. downsi* intensity was 50. This result is comparable to the mean of 51 parasites found in large tree finch nests (n=5) on Santa Cruz Island (Dudaniec et al., 2007). Although the parasite intensity for the single large tree finch on Floreana Island was slightly lower than for the 13 medium tree finch nests, the linear contrast for overall parasite intensity by body size among the three tree finch species was significant (t_{27}=2.11, r_{pb}=0.38, P=0.04). We used ANOVA with contrasts to examine total and mean parasite intensity in relation to nesting outcome in the 15 tree finch nests (all species combined) that were not predated (fledged=+1, partial failure=0, and total failure= -1). Nesting outcome was not significantly related to total parasite intensity ($t_{1,13}$=1.00, r_{pb}= 0.27, P=0.34) or mean intensity ($t_{1,12}$= 1.14, r_{pb}= 0.30, P=0.28). It should be noted that these non-significant results were anticipated given the expected size of the depredation effect (r= -0.19, n=96), computed from Dudaniec et al. (2007), and our modest sample size (n=15), which had only 10% power to detect the expected effect.

Rainfall and *P. downsi* intensity

The highlands of Floreana received little rain (13.5mm) during the sampling period in 2006, but heavy rains (297mm) during the 2008 study period. In the medium tree finch, both total and mean parasite intensity were higher in 2008, which was a wet year, than in 2006, which was a dry year (Table 3.1), although this difference was also not significant (independent Student's t-test, total parasite intensity: t_{11}= 1.67, r_{pb}= 0.45, P=0.12, mean parasite intensity: t_{11}= 1.00, r_{pb}= 0.29, P=0.34). It is worth noting, however, that mean effect size obtained for our two measures of parasite intensity in the medium tree finch (r_{pb}=0.37) was reasonably similar to the effect size derived from data published by Dudaniec et al. (2007) in their review of parasite intensity and rainfall levels in the highlands on Santa Cruz Island from 1998 to 2005. In addition, after combining the findings for all seven species analysed in these two studies as a whole, the mean-weighted effect size for the relationship between mean parasite intensity and highland rainfall was significant (r_{pb}=0.30, n=110, P=0.002).

Nesting outcome in medium tree finches

Less than half of all males singing at display nests attracted a female and formed a pair (Table 3.1). Only 17 medium tree finch nests contained nestlings, and all of these nests were infested with *P. downsi*. All nestlings found dead in the nest had large open wounds on their bodies and significant loss of blood/body fluids, which are signs of *P. downsi* parasitism. Most nests failed to produce fledglings, as all nestlings died in 65% (11/17) of medium tree finch nests (Figure 3.3). Across years, *P. downsi* parasitism was the main cause of nestling mortality (partial or total brood mortality) in 9 of 17 nests (53%). Another 29% (5/17) of nests with nestlings were depredated. Only 18% (3/17) of nests

experienced total fledging success (Figure 3.3). Egg depredation occurred for 22% of nests in 2008, but was not observed in 2006. The percentage of depredated nests (egg or nestling depredation) was comparable across years (Likelihood ratio=1.31, *P*=0.25; see Table 3.1). The precise nesting outcome is known for 53 individual nestlings from 17 medium tree finch nests across both study years and was categorised as: (1) fledged, (2) depredated, and (3) mortality from parasitism. For both years combined: 25% of nestlings fledged, 28% were depredated, 41% died in the nests due to *P. downsi* parasitism, and 6% died when a tree fell directly on the nest (Figure 3.4). There was no significant difference in nesting outcome (fledged, depredated or parasitised) for the medium tree finch across years (Likelihood ratio=0.78, *P*=0.68). We found no evidence of nestling mortality due to depredation by the introduced small fire ant (*Wasmannia auropunctata*).

3.5 Discussion

P. downsi intensity co-varies with host body size

This is the first systematic study of parasite intensity and nesting outcome of Darwin's tree finches on Floreana Island. A previous study showed that parasite intensity across 13 islands in the Galápagos Archipelago was highest on Santa Cruz Island (Wiedenfeld et al., 2007). Here we show high parasite intensity in all three tree finch species on Floreana Island. Perhaps surprisingly, the high parasite intensity found in the highlands of Floreana Island was comparable to Santa Cruz Island (Dudaniec and Kleindorfer, 2006, Dudaniec et al., 2007), particularly as molecular evidence suggests that *P. downsi* dispersal is somewhat restricted between Floreana and the potential source population on Santa Cruz (Dudaniec et al., 2008). As predicted, *P. downsi* intensity differed across the three tree

finch species according to host body size. Total parasite intensity per nest and mean parasite intensity per nestling were highest in the larger bodied tree finches (medium and large tree finch), and lowest in the small tree finch (Figure 3.2). These findings confirm the positive correlation found between host body mass and *P. downsi* intensity in six species of Darwin's finches on Santa Cruz Island (Dudaniec et al., 2007). Host body mass is a strong predictor for *P. downsi* intensity, but, independently of body mass, large nest size and high nesting density can also increase *P. downsi* intensity and were not tested in this study (see Kleindorfer and Dudaniec, 2009). Population count and mist netting surveys from Floreana Island (O'Connor et al. unpublished data), along with historical records (Kleindorfer and Sulloway, unpublished data) have found that the small tree finch is common, the medium tree finch population is moderate, but declining, and the large tree finch has always been rare. High *P. downsi* intensity thus has potentially severe implications for the conservation of these larger bodied tree finch species that are rare or declining. Future studies could examine fledging success in relation to the number of parasites per gram of nestling tissue available within each nest. Although larger bodied finches have higher parasite intensity (Dudaniec et al. 2007), smaller-bodied finch species usually have smaller nestlings, which may have greater parasite intensity per gram of nestling tissue (Dudaniec et al., 2007, Kleindorfer and Dudaniec, 2009).

Medium tree finch nests had the highest recorded parasite intensity per nest of any finch species from Floreana Island (compared with the other two tree finches as well as the small ground finches from the same highland site; (Dudaniec et al., 2006, O'Connor et al. unpublished data). *P. downsi* intensity in the medium tree finch was 1.73 times greater than that found in the small tree finch (Figure 3.2), which is somewhat higher than would be expected based on body size alone (1.23 to 1). Using data for more than one year,

medium tree finch nests had the second highest parasite intensity recorded to date in the entire archipelago, with a mean 52 parasites per nest (*n*=13), compared to 67 parasites per nest (*n*=27) in the much larger woodpecker finch (*Cactospiza pallida*) on Santa Cruz Island (Dudaniec et al., 2007). It is important to note, however, that not all Darwin's finch species have been surveyed equally for *P. downsi* parasite intensity. The largest of Darwin's finches, the vegetarian finch (*Platyspiza crassirostris*), weighs about 34 grams (Grant, 1999) and based on prior findings, should therefore have the highest parasite intensity, but this species has not yet been sampled for *P. downsi*. Large tree finches are larger bodied (at 18 g) than medium tree finches (16 g) and should similarly have higher *P. downsi* intensity, which was not reflected in this study possibly due to modest sample sizes in these less common species (medium tree finch= 13, large tree finch=1). Additional sampling across nests of the four tree finch species on Floreana Island (small, medium and large tree finches, and the vegetarian finch) would help to clarify the parasite intensity and host body size relationship. However, only small tree finch nests are found at high density on Floreana Island: nests rarely reached the nestling stage in the medium (*n*=16) or large tree finches (*n*=4). We did not find any vegetarian finch nests, nor catch any individuals of this species during extensive nest-searching and mist-netting surveys of the Cerro Pajas site in 2004-2006 and 2008.

Rainfall and *P. downsi* intensity

The distribution, prevalence, and intensity of *P. downsi* may be linked with moisture availability and rainfall in the Galápagos Islands (discussed in Wiedenfeld et al., 2007). Despite higher mean *P. downsi* intensity in the medium tree finch in a year with high rainfall, the difference across years was not significant in our sample, which has

relatively low statistical power to detect the modest effect size that is expected based on previous findings (Dudaniec et al., 2007). However, the documented trend was significant as part of a meta-analytic compilation of data from our own study and that by Dudaniec et al. (2007), for which the combined statistical power is sufficient to detect the expected effect. The highland forests are always somewhat moist irrespective of the rainfall, and hence *P. downsi* can always coexist with the medium tree finch. Parasite intensity per medium tree finch nest ranged from 8-91 in 2006 (a dry year), with a mean of 43.12, but in 2008 (a wet year) this range was much smaller, and *all* nests had high intensity (49-96 parasites, with a mean of 65.60). A study by Dudaniec et al. (2007) on Santa Cruz Island found that *P. downsi* intensity was highest in the ENSO year of 1998, but did not vary much in relation to smaller rainfall fluctuations in other years. Like other species of Darwin's finches that increase clutch size by about one egg in a year with higher rainfall (Price, 1985, Kleindorfer, 2007b), we found this same pattern in the medium tree finch. However, we did not find significantly higher parasite intensity in the wet year, either per nest or per nestling, although the trend was in the anticipated direction and of the expected size.

P. downsi intensity and nestling mortality in the medium tree finch

Of 63 active medium tree finch nests with singing males, only 47% of males with display nests attracted a female mate (Table 3.1), which may indicate an unbalanced sex ratio in the population (in favour of males). Of 47 medium tree finches caught in mist-nets (2004-6 and 2008) only 11 were females, and we rarely observed unpaired females. Adult populations of monogamous birds are commonly male-biased (Breitwisch, 1989) because females often have a higher probability of mortality via mechanisms such as higher

parental investment (Trivers, 1972), or natural selection for larger-bodied birds (usually male) during migration (Breitwisch 1989), or harsh environmental conditions (Boag and Grant 1984). Female Darwin's finches are uniparental incubators and thus should be more vulnerable to depredation by native short-eared owls, which will take entire nests (personal observation Kleindorfer and O'Connor). Female nestlings may also be experiencing greater mortality because they are smaller and thus more vulnerable to *P. downsi* parasitism because they are less able to defend themselves or compete for parental resources. Future genetic studies of sex ratios in nestling, juvenile and adult Darwin's finches could help us understand the processes that shape male-biased population sex ratios.

In this study involving small, medium, and large tree finch nests, *P. downsi* larvae or pupae were found in every nest, and every nestling showed signs of parasitism. We regularly found dead nestlings with characteristics of *P. downsi* parasitism, including: grossly enlarged nares, deteriorated and cavernous beaks, and large open body cavities with significant tissue loss (see Fessl et al., 2006b). Although parasitism was a major cause of nestling mortality, parasite intensity had no measurable effect on nesting outcome (fledged, partially fledged, or total mortality) in the three species we studied on Floreana--a circumstance attributable to modest statistical power to detect the expected effect, given the uniformly high rates of parasitism in this study. Although *P. downsi* parasitism was responsible for the majority of nestling mortalities, introduced predators, highland habitat destruction for agriculture, and habitat degradation from introduced herbivores and plants are important factors affecting the survival of Darwin's finches in the Floreana highlands and require further investigation.

Impacts of nest predators on nesting success

Medium tree finch nests were depredated more frequently in the nestling stage compared to the incubation stage. Rates of nest depredation vary among tropical birds (Ricklefs, 1969, Martin, 1992) and can be affected by factors such as parental nest-visitation, choice of nest-site (Martin and Menge, 2000), and the amplitude and frequency of nestling begging calls (Briskie et al., 1999). Darwin's finch nests should be more conspicuous to predators during the nestling stage because: (1) they are bi-parental feeders, which increases behavioural conspicuousness at the nest, and (2) their nestlings produce loud, easily locatable begging and feeding noises. Although we could not conclusively determine the identity of nest predators in this study, the native diurnal Galápagos short-eared owl (*Asio flammeus galapagoensis*) and introduced black rats (*Rattus rattus*) are known predators of Darwin's finches and were probably responsible for most depredation events. In 2008, a rat was observed depredating eggs from a small ground finch nest just metres from an active medium tree finch nest (from which eggs were depredated two weeks later). Other potential nest predators include introduced species such as mice (*Mus musculus*), cats (*Felis catus*), dogs (*Canis lupus familiaris*) and smooth-billed ani (*Crotophaga ani*). The close proximity of agricultural areas to tree finch breeding sites probably encourages the persistence and dispersal of introduced predators, particularly rodents (see Figure 3.1). The Galápagos National Parks service distributes baited rat tunnels within the central cone of Cerro Pajas (personal observation Jody O'Connor): the main breeding area on the island for the critically endangered dark-rumped petrel, *Pterodroma phaeopygia* (Cruz and Cruz, 1990). Nest depredation by rats may thus be even higher in other, unbaited highland forest areas. Further studies could attempt to separate the relative impacts of the native predator (Galápagos short-eared owl),

introduced rodent predators, and *P. downsi* parasitism by comparing finch nesting outcome in rodent-reduced (extensively baited) and rodent-affected (unbaited) highland sites. Nest depredation may be lower in areas with few rodent predators. However, since all highland nests are infested with *P. downsi*, more non-depredated nestlings would ultimately die from parasitism in these areas.

3.6 Conclusion

This study shows that the larger bodied medium and large tree finch on Floreana Island experienced higher levels of *P. downsi* parasite infestation than the smaller-bodied small tree finch. The medium and especially the large tree finch are comparatively rare on this island (Lack, 1947, BirdLife, 2009, O'Connor et al. unpublished data). There are three main concerns for the conservation of the medium tree finch: (1) the species only occurs in the degraded highlands of Floreana Island; (2) historical records suggest it is declining; and (3) its parasite intensity appears to be somewhat higher than expected based on the average body mass of this species. Notably, the *P. downsi* parasite has been identified as one of the biggest threats to the survival of endemic Galápagos birds (Causton et al., 2006), and has subsequently been added to the International Union for Conservation of Nature's 'Global Invasive Species Database'. Floreana Island has the longest history of human settlement and local bird extinctions in the Galápagos and the current impacts of the introduced parasite *P. downsi*, nest predators, and habitat degradation may result in future species losses, particularly in Darwin's finches. To date, no finch species has become extinct in the Galápagos Archipelago, and we hope the medium tree finch will not be the first instance. It is now essential that we develop and implement an effective conservation program to ensure the survival of Darwin's medium tree finch.

3.7 Acknowledgments

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 Flinders University (Research Establishment Grant to SK), Conservation International, and the American Bird Conservancy with awards to SK and also the Royal Zoological Society of South Australia with an award to JO'C. TAME airlines provided reduced airfares. We thank Rebekah Christensen and Santos Humberto for field assistance, and Rachael Dudaniec for field assistance and comments on the manuscript. We extend special thanks to the community of Floreana Island, and the local National Parks team for their invaluable assistance and support.

Table 3.1 Nesting outcome in medium tree finches. Data were collected in 2006 (dry year) and 2008 (wet year).

	2006	**n**	**2008**	**n**	**Total n**
Rainfall	13mm		297mm		
Total # active display nests found with a singing male	27	27	36	36	63
Active nests with pair[a]	14	14	16	16	30
Clutch size (mean ± s.e.)	2.92 (±s.e. 0.08)	12	4.00 (± s.e. 0.16)	9	21
Clutch size range	2-3	12	3-5	9	21
% nests depredated with eggs	0%	0	22%	2	2
Abandoned nests[b]	2	2	5	5	7
Nests with nestlings	10	10	7	7	17
% nests depredated with chicks[c]	30%	3	29%	2	5
% nests failed: other reason[d]	10%	1	0	0	1
% nests fledged[e]	40%	4	29%	2	6
% *P. downsi* prevalence[f]	100%	8	100%	5	13
Partial brood loss due to parasitism[g]	40%	4	0%	0	4
Total brood loss due to parasitism[g]	20%	2	38%	3	5
Total parasite intensity (mean ± s.e.)	43.12 (±10.72)	8	65.60 (±8.46)	5	13
Mean parasite intensity (mean ± s.e.)	16.27 (±5.00)	8	19.65 (±3.91)	5	13
Total parasite intensity range	8-91	8	49-96	5	13

[a] Number of males that attracted a female mate to their display nest.
[b] Abandoned before completion of egg laying.
[c] 1 nest was depredated after already experiencing partial brood loss (2008).
[d] 1 nest failed after a tree fell onto the nest and crushed the nestlings (*P. downsi* intensity was not determined from this nest).
[e] Some or all fledged.
[f] Percentage of nests containing nestlings and *P. downsi* parasites.
[g] Total or partial brood either (1) found dead in nest with signs of *P. downsi* parasitism: large, open wounds on body, and significant loss of blood/body fluids, or (2) removed individually from nest by parent birds after death due to parasitism.

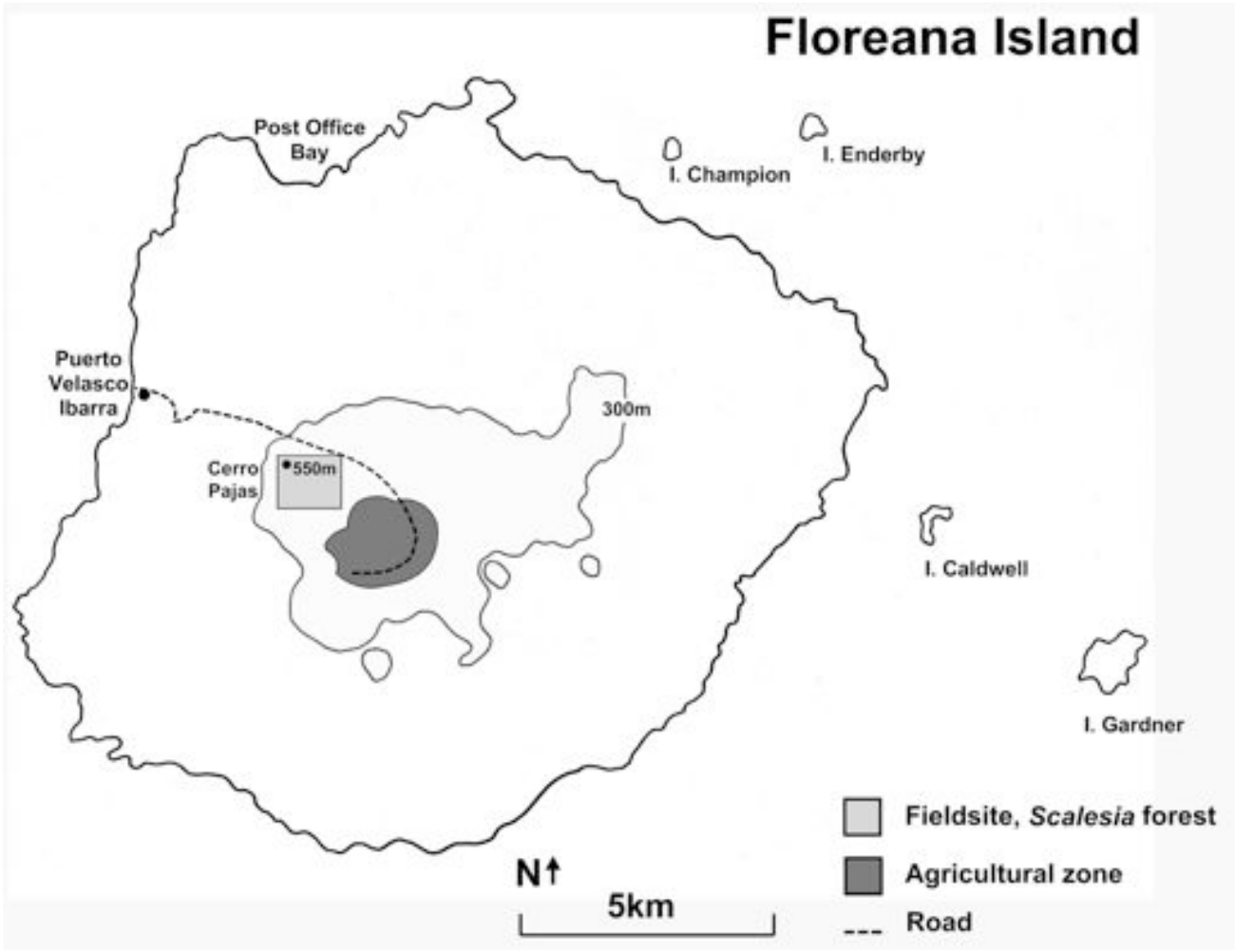

Figure 3.1 Map of Floreana Island, Galápagos Archipelago, Ecuador. The highland zone includes elevations above the 300m contour, within which the largest area of prime *Scalesia* habitat was chosen as our field site (base of Cerro Pajas volcano). The site was accessed via the road leading from the town of Puerto Velasco Ibarra to the agricultural area.

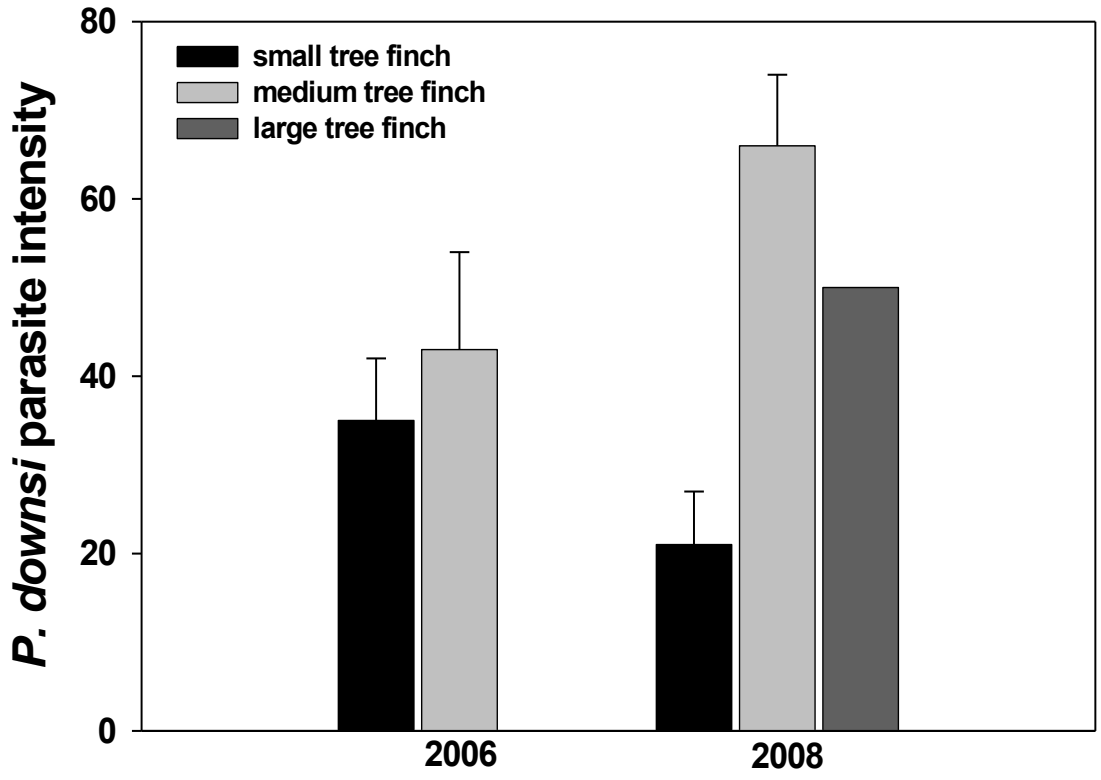

Figure 3.2 Total *P. downsi* intensity (per nest) (shown as mean±se) in three tree finch species on Floreana Island in which nestlings were at least 6 days old (*n*=29): (1) small tree finch, *C. parvulus,* 29.93 ± 5.29, (2006, *n*=10; 2008, *n*=5), (2) medium tree finch, *C. pauper,* 51.77 ± 7.77, (2006, *n*=8; 2008, *n*=5), (3) large tree finch, *C. psittacula,* 50, (2008, *n*=1), which has been included in the figure for illustrative purposes only and was not included in the ANOVA.

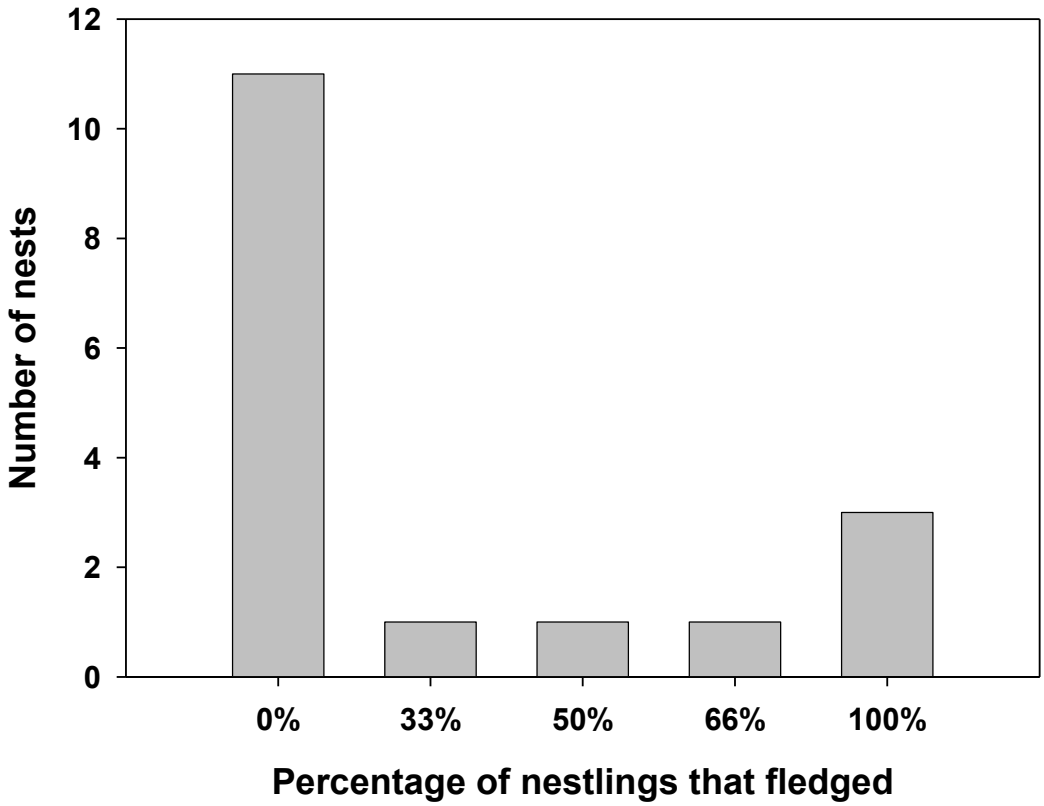

Figure 3.3 Number of medium tree finch nests across years that fledged (1) 0% of hatchlings (*n*=11), (2) 33% of hatchlings (*n*=1), (3) 50% of hatchlings (*n*=1), (4) 66% of hatchlings (*n*=1), or (5) 100% of hatchlings (*n*=3). Causes of 0% fledging success in nests are as follows; total brood loss due to parasitism (*n*=5), nestling predation (*n*=3), partial brood loss due to parasitism followed by nestling predation (*n*=1), tree falling on nest (*n*=1). Partial brood loss due to parasitism was responsible for all other nestling deaths leading to categories of 33, 50 and 66% fledging success.

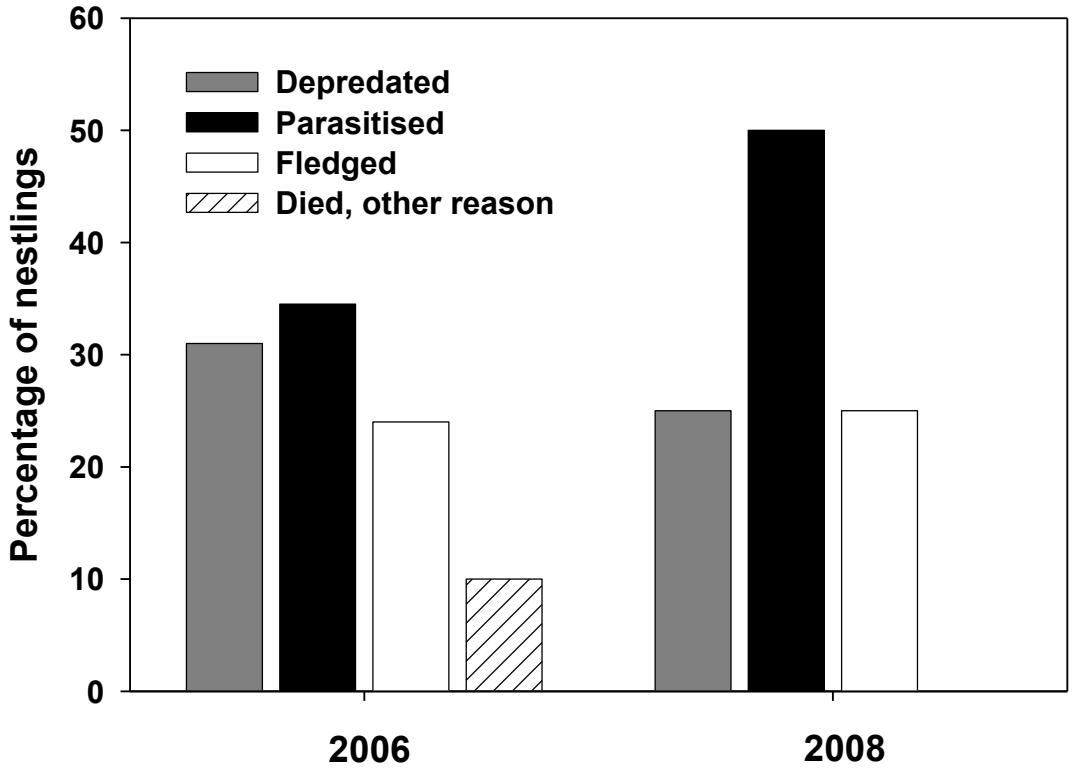

Figure 3.4 The percentage of medium tree finch nestlings that died due to (1) nest predation (2006, n=9; 2008, n=6), or (2) *P. downsi* parasitism (2006 n=10; 2008, n=12). 13 nestlings fledged (2006, n=7; 2008, n=6), and 3 nestlings died when a tree fell on the nest (2006). The sample size was 53 nestlings from 17 nests. For this figure, each nestling was treated independently because some nests had partial brood mortality due to parasitism and partial fledging success.

4 Parasite infestation and predation in Darwin's small ground finch: contrasting two elevational habitats between islands

Jody A. O'Connor, Rachael Y. Dudaniec, and Sonia Kleindorfer

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4.1 Abstract

Contrasting ecological conditions may affect the distribution, abundance and impact of parasites and predators throughout the ranges of hosts and prey. Such patterns are evident on the archipelagos of Hawaii and the Galápagos, which vary in their distribution and abundance of avian parasites within and across islands. Previous research has documented higher intensity of parasitic fly larvae (*Philornis downsi*) in nests of Darwin's finches on elevated islands of the Galápagos. Here we examine *P. downsi* intensity and predation in 71 nests of Darwin's small ground finch (*Geospiza fuliginosa*) on Floreana Island. We found significant differences in parasite intensity, nest predation and clutch size between the lowland (0-100 m) and highland (300-400 m) habitats. Lowland finch nests had few *P. downsi* parasites (mean of 8 per nest), high nest predation (44% of nests), and large clutch size (3.4). Highland finch nests showed the opposite pattern, with many *P. downsi* parasites (40 per nest), low nest predation (17%), and small clutch size (2.5). This study suggests that the impacts of an introduced parasite are limited by its niche requirements and resource availability within and across islands. Our findings also imply that the vulnerability of bird populations to introduced parasites and predators is linked with variation in life history strategies across habitats.

4.2 Introduction

Island species are particularly vulnerable to the impacts of introduced parasites and diseases because they have typically evolved in isolated environments with low pathogen diversity (Hochberg and Møller, 2001, Murray, 2001, Wikelski et al., 2004). Elevated islands can favour the establishment of invasive parasites, diseases, and their vectors by providing contrasting habitats and microclimates at different altitudes (Loope et al., 2001). Certain pathogens such as the introduced avian pox-virus (*Poxvirus avium*), for example, have higher prevalence in the lowlands of the Galápagos Islands (Kleindorfer and Dudaniec, 2006). The prevalence of the introduced avian ectoparasite *Philornis downsi*, however, differs across islands of the Galápagos (Wiedenfeld et al., 2007), but is similar between habitats on the central island of Santa Cruz (Dudaniec et al., 2007). Adult *P. downsi* flies are non-parasitic, but its larvae reside in the nest base and feed on the blood and tissues of nestlings (Fessl et al., 2006b, O'Connor et al., 2010b). The parasite causes significant mortality (16%-95% across years) in Darwin's finch nestlings (Fessl and Tebbich, 2002, Fessl et al., 2006b, O'Connor et al., 2010d) and is in the highest risk category for invasive species affecting endemic Galápagos biota (Causton et al., 2006).

A survey of the 13 main islands in the Galápagos archipelago found more *P. downsi* parasites in nests from elevated islands (maximum elevation >400 m) containing forested highlands compared to low-elevation islands (<200 m), however, intra-island site effects and site-specific variables were not evaluated (Wiedenfeld et al., 2007). The wet, elevated highlands of the Galápagos are predicted to provide more favourable conditions for *P. downsi*, with abundant year-round resources for the fly's persistence within and

across years (Dudaniec et al., 2007, Wiedenfeld et al., 2007, Kleindorfer and Dudaniec, 2009, Kleindorfer and Mitchell, 2009). The arid lowlands are predicted to be less suitable for reproduction and survival of *P. downsi* as the habitat is drier and host nesting-density is lower. Dudaniec et al. (2007) found no significant difference in the number of *P. downsi* in nests across lowland and highland habitats on Santa Cruz Island. Santa Cruz Island has the largest human settlement in the archipelago and is a busy central port for tourists and shipped supplies from the mainland. It is suspected that *P. downsi* was initially introduced to Santa Cruz Island where it established a large population due to the abundance of resources for both the adult and larval stages (artificial water sources, fruits and vegetables, nesting birds). Here we examine the difference in numbers of *P. downsi* parasites in Darwin's finch nests across lowland and highland habitats on Floreana Island, which may have been more recently colonised by *P. downsi* (Dudaniec et al., 2008). Geographic variation in habitat characteristics may also influence the distribution and impacts of avian predators on islands (Martin and Menge, 2000, Wiles et al., 2003). Such patterns may cause local declines or extinctions of endemic island birds that may be dependent upon prey life history or body size (Martin and Menge, 2000, Wiles et al., 2003).

In this study, we investigate habitat-specific effects of predation on *Geospiza fuliginosa*, in relation to clutch-size variation and examine the number of *P. downsi* parasites in *G. fuliginosa* nests across lowland and highland habitats on Floreana Island. We predict that the impact and number of parasites will be higher in highland nests than lowland nests due to increased host nesting density (including multiple avian host species), rainfall, and resources for adult *P. downsi* flies. We compare our findings on parasite intensity for Floreana Island with those of Dudaniec et al. (2007) for Santa Cruz Island. We also

examine patterns of clutch size, nesting outcome, and nest predation in *G. fuliginosa* across habitats and predict that larger broods will be depredated more frequently due to greater behavioural conspicuousness.

4.3 Methods

Study species

In this study, we use the small ground finch, *G. fuliginosa* as an 'indicator' species of current parasite pressure (Galligan and Kleindorfer, 2009) across elevational habitats on Floreana Island. *Geospiza fuliginosa* is a small-bodied (~13 g) finch that is found on most islands in the Galápagos archipelago (Grant, 1999) in both lowland and highland habitats (Kleindorfer et al., 2006, Kleindorfer and Mitchell, 2009). After sufficient rains males will build display nests within their territories and sing to attract mates (Kleindorfer, 2007a). Clutch sizes range from 2-5 eggs, the incubation phase is 10-12 d, and nestlings fledge from the nest after approximately 12-14 d (Kleindorfer, 2007a).

Study site

Floreana Island (1°17 S, 90°26W) has a maximum elevation of 600 m and an area of 170 km^2 (Wiedenfeld et al., 2007). Approximately 95% of Floreana Island is protected by National Parks (Edwin Egas, Galápagos National Parks, pers. comm.). We collected data on nesting outcome in *G. fuliginosa* across habitats (lowlands, highlands) during three breeding seasons: February 2004, February 2005 and February-April 2006. The highland study area (1°17 S, 90°27W) was located at the base of Cerro Pajas; the lowland study area (1°16S, 90°29W) was located adjacent to the town of Puerto Velasco Ibarra (human

population ~100). We sampled from an area of approximately 6 km^2 in the lowlands (at elevations of 0-100 m asl) where nesting density was low, and in four 200 × 200-m study plots within a 2.5-km^2 patch of highland forest where nesting density was high (at elevations of 300-400 m asl).

The lowlands and highlands on Floreana Island are ecologically distinct habitats (described in Dudaniec et al. 2008). Rainfall is much lower in the lowlands than highlands of the Galápagos islands in general (Dudaniec et al., 2007), although no quantitative rainfall data are available for Floreana Island across habitats for the years of 2004, 2005 or 2006, which were all years of very low rainfall (Edwin Egas pers. comm.). We first collected rainfall data across both habitats on Floreana Island in 2008: within our highland study site at an elevation of 343 m (1°17'48.4"S, 90°27'07.0"W), and within our lowland site at an elevation of 5 m (1°16'37.8"S, 90° 29'18.4"W). Rainfall data were collected daily with a rain gauge for 50 d between 26 February and 7 April 2008 and showed that the highlands received over twice as much rain (388 mm) as the lowlands (182 mm). We collected 13 mm of rainfall from the same location in our highland site in 2006 over 38 d (2 March–8 April 2006).

Nest monitoring

Geospiza fuliginosa nests were located by systematically searching study plots for evidence of singing males, nest-building behaviour, or pair activity at a nest. Nesting activity was monitored using 20-min continuous focal sampling, or by visually checking inside nests every 2 d to determine the status of the nest. In 2004 and 2005, we inferred clutch size either from the onset of nest activity, or from the maximum number of eggs or

nestlings recorded during nest monitoring. In 2006, we monitored nests from the onset of rain and nest building, and clutch size was checked daily or after incubation was first observed. We checked clutch size again on day 7-10 of the incubation phase or until egg hatching. The sample size was 34 nests in the lowlands and 37 nests in the highlands.

Nesting outcome was categorised into five possible outcomes at the end of the nesting event: (1) abandoned (no parental activity, but the nest still contains eggs or nestlings); (2) fledged; (3) depredated; (4) partial or total brood loss; (5) unknown outcome. Fledging was either observed directly (fledglings present near nest) or inferred when all of the following conditions were met: (1) nests were empty with no signs of predation or parasitism, (2) there was evidence of parental activity, and (3) nestlings had reached at least the ninth day from hatching. Predation was inferred when nests were empty, there was no sign of parental activity, and where nestlings had not reached an age of possible fledging (≤ 8 d old). Finches were not observed to re-nest in the current study, though on Santa Cruz Island highland birds were found to re-nest after 11 d, whereas lowland birds were never observed to do so during drought years (Kleindorfer 2007a).

Parasites

The intensity of *P. downsi* per nest was examined using methods established by Fessl & Tebbich (2002) and Dudaniec et al. (2006). All larvae, pupae and pupae cases were counted to quantify the total *P. downsi* nest intensity and were preserved in 95% ethanol. Total intensity refers to the number of *P. downsi* per nest, whereas mean intensity refers to the number of *P. downsi* per nestling in each nest. These definitions are based on those proposed by Bush et al. (1997), and have been modified to incorporate the nest as the unit

containing parasites. Total parasite intensity is a function of nestling age (Fessl and Tebbich, 2002): nests with older nestlings have higher parasite intensity than those with younger nestlings (Fessl et al., 2006b). In accordance with previous studies (Dudaniec et al., 2006, Fessl et al., 2006a), we analyse parasite intensity (total and mean) for nests at which nestling survival was ≥ 6 d post hatching.

Predators

Introduced nest predators on Floreana Island include the black rat, *Rattus rattus*, house mouse, *Mus musculus*, cat, *Felis catus*, dog, *Canis lupus familiaris*, and smooth-billed ani, *Crotophaga ani*. Approximately 30 cats and 20 dogs are kept as pets in both the town of Puerto Velasco Ibarra and the highland agricultural zone and have free roam of the areas (J. O'C. pers. obs.). The diurnal Galápagos short-eared owl *Asio flammeus galapagoensis* Gould is the only known native predator left on Floreana Island following local extinctions of native predators such as the Galápagos hawk, *Buteo galapagoensis* Gould, and Floreana mockingbird *Mimus trifasciatus* Gould. The barn owl, *Tyto alba punctatissima* Gray, feeds mostly on rodents and not birds (Curio, 1969).

Statistical analysis

We analysed data for 2004 and 2006 only and provide descriptive data for 2005 due to low sample size caused by drought conditions. All analyses were performed using SPSS 14.0 for Windows. Summary statistics are presented as mean ± SE. Analyses were conducted on nests rather than nestlings to avoid pseudoreplication. We used ANOVA to examine inter-habitat and inter-annual variation in clutch size, fledging success, *P*.

downsi intensity, and predation. Fledging success was arcsine square root-transformed for analyses. Chi- square analyses were used to examine the stage of nest predation (i.e. eggs or nestlings) in relation to clutch size across highlands and lowlands on Floreana Island for 2004 and 2006.

4.4 Results

Philornis downsi parasitism

In highland nests, total *P. downsi* intensity was approximately four times greater than in lowland nests, and this was independent of clutch size (Table 4.2) (ANOVA: habitat: $F_{1, 43} = 21.5$, $P < 0.001$; clutch size: $F_{3,43} = 1.5$, $P > 0.2$; interaction term: $F_{1, 43} = 0.8$, $P > 0.3$). Mean *P. downsi* intensity was also significantly higher in highland than lowland nests and did not differ across clutch sizes (Table 4.1, 4.2) (ANOVA: habitat: $F_{1, 40} = 15.9$, $P < 0.001$; clutch size: $F_{3,40} = 0.4$, $P > 0.7$; interaction term: $F_{1, 40} = 0.3$, $P > 0.5$). In the lowlands, total *P. downsi* intensity was not significantly related to fledging success and did not vary across years (ANOVA: fledging category $F_{2, 6} = 3.6$, $P = 0.09$; year $F_{1, 6} = 0.03$, $P = 0.86$). We found the same pattern for mean *P. downsi* intensity (ANOVA: fledging category $F_{1, 6} = 0.13$, $P = 0.73$; year $F_{1, 6} = 0.02$, $P = 0.97$). In the highlands, total and mean *P. downsi* intensity were related to fledging success: higher *P. downsi* intensity resulted in fewer fledglings. We found no significant effect of year on total or mean *P. downsi* intensity, but an effect of the interaction term fledging category × year (total *P. downsi*: fledging category: $F_{2, 23} = 6.4$, $P = 0.008$; year: $F_{1, 23} = 1.1$, $P > 0.7$; interaction term: $F_{2, 23} = 3.4$, $P = 0.056$; mean *P. downsi*: fledging category: $F_{2, 23} = 4.3$, $P = 0.030$; year: $F_{1, 23} = 0.52$, $P > 0.8$; interaction term: $F_{2, 23} = 4.6$, $P = 0.024$).

Nest predation and brood loss

Nest predation was two-fold higher in the lowlands than highlands (Table 4.3) (Likelihood ratio = 23.8, df = 1, P < 0.001). Patterns of egg predation were comparable between habitats (Likelihood ratio = 0.094, df = 1, P = 0.76), but nestling predation was only observed in the lowlands (42.3% of all nests were depredated during the nestling phase) (Likelihood ratio = 12.8, df = 1, P < 0.001) (Table 4.3). Lowland nests with large clutch size had higher nest predation than nests with small clutch size (Likelihood ratio = 7.14, df = 2, P = 0.028). Nest predation at highland nests was not significantly related to clutch size (ANOVA: $F_{1, 27} = 0.29$, P = 0.60). The proportion of nests that were depredated in the lowlands did not differ across years (Likelihood ratio = 1.67, df = 1, P = 0.20) or highlands (Likelihood ratio = 0.06, df = 1, P = 0.81).

Clutch size and fledging success

Lowland clutch size (mean ± SE) was significantly larger (3.4 ± 0.2 eggs) than highland clutch size (2.5 ± 0.1 eggs) and did not differ significantly across years (ANOVA: year: $F_{1, 67} = 0.12$, P > 0.7; habitat: $F_{1, 67} = 26.0$, P < 0.001; interaction term: $F_{1, 67} = 6.21$, P = 0.015) (Table 4.1). The range for clutch size was 3-5 eggs in the lowlands and 2-4 eggs in the highlands. The percentage of nests to produce fledglings was comparable between the lowlands and highlands (t = -1.3, P > 0.1, df = 54) (Table 4.1). However, on average, the number of fledglings produced per nest was higher in the lowlands (3.0 ± 0.4) than highlands (1.6 ± 0.2) (t = 3.7, P < 0.002, df = 16) (Table 4.1).

Descriptive results for 2005

Fledging success was 0% in 2005, presumably due to prevailing drought conditions. No nests were found with eggs in the lowlands, but 16 highland nests had eggs (9 nests had two eggs; 7 nests had three eggs). We have data on nesting outcome for 11 out of these 16 nests: four nests were depredated, three were abandoned with eggs, one had partial brood loss (dead nestling) followed by predation, and four nests contained dead nestlings (mortality due to *P. downsi* suspected). Total *P. downsi* intensity for three *G. fuliginosa* nests in which nestlings survived ≥ 6 d was 24, 31, and 51 respectively.

4.5 Discussion

We demonstrate that environmental variation within the range of a single species can affect mortality impacts from both parasites and predators in an island ecosystem. Variation in the causes of habitat-specific mortality point to trade-offs between contrasting selection pressures that may shape reproductive investment strategies in birds (Garant et al., 2007).

Parasitism across habitats

Parasites may be more successful under particular abiotic or biotic conditions. For example, many endemic Hawaiian birds are now restricted to parts of high-elevation forest where cooler temperatures and a lack of water pools limit the mosquito vector for both malaria and pox-virus (van Riper et al., 1986, Freed et al., 2005). On Floreana Island, lowland host nesting density and parasite intensity were low, while climatic conditions were hot and dry. This was in contrast to the highlands, where host nesting

density and parasite intensity were high and conditions were wet and humid. *P. downsi* abundance may be limited in the lowlands due to lower host breeding density. This is supported by Dudaniec et al. (2009) who found a reduced number of ovipositing female *P. downsi* and higher genetic relatedness of larvae in lowland versus highland nests on Floreana Island. Molecular evidence suggests some genetic divergence in *P. downsi* on Floreana Island, possibly indicating restricted gene flow between other islands, a recent colonization event, or a distinct founding population (Dudaniec et al., 2008).

Anthropogenic habitat change (e.g. agriculture) may alter parasite distribution and impact on wild bird populations (Chasar et al., 2009). We observed higher *P. downsi* intensity in the highlands, where an agricultural zone was present, than in the lowlands (agricultural zone absent). On Santa Cruz Island, there was no difference in parasite intensity across habitats (Dudaniec et al., 2007). However this island has an extensive agricultural 'belt' across its mid-elevations (100-500 m) that may provide consistent nutritional resources and water for adult flies and may facilitate parasite dispersal between the lowlands and highlands. Agricultural areas on Floreana Island are restricted to the inner highland plains (~300 m elevation), therefore a connecting area containing adult fly resources is not present to aid *P. downsi* inter-habitat dispersal. The influence of habitat alteration on host-parasite interactions in wildlife raises many mechanistic questions (Chasar et al., 2009), while our findings point towards a potential relationship between habitat alteration and fly dispersal in the *P. downsi*-Darwin's finch system. Future studies could measure *P. downsi* intensity and habitat variables across a wider range of elevational gradients to identify the individual and combined effects of rainfall, habitat type, agricultural crops, elevation and nesting density.

Nest predation across habitats

The abundance and impact of predators can vary according to habitat and prey distributions (Martin et al., 2000, Wiles et al., 2003), potentially altering selection pressures on prey reproductive investment (Garant et al., 2007). Nest predation in *Geospiza fuliginosa* occurred more frequently in the lowlands (Table 4.2), where clutch size was larger, while clutches of 4-5 eggs were more frequently depredated than those with 3 eggs. Predation may be higher at nests with larger clutch size due to greater nest or nestling conspicuousness from auditory or visual cues (Slagsvold, 1982, Skutch, 1985). Our results support an effect of clutch size on susceptibility to predation across two contrasting habitats.

The visibility and accessibility of a nest to predators is generally associated with predation rates in birds, and has been shown to be important in Darwin's finches that inhabit highland forest (Kleindorfer, 2007b). Kleindorfer (2007b) showed that males that built well-concealed nests in Darwin's small tree finch (*Camarhynchus parvulus*) had higher pairing success, higher fledging success, and lower nest predation in the highlands of Santa Cruz. On Santa Cruz, the lowland *Opuntia* cactus is a preferred nesting substrate for ground finches and appears to confer protection from predators (Kleindorfer 2007a, Table 4.4). *Opuntia* cacti are now rare on Floreana Island, most likely because of destruction by introduced mammals and rodents (Curry, 1986, Hicks and Mauchamp, 1995). As a result of the scarcity of *Opuntia* cacti, we found just 9% (3/34 nests) of active lowland *G. fuliginosa* nests in *Opuntia.* All hatchlings from nests in *Opuntia* fledged, despite high overall lowland nest predation (44%). Loss of *Opuntia* cacti on Floreana Island may contribute to lower finch nesting density from increased predation in the

lowlands, and lower host nesting density may be linked with reduced *P. downsi* prevalence (Kleindorfer and Dudaniec, 2009).

As is common throughout island ecosystems (Martin and Menge, 2000, Loope et al., 2001), predation pressure on Floreana Island finches is largely from a suite of introduced fauna. The largely intact condition of nests following predation suggests that the predators were introduced rats, mice or smooth-billed anis because native short-eared owls rip and/or remove entire nests. Smooth-billed anis are known to be voracious predators of all life stages of birds (Gill and Stokes, 1971, Olivares and Munves, 1973) and cause mortality by attacking adult Darwin's finches in groups (Edwin Egas pers. comm.). Floreana did not historically support a native rat population, hence the avifauna are likely more vulnerable to the impacts of introduced black rats (Curry, 1986), which are common on Floreana and have been implicated in the local extinction of the Floreana mockingbird (Curry, 1986). Our results show that lowland birds on Floreana are at greater risk from introduced predators than highland birds, which are more vulnerable to parasitism.

Clutch-size variation

Conspecifics may show variation in reproductive investment across habitats subject to differing selection pressures (Garant et al., 2007). Nest predation and ectoparasites are recognised as major selective factors that shape clutch-size variation in birds (Slagsvold, 1982, Richner and Heeb, 1995). Smaller clutch sizes may confer several advantages in habitats with high nest predation as they have decreased visual and auditory conspicuousness due to reduced parental feeding visits (Slagsvold, 1982, Skutch, 1985). A larger clutch size should be advantageous in habitats with high parasite intensity as the

impact of nest ectoparasites is diluted across larger broods (Richner and Heeb, 1995, Fessl and Tebbich, 2002). We document habitat-specific patterns of reproductive investment in nests of *G. fuliginosa*. Clutch size followed the same pattern across habitats on both Floreana and Santa Cruz Islands (small in the highlands, large in the lowlands) despite significant inter-island variation in parasite intensity and rates of nest predation. The comparable pattern of clutch size may reflect similar evolutionary pressures for phenotypic clutch size variation or shared ancestry, although habitat-mediated selection may also be at play. The high levels of nestling mortality caused by introduced predators and parasites may be a sufficient selective pressure to influence changes in clutch size in future generations of Darwin's finches.

Conclusion

Island species are particularly vulnerable to the effects of invasive species because they typically existed in predator- and parasite-sparse environments prior to human settlement. Darwin's finches are known to show remarkable phenotypic plasticity in life-history traits due to climatic and habitat variation, but are not adapted to current conditions of increased predator risk or nestling parasitism. The establishment and range expansion of *P. downsi* parasites in the Galápagos Islands appears to be mediated by both biotic factors (e.g. host density) and landscape features (e.g. availability of water and agricultural crops). The impacts of parasitism are more severe for birds nesting in highland areas that support both the larval and adult stages of *P. downsi*. Furthering our understanding of how and why spatial variation in ecological factors affects invasion success on islands will assist current efforts to conserve threatened ecosystems.

4.6 Acknowledgements

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Table 4.1 Overview of *Geospiza fuliginosa* nesting outcome on Floreana Island (2004 and 2006). Brood loss refers to nestlings found dead in the nest. The causes of partial or total brood mortality are unknown, although indirect evidence suggests mortality due to *Philornis downsi.* Nests in which some or all nestlings fledged are within '% nests with fledglings'. Sample size is shown in parentheses, and percentages were calculated in relation to the number of nests with eggs with known outcomes in each habitat. Means are shown ± SE. P-values are reported from either: student's t-test analyses (t-test) or chi-squared analyses ($\chi 2$) (Likelihood ratio), and are denoted as * P < 0.05, ** P < 0.01, *** P < 0.001, or ns (not significant).

	Lowlands	Highlands	P value ($\chi 2$)	P value (t-test)
Number of nests with eggs	34	37		
				**
Clutch size (range)	3-5	2-4		
Mean clutch size	3.44 ± 0.17	2.53 ± 0.11		
% Nests with unknown outcome	3% (1)	21% (8)	ns	
% Nests with partial brood loss	5.8% (2)	10.8% (4)	ns	
% Nests with total brood loss	3% (1)	33.4% (12)	***	
Total parasite intensity	8.0 ± 1.6 (15)	39.30 ± 4.6 (24)		***
Mean parasite intensity	2.7 ± 0.7 (15)	15.8 ± 1.7 (24)		***
% Nests with fledglings	29.4% (10)	43.2% (16)	ns	
Mean number of fledglings per nest	3.0 ± 0.4 (4)	1.6 ± 0.2 (7)		***

Table 4.2 Mean *Philornis downsi* intensity (defined as the number of *P. downsi* per nestling) and range in total *P. downsi* intensity (defined as the number of *P. downsi* per nest) in *Geospiza fuliginosa* for each clutch size (with nestlings ≥ 6 d old) observed across habitats (lowlands, highlands) for 2004 and 2006 (sample size). N = sample size of nests.

	Lowlands		Highlands	
Clutch Size	Mean intensity ± SE (N)	Total intensity range	Mean intensity ± SE (N)	Total intensity range
2			16.9 ± 2.7 (13)	35-54
3	1.1 ± 0.42 (9)	0-7	14.6 ± 2.9 (11)	8-78
4	3.8 ± 1.25 (8)	8-23	14.0 ± 2.65 (4)	36-72
5	1.3 ± 1.3 (3)	0-13		

Table 4.3 Type of nest predation in *Geospiza fuliginosa* across the lowlands and highlands on Floreana Island for 2004 and 2006. Sample size is shown in parentheses, and percentages were calculated in relation to the number of nests with eggs with known outcomes in each habitat (23 nests in the lowlands; 17 nests in the highlands). P-values are reported from chi-squared analyses (Likelihood ratios) to test for differences between habitats. Significance is denoted as: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	Lowlands	Highlands	P value
% Depredated (total nests)	43.8% (14)	17.2% (5)	**
% Egg predation	14.3% (4)	17.2% (5)	ns
% Nestling predation	42.3% (11)	0% (0)	***
% Abandoned with eggs	30.4% (7)	0%	**
% Abandoned with nestlings	0%	0%	ns

Table 4.4 Overview of clutch size and nesting success in *Geospiza fuliginosa* in the lowlands and highlands of Santa Cruz Island (from Kleindorfer 2007a) from 2000 to 2004, and Floreana Island in 2004 and 2006. Data for Santa Cruz Island parasite intensity in *G. fuliginosa* (1998-2005) are from Dudaniec *et al.* (2007). For some nests, nesting outcome was unknown.

	Santa Cruz Island		Floreana Island	
	Lowlands	Highlands	Lowlands	Highlands
Mean clutch size	3.54 ± 0.18	2.50 ± 0.10	3.44 ± 0.17	2.53 ± 0.11
Predation	5%	52%	43.8%	17.2%
Total parasite intensity	40.4 ± 6.0	29.3 ± 3.44	8.0 ± 1.60	39.3 ± 4.60
Partial brood loss	30%	6%	5.8%	10.8%
Total brood Loss	8%	12%	3%	33.4%
Fledging success	85%	32%	29%	43%

5 Video analysis of host-parasite interactions in Darwin's finch nests.

Jody A. O'Connor, Jeremy Robertson, and Sonia Kleindorfer.

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5.1 Abstract

Parasites place their hosts under strong selection for adaptive traits that increase parasite resistance. The initial impact of invasive parasites has rarely been observed and can be particularly strong on naïve hosts with limited prior exposure to parasites. *Philornis downsi* is an introduced fly to the Galápagos Islands whose parasitic larvae cause high mortality in Darwin's finch nestlings. We used a within-nest camera system and nest monitoring data to examine this new host-parasite interaction in the wild. Many *P. downsi* flies entered finch nests with incubated eggs or nestlings, but only when parent finches were not present. Parasitic *P. downsi* larvae were observed to emerge from the nest-base at night to feed both internally and externally on nestlings. Adult and nestling Darwin's finches exhibit grooming and avoidance behaviours in the presence of *P. downsi* parasites. Specifically, in nests with high parasite intensity, nestlings increased self-preening behaviour, ate larvae, and stood on top of one another. Female finches probed into their nestling's nares (1st instar larvae reside in the nares) and probed into the nest base (2nd and 3rd larvae reside in the nest base during the day). These findings shed light on the emergence of anti-parasite behaviour as well as host/parasite relationships after recent parasitism in a naïve host.

Key words: bird, camera, larvae, mortality, nest, *Philornis downsi*, preening, video

This paper contains supplementary material that can be found online at http://journals.cambridge.org/action/journalAllSuppMaterial?jid=orx

5.2 Introduction

Birds can develop and adapt their parasite defences under long-term, continued exposure to a particular parasite (Jarvi et al., 2001, Foster et al., 2007), but can be extremely vulnerable on initial contact with a novel parasite (Warner, 1968, van Riper et al., 1986). Island taxa are particularly vulnerable to introduced pathogens because they evolve in isolated, often pathogen-depauperate environments, with little need for defences until parasites and diseases are introduced (Wikelski et al., 2004). The majority of infectious avian pathogens in the Galápagos Islands have been introduced via the importation of poultry and pigeons (Gottdenker et al., 2005, Wiedenfeld et al., 2007). Of the ~34 avian pathogens currently identified in the Galápagos (Fessl et al., 2001, Wikelski et al., 2004, Dudaniec et al., 2005, Gottdenker et al., 2005, Soos et al., 2008), the invasive parasite *Philornis downsi* presents the most imminent threat to the survival of Darwin's finches. In 1997, blood-filled larvae of the introduced fly, *P. downsi*, were discovered in the nests of Darwin's finches (Fessl et al., 2001). Retrospective examination of insect collections has found that the fly was present in the Galápagos Islands as early as 1964 (Causton et al., 2006). Adult *P. downsi* flies are vegetarian and lay their eggs in bird nests, where the three larval stages are free-ranging and feed on the blood and tissues of nestling birds (Figure 5.1) (Fessl and Tebbich, 2002, Dudaniec and Kleindorfer, 2006, Fessl et al., 2006b). On average, finch nests are infested with 30-50 *P. downsi* larvae (Fessl and Tebbich, 2002, Dudaniec et al., 2007), but up to 182 parasites have been found in a single nest (Fessl and Tebbich, 2002). For Darwin's finches, the fitness costs of *P. downsi* are severe, with 16-95% brood mortality from 1998-2008 (Dudaniec and Kleindorfer, 2006, Fessl et al., 2006b, Huber, 2008), reduced blood haemoglobin concentrations (Dudaniec et al., 2006), multiple body wounds and infections, substantial blood loss (18-55%) (Fessl

et al., 2006b), grossly deformed nasal openings (nares) (Galligan and Kleindorfer, in press), and reduced growth rates and fledging success (shown experimentally)(Fessl et al., 2006a). Host responses such as increased parental care and nestling defensive behaviours are yet to be tested between Darwin's finches and *P. downsi* (Huber, 2008), but may represent an important dynamic in this new host-parasite interaction. For example, when parasitism is specific to the nestling phase of the host, critical anti-parasite defences are usually underdeveloped (Lung et al., 1996, Smits and Bortolotti, 2008) and host parents typically provide extra care in the form of increased preening and feeding (Christe et al., 1996, Tripet and Richner, 1997, Hurtrez-Boussès et al., 1998, Pacheco et al., 2008).

The detrimental impacts of *P. downsi* on Darwin's finches are well documented, but because larval parasitism occurs within finch nests at night (Fessl et al., 2006a) we have very few observations of the host-parasite interaction in the wild. Developing effective control methods requires a more detailed understanding of within-nest activity such as the fly's reproductive characteristics, larval feeding strategies, and finch anti-parasite defences. Meanwhile, the threat this parasite poses to endemic birds is steadily increasing Since first being discovered on Santa Cruz Island, *P. downsi* has now spread to 12 Galápagos islands (Wiedenfeld et al., 2007 also Sarah Huber & Rosemary Grant personal communication), and larvae have been found in 64-100% of nests (Fessl and Tebbich, 2002, Fessl et al., 2006b, Dudaniec et al., 2007, Huber, 2008) of 11 of the 14 species of Darwin's finches in the Galápagos (Wiedenfeld et al., 2007). Here, we use infra-red video cameras inside nests to monitor fly visitation and finch responses to the presence and activity of the fly and larvae. We provide the first observational data of within-nest interactions between Darwin's finches and *P. downsi* in the wild.

5.3 Methods

Study area and study species

Flies and finches were studied at the height of the finch breeding season between February and April 2008 in the arid zone (00°44 S, 090°18W) of Santa Cruz Island and both the arid (01°16S, 090°29W) and humid highland (01°17 S, 090°27W) zones of Floreana Island. We monitored nests of three common species that have comparable inter-species variation in *P. downsi* intensity (number of parasites/nest) (Dudaniec et al., 2007): the small ground-finch *Geospiza fuliginosa*, medium ground-finch *G. fortis*, and small tree-finch *Camarhynchus parvulus.* The location, general characteristics and video recording details of each nest are shown in Table 5.1.

Video monitoring system

We monitored nest activity with a battery-powered video monitoring system that included four cameras, a multiplexer and a digital video recorder (DVR). Each of the Jaycar monochrome CCD security cameras were fitted with two infrared light-emitting diodes with shaven ends to diffuse light more evenly within the nest. This light is not visible and does not affect nest activity or predation (Delaney et al., 1998, Pierce and Pobprasert, 2007) but enables cameras to function day and night. A 15mm diameter hole was cut through the roof material of the dome-shaped nests to insert the camera lens and infrared LEDs (lights), leaving the small camera body (60g) outside and supported by remaining roof material. Camera insertion caused no structural damage, gaps were sealed with waterproof material, and video and power cables were firmly secured to branches to avoid weighing down the nest. Each camera was connected by video cable to a

multiplexer that combines up to four signals into a single quad split-screen input recorded onto an Archos 605 DVR that was programmed to record continuously in two-hour segments. DVR and cable malfunctions interrupted video recordings between 1 and 12 hours at different nests (Table 5.1), and in a complete DVR breakdown a Sony digital camcorder was used to record an LCD monitor displaying the camera outputs. The remoteness of our field sites prevented the possibility of repair or equipment replacement. Recordings were downloaded to an Apple MacBook Pro laptop for later analysis with Quicktime Pro 7.4.

Parasite intensity

The intensity of *P. downsi* per nest was determined using established methods (Fessl and Tebbich, 2002, Dudaniec et al., 2006). Empty nests or those containing dead nestlings were considered inactive and were removed from the nesting tree, sealed in plastic bags, and later dismantled. All larvae, pupae and pupae cases were preserved in 95% ethanol and summed for total *P. downsi* intensity.

All parasite/host behaviours were counted from either one-hour of video recording, or if there was sufficient video footage, behaviour frequency was averaged over two randomly selected hours during the day and/or night. Table 5.2 provides an overview of the behaviours inside the nest that were observed and quantified for *P. downsi* flies, fly larvae, nestlings, and parent birds. For statistical analysis, nests were categorised according to *P. downsi* intensity and nestling age. The three categories of total *P. downsi* intensity were classed as: low (0-9), medium (18-25), and high (52-74). No nests contained a parasite intensity that was either between or above these categories. Nestlings

were classed as: young (1-4 days) or old (>7 days). We did not film any nestlings aged 5-7 days because either (1) nestlings had died before day 5, or (2) cameras were placed in nests when nestlings were already >7 days old.

5.4 Results

Adult fly activity

Of the nests monitored, *P. downsi* flies were videoed entering one of two nests with incubating eggs and seven of nine nests with nestlings (Table 5.2). No fly activity was observed in: (1) a nest with recently abandoned eggs; (2) in the hour before or 24 hours after nestlings had fledged from 4 nests; (3) 1 nest with 10 day old nestlings in the Floreana arid zone, where *P. downsi* intensity was low (8 larvae in the nest). Flies walked over all inner nest surfaces and remained in a nest for up to ten minutes. Mean duration of fly activity in nests was 1.34 minutes (± s.e. 0.43), and was similar for nests with eggs and nestlings. Flies were only observed entering nests with young nestlings during the day (mean entry 0.63 h^{-1} ± s.e. 0.18, n=8), and nests with old nestlings during the night (mean entry 1.6 h^{-1} ± s.e. 1.6, n=5), when adult finches were absent. Finches did not display fly-repelling behaviours (Hart, 1997). White eggs were observed at the rear of female flies in two nests, and were deposited on the base of a nest with eggs and a nest with 2-3 day old nestlings. Oviposition probably occurred in all eight nests with fly activity, but could not always be confirmed due to the angle of our camera lens. Flies barely touched nestlings or finch eggs (max 3-second contact per nest visit). We did not observe egg-laying directly on the nares of nestlings where 1st instar larvae are first found (Fessl et al., 2006b) but 13 fly eggs were found clumped on the naris of a <1 day old

chick in an unfilmed nest. *Philornis* spp. larvae can hatch within a few hours following hatching of host eggs (Spalding et al., 2002) and navigate to the nares of nestlings to begin feeding hence it may not be necessary for flies to lay their eggs directly on the nares of nestlings. Fly mating was not observed.

Larval activity

Larvae were only observed in nests with nestlings, and were not observed in nests during the 24 hours post fledging. Larval activity was observed at the surface of the nest base between nightfall (1800hrs) and sunrise (0600hrs) (Video 1) when parent finches did not visit the nest, although larvae were occasionally seen for short periods during the day. Larvae were observed crawling over and between young nestlings that were being brooded by their mother at night. A maximum of 40 large larvae were seen emerged from the base of nests with old nestlings at any one time. Larvae spent a mean 14.3 minutes squeezing in and out of nares (n=17 larvae, 2 nests) (Video 2), and a maximum of 5 large larvae emerged from the nares of one nestling within a 10-minute period. The larvae had presumably resided in the nestling for at least one hour, as the larvae were not observed externally. Larvae attached to nestlings for external feeding for 1-3 minutes, and entered nares of nestlings an average 2.5 times per hour (± s.e. 0.5, n=2) (Table 5.2).

After killing one >8 day old nestling, larvae ate a hole through the rear of its body and consumed most of its internal tissues within two hours (saprophagous feeding). After this time, the larvae moved away from the dead nestling and congregated around the feet of the surviving nestling, after which the nestling perched at the nest entrance.

Nestling evasive behaviour

Night-time nestling evasive behaviour could be quantified from two nests with >8 day old nestlings that had markedly different parasite intensities. In a nest with low *P. downsi* intensity (8 parasites in a nest with 4 nestlings, Floreana arid zone), the nestlings spent 98% of the night resting, 2% repositioning, and preened themselves an average of once per hour. In contrast, in a nest with high *P. downsi* intensity (74 parasites in a nest with 2 nestlings, Santa Cruz arid zone), nestlings spent 10% of the night resting, 90% repositioning, and preened themselves a mean of 28.5 times per hour (Video 3). Furthermore, in the nest with high parasite intensity the older nestling frequently trampled on top of the younger nestling (whilst alive, and for two hours after its death), forming a 'buffer' between itself and the larvae (Table 5.2). On one occasion, a nestling (>8 day old) was observed to pick a larva from under its wing and eat it. Nestlings used their beaks for preening but were never observed to use a foot to scratch their heads and reach ectoparasites inaccessible to their beaks (Moyer and Clayton, 2003).

Parental care

Female finches preened their nestlings' feathers, probed within nestlings' nares (Video 4), probed nest material (Video 5), and probed between nestlings probably in an attempt to remove larvae from the nest (Table 5.2). Female finches will remove dead nestlings from the nest (Jody O'Connor pers. obs.), which would also discard any larvae in the nestling. There was no significant correlation between *P. downsi* intensity and the rate at which parents fed their nestlings (Spearman's rank order correlation $r=0.25$, $n=8$, $P>0.5$).

Parasitism and fledging success in filmed nests

In the nine nests, *P. downsi* intensity ranged from 4-74 parasites per nest (mean 27.13, ± s.e. 8.5) and only 20.8% of nestlings fledged (5/24). However the relationship between parasite intensity and fledging success was not clear-cut (χ^2 =1.2, df=8, P=0.15). No fledglings left the nest before the expected minimum 14 days. Of the five fledglings, four were from a nest with only eight larvae, while only one fledgling survived from a heavily parasitised nest (74 parasites), presumably because it perched on top of its younger sibling before and after it died. Only one of the nine nests was free of *P. downsi* larvae and pupae and those chicks were found dead and covered with fire ants (*Wasmannia auropunctata)* within a day of hatching. It is possible that larvae were removed by ants in this nest, but cannot be confirmed because video recording stopped before the nestlings died. In another nest, small ants were seen removing small *P. downsi* larvae from nesting material during the day and large ants were seen inspecting nares of live and dead chicks and removing small larvae at night.

5.5 Discussion

Our new observations on the behaviour of *P. downsi* flies, larvae, Darwin's finch nestlings, and parental care will facilitate strategies to control *P. downsi*.

Most nests had multiple fly visitations throughout the finch incubation and nestling period (Table 5.2), which would contribute to high parasite numbers from several flies accumulating within the same finch nest. *P. carinatus* and *Protocalliphora* botflies have similarly been observed to randomly enter and oviposit in bird nests regardless of host nestling age (Gold and Dahlsten, 1983, Young, 1993). Recent microsatellite analyses

provide genetic evidence that up to five *P. downsi* females contribute to the larvae within a single nest (Dudaniec et al., 2008).

Birds can reduce the impact of high ectoparasite intensity by preening (Cotgreave and Clayton, 1994). Female finches directed anti-parasite behaviour at areas of larval infestation by using their beak to probe: (1) directly into the nest base; and (2) within the enlarged nares and between feathers of parasitised nestlings. Nestlings rely on maternal anti-parasite defences for at least the first four days after hatching, when they are blind, featherless and have rudimentary motor control skills. Subsequently, older nestlings (>8 day old) had to undertake their own anti-parasite behaviours because their parents did not visit the nests at night. Adults did not alter feeding rates to compensate for the effects of parasitism, which contrasts with studies of blue tits (*Parus caeruleus*) in which parents increased food provisioning to parasitised nestlings (Christe et al., 1996, Tripet and Richner, 1997, Hurtrez-Boussès et al., 1998). We recommend further within-nest studies of Darwin's finches to examine the role of host species, island, and parasite intensity on host-parasite behaviours.

Larvae of most *Philornis* species are subcutaneous feeders, which feed under the skin of nestling hosts (Dudaniec and Kleindorfer, 2006). *P. downsi* larvae are known as free-living semi-haematophagous feeders that feed externally on their host (Dudaniec and Kleindorfer, 2006), but our study has found that they also enter through the nares of nestlings to feed internally. Repeated larval movement through the nares is no doubt the cause of the gross enlargement of nasal openings observed in Darwin's finches (see Fessl *et al*. 2006b; Galligan and Kleindorfer, in press). In addition to external evidence of naris

damage, we found many nestlings with an empty, cavernous inner beak (Figure 5.1), devoid of a nasal septum, and lacking the ciliated, mucosa-covered turbinate projections that increase surface area to humidify respired air (Geist, 2000) and filter large particulate matter, which may increase the likelihood of dehydration during expiration and contracting respiratory diseases. Beak deformation is also associated with high ectoparasite infestations and decreased preening efficiency in studies of other bird species (Ledger, 1969, Clayton, 1991, Clayton et al., 1999).

A nestling's beak is essentially its only means for removing larvae: once larvae have entered the nares, nestlings are unable to prevent or obstruct their progress. Nestlings weakened by blood loss and constant repositioning often collapsed, and subsequently their beak or face rested on the nest base, which facilitated larval attachment to the nape or entry into the nares. After a night attempting to avoid consumption by larvae, weaker nestlings may be unable to effectively beg for food when competing with stronger nestlings, and thus further lose body condition. Simon *et al*. (2003) showed that weakened blue tit nestlings with lowered immunocompetence attract more feeding attacks by fly larvae, providing evidence for the 'tasty chick hypothesis'. Larval preference for weaker Darwin's finch nestlings could therefore select for the survival of nestlings that have strong immune defences and are competent at avoiding parasite attachment/naris entry.

5.6 Conclusion

Newly formed avian host-parasite systems are commonly characterised by large ectoparasite numbers with high fitness costs to the host (Clayton, 1991). This pattern is being found in the effects of the recently introduced *P. downsi* on Darwin's finches in the

Galápagos archipelago. The past ten years of research have documented the spread of the parasite (Wiedenfeld et al., 2007), parasite intensity and finch mortality (Dudaniec et al., 2007) and here we provide detailed observations of the behaviour of parasites and the responses of nestlings and parents. It is clear that if Darwin's finches are to persist the fly needs to be controlled or eradicated.

5.7 Acknowledgements

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Table 5.1 Description of nests fitted with in-nest cameras on Santa Cruz (SC) or Floreana (F) Island in 2008.

Species	G. fortis	C. parvulus	***G. fuliginosa***								
Nest number (ID)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
# Young chicks in nest	-	2	-	-	3	2	4	2	-	-	6
# Old chicks in nest	2	-	-	-	-	-	-	-	2	4	-
Eggs (incubated or unhatched)	-	1	-	5	2	2	-	-	-	-	-
Eggs (abandoned)	-	-	3	-	-	-	-	-	-	-	-
Hours of footage	55hr	1hr	40 hr	1hr	14 hr	14 hr	6hr	4 hr	2hr	12hr	12hr
Footage taken during Day or Night	Day/ Night	Day	Day/ Night	Day	Day/ Night	Day/ Night	Day	Day/ Night	Day	Night	Night
Island	S C	F	SC	F	F	F	F	F	F	F	F
Habitat	Low	High	Low	High	High	High	High	High	Low	Low	Low
Total *P. downsi*	74	N/A	0	1	21	22	52	33	4	8	18

Table 5.2 Overview of Darwin's finch and *P. downsi* host/parasite interactions observed in video during the day and night. We filmed at two nests with eggs and nine nests with nestlings (total No. nests=11). We have identified the number of nests at which each behaviour was observed. Corresponding nest ID details and nest characteristics are given in Table 5.1.

	Actor	Activity	No. nests	ID of nest(s)	Frequency (Mean/hr)	Duration (Mean mins/hr)
Day	Adult finch	**Probe nest**	**6**	**1,2,5,6,8,11**	**4.3**	
		Nest sanitation	**3**	**5,6,11**		**5.7**
		Preen chick feathers	**2**	**1,11**	**23.5**	
		Preen chick nares	**1**	**1**	**11**	
	Adult fly	**Enter nest (day)**	**6**	**2,4,5,7,8,11**	**1.2**	
		Land on chick	**5**	**2,4,5,8,11**	**1**	
		Land on eggs	**3**	**2,4,5**	**1**	
		Land on nest material	**7**	**2,4,5,7,8**	**1**	
		Carry egg sack	**2**	**2,8**	**1**	
Night	Adult finch	**Probe nest**	**4**	**5,6,8,11**	**17**	
	Finch nestling	**Self preen**	**2**	**1,10**	**30.5**	
		Stand on top of sibling(s)	**1**	**1**	**10**	**9.6**
	Adult fly	**Enter nest (night)**	**1**	**1**	**8**	
		Land on chick	**1**	**1**	**4.5**	
		Land on nest material	**2**	**1,11**	**1**	
	Fly larvae	**Enter chick nares**	**2**	**1,7**	**1.8**	
		Minutes feeding in naris	**2**	**1,7**		**16.4**
		Minutes attached to chick	**3**	**1,5,11**		**1.9**

5.8 Supporting Information

The following Supporting Information is available for this article online at the following two addresses:

http://bioweb.bio.flinders.edu.au/ocon0124/

http://journals.cambridge.org/action/journalAllSuppMaterial?jid=orx

These videos are from a nest containing two >8 day old *G. fortis* nestlings, 10-11 February 2008 on Santa Cruz Island.

Video 1

High *P. downsi* larval activity in the nest base (time: 2000 hours).

Video 2

P. downsi larvae emerging from the naris of a nestling (time: 0430 hours).

Video 3

Nestling preening its wing and eating a *P. downsi* larvae (time: 1930 hours).

Video 4

Female finch probes into nares of nestling (time: 0730 hours).

Video 5

Female finch probes into inner base of nest where larvae are found (time: 0800 hours).

Figure 5.1 Recently deceased nestling with larval damage to beak. 1st instar larvae present feeding in beak cavity.

6 Begging does not signal need in parasitised Darwin's finch chicks, but it does stimulate parental feeding

Jody A. O'Connor, Jeremy Robertson, and Sonia Kleindorfer.

IBIS (in review)

6.1 Abstract

We tested predictions of the parental food compensation hypothesis. The hypothesis rests on observations that altricial chicks can experience small or negligible impacts of hematophagous nest parasites, and the prediction is that parents increase their provisioning to compensate for the costs of parasitism. One possible mechanism for increased parental feeding is chick begging behaviour, which may be an honest signal of need to parents and therefore trigger increased feeding. We used an experimental approach to test whether the presence of *Philornis downsi* fly larvae in nests of Darwin's small ground finch (*Geospiza fuliginosa*) was associated with increased chick begging intensity, higher parental food provisioning, and reduced chick size and growth. To determine parental food allocation to individual chicks in relation to strength of begging behavior, we used video cameras to record within-nest activity in two experimental groups: naturally parasite infested (mean 27 parasites) and experimentally parasite-free (pyrethrin-treated, mean 0.2 parasites). Chicks were measured for body size and mass every second day until they died or fledged from the nest. We observed no difference in chick begging intensity across treatments. Parents did not increase the frequency of feeding visits to parasitised nests, but instead regurgitated food into parasitised chick beaks nearly twice as often as they did for parasite-free chicks per feeding visit. Strongly begging chicks were allocated more food. Parasitised chicks did not experience reduced body mass, size or growth rates compared to parasite-free chicks, which supports the hypothesis that increased parental food provisioning can maintain growth rates for parasitised chicks. Despite the compensatory effect of increased parental feeding on chick growth rates in parasitised nests, chicks died after a mean 3.2 days with *P. downsi* parasitism causing 80% of chick mortality. Ultimately, we show that *P. downsi* larval feeding is costly and prompts adaptive responses in parent birds, which are not sufficient to compensate for the negative impacts of parasitism.

6.2 Introduction

Hematophagous nest parasites such as fly larvae, fleas and mites consume the blood of altricial chicks, and can reduce the growth, health, and fledging success of their hosts (Möller, 1990, Richner et al., 1993, Hurtez- Boussès et al., 1997, Morrison and Johnson, 2002, Dudaniec et al., 2006, Fessl et al., 2006a). But the effects of nest parasites can be highly variable and sometimes negligible (Gold and Dahlsten, 1983, Roby et al., 1992, Johnson and Albrecht, 1993, Rendell and Verbeek, 1996, Miller and Fair, 1997, Thomas and Shutler, 2001), especially when biotic conditions and host adaptations reduce parasite success (see Clark and Mason, 1988, Merino and Potti, 1996). For example, the parental food compensation hypothesis predicts that when there are sufficient resources, parents increase food provisioning to their young to compensate for the costs of parasitism and thereby maintain chick growth rates (Johnson and Albrecht, 1993, Tripet and Richner, 1997, Tripet et al., 2002). Food delivery to chicks can be underestimated in the absence of within-nest video monitoring, especially for multiple prey loaders While the parental food compensation hypothesis can be generally tested by monitoring feeding visits to nests, complementary hypotheses that predict parental allocation decisions are important to understand food distribution per chick, and therefore partial nesting success.

We use Darwin's small ground finch (*Geospiza fuliginosa*) on Floreana Island, Galápagos Archipelago as a model system to test predictions of the parental food compensation hypothesis. Darwin's finches experience high fitness costs from introduced *Philornis downsi* fly larvae parasites, which reside in the nest material and emerge to feed on the blood and flesh of developing chicks (Fessl et al., 2006b, O'Connor et al., 2010b). Chick mortality due to parasitism varies between 13-100% (Fessl and Tebbich, 2002, Dudaniec and Kleindorfer, 2006, Fessl et al., 2006a, Dudaniec et al., 2007, Huber, 2008, O'Connor et al., 2010a, O'Connor et al., 2010d), but interestingly it is not always predicted by the number of parasites

per nest (Huber, 2008, O'Connor et al., 2010a, O'Connor et al., 2010b). For example, some chicks will fledge from heavily infested nests containing 60-90 larvae, whereas others die in nests containing fewer than 20 larvae (O'Connor et al., 2010b, O'Connor and Kleindorfer unpublished data) We also found considerable variation in age of death for sibling chicks under equal levels of parasitism (O'Connor and Kleindorfer unpublished data, Fessl & Tebbich, 2002), indicating that some chicks are able to survive the effects of *P. downsi* longer than their siblings. The differential mortality of parasitised chicks may be explained by variation in levels of parental food provisioning if increased feeding leads to increased survival. We predict that food distribution to individual parasitised chicks should depend on sibling conflict as expressed by the strength of begging behaviour (see Kilner and Johnstone, 1997). If heavily parasitised individuals are weakened and reduce the intensity of their begging behaviour, they should receive less food from their parents (see Christe et al., 1996). Chicks that beg strongly should be allocated more food by their parents and therefore survive the effects of parasitism longer than weaker siblings. In addition to sibling rivalry for parental allocation, theory predicts conflict between the parents about who will provide costly parental care (Trivers, 1972, Dor and Lotem, 2010).

We use within-nest video to monitor parental food delivery to chicks in naturally parasitised and experimentally parasite-free nests of Darwin's small ground finch. We compare chick growth, development, and mortality per day in nests with and without parasites, and the effect of parasite intensity on finch nesting success. We predict: (1) increased food provisioning at parasitised nests; (2) more parental feeding of strongly begging chicks; and (3) the age of chick death will correlate with parental food provisioning.

6.3 Methods

Study site and species

Nests were monitored in Feb to April, 2010 at two study sites on Floreana Island, Galápagos: in lowland scrub around the township of Puerto Velasco Ibarra (1º 16'28S, 90º 29'13W); and in highland forest at the base of Cerro Pajas volcano (1º 17'46S, 90º 27'06W) (sites fully described in O'Connor et al. (2010a)). The small ground finch is common on elevated islands of the archipelago (Grant, 1999, Kleindorfer, 2007a) and is the most abundant finch on Floreana Island (O'Connor et al., 2010c). They tend to build nests in lower vegetation (1-4 m), which were accessible for the nest-camera surveillance. We recorded rainfall at an elevation of 6m in our lowland study site (1º 16'20.5" S, 90º 29'16.5" W) and 343m in our highland study site (1º 17'48.4" S, 90º 27'07.0" W) between February 19 and April 14. There was comparatively high rainfall in 2010, lowland: 306mm, highland: 504mm. Chicks of most Darwin's finches feed on seeds and caterpillars (Grant, 1999), both of which were abundant due to the high rainfall during this study (O'Connor pers obs).

Philornis downsi life cycle

The adult fly life-stage of *P. downsi* is vegetarian and only the larval stage is parasitic to birds (Fessl et al., 2006b). Multiple *P. downsi* flies will enter active finch nests (containing eggs or chicks) and lay eggs on inner nest surfaces when parent Darwin's finches are absent (Dudaniec et al., 2010, O'Connor et al., 2010b). *P. downsi* eggs hatch into parasitic larvae, which in 1st and early 2nd instar stages feed within the nares of chicks (Fessl et al., 2006b). Late instar larvae (2nd and 3rd) reside in the nest base during the day, and emerge at night to feed on the blood and tissues of chicks by external attachment and by entering through the nares to feed internally (O'Connor et al., 2010b). Larvae pupate in the nest base after 4-7 days of feeding on chicks (O'Connor and Kleindorfer unpublished data) and emerge as flies after

7-18 days (P. Lincago and C. Causton, unpublished data). The within-nest video showed that parasitised chicks can appear healthy right up to their death; we observed chicks to die after one night of intense larval parasitism (O'Connor et al 2010b, and this study). Dead chicks are commonly found with flesh wounds, damage to vital internal organs, open body cavities sometimes devoid of any blood or flesh, enlarged nares and loss of internal beak structure (Fessl and Tebbich, 2002, Fessl et al., 2006b, Huber, 2008, O'Connor et al., 2010b, O'Connor et al., 2010d).

Nest monitoring

We located 22 active small ground finch nests in the highlands, and one nest in the lowlands. We monitored nests every day to determine the nesting activity and the age of chick death. The average nestling period for Darwin's finches is 14 days (Grant, 1999). After all chicks had died or fledged from a nest, the nesting material was dismantled and all *P. downsi* larvae, pupae and pupae cases were counted to calculate the total number of parasites per nest (see Fessl and Tebbich, 2002, Dudaniec et al., 2006). Chicks that had recently died were immersed in alcohol so that larvae feeding within the nares and body wounds would float out and could be counted. The mean number of parasites per chick was calculated by dividing the total number of parasites in the nest by the brood size. Parasite intensity could not be determined in two nests: one nest was completely depredated (whole nest was missing) by an owl, the other nest was depredated by fireants (*Wasmannia auropunctata*) (N=1), which remove *P. downsi* larvae from the nest (O'Connor et al., 2010b)

Chick growth and begging behaviour

Bird and parasite activity was monitored using video cameras at 16 nests in 2010, nine were experimentally parasite-free and seven were naturally parasitised. The nine parasite-free nests were treated by removing the 1-2 day old chicks while the nest interior was sprayed with 1%

pyrethrin solution, which is non-toxic to birds. The pyrethrin insecticide virtually eliminates larvae that are already present in the nest (Fessl et al., 2006a) and prevents further infestations because *P. downsi* flies do not enter treated nests (O'Connor pers obs). The sole lowland nest (brood size =4) was included in the parasite-free group. The seven parasitised nests were naturally infested with *P. downsi*; in this group, we removed 1-2 day old chicks and sprayed the nest interior with water.

To film within nests, the lens of a small surveillance camera was inserted through the roof of each nest to continuously record all within-nest activity, until the chicks either died or fledged. We used an Archos 605 180GB media device and Archos 5 250GB media tablet with the Archos DVR station (O'Connor et al., 2010b). Chicks in videoed nests were uniquely marked by colouring parts of the beak and toes with non-toxic black marker. These marks allowed us to identify individual chicks when quantifying begging intensity and parental care from video recordings, and for identification of individuals when taking morphological measurements. In 2010, all chicks in 14 small ground finch nests (six parasitised and eight parasite-free) were measured every second day for the following measurements: body mass (grams), naris diameter, wing length, tarsus, beak length and beak tip-back of head (beak-head). Growth rates were determined by calculating the change in mass for individual nestlings over the two days since the last measurement. To determine intra-brood variance in chick mass, we calculated the difference between the lightest and heaviest chick in each brood at day 4 from hatching.

Chick begging and parental care behaviour was quantified from video recordings of the first five feeding visits of the day in nests that contained 3-5 day old chicks. We calculated the frequency of parental visits to the nests per hour and quantified the number of feeds provided to each chick per feeding event. For every feeding event, each chick was assigned to one of three begging intensity categories: (1) weak - chick's body was not extended and gape

opened <30% of maximal capacity; (2) medium - chick's body was partially extended and gape opened ~50-70% of maximal capacity; or (3) strong - chick's body was fully extended and gape opened to 100% of maximal capacity. To determine intra-brood variance in food allocation for each feeding event, we calculated the difference between the least and the most number of feeds provisioned to a single chick within the brood. To estimate the mean length of a food transfer (a single feed to one chick), we divided the length of the entire feeding visit to the nest (in seconds) by the total number of feeds to all chicks during that event.

Statistical analyses

All analyses were performed with SPSS 17.0 for Mac. An independent samples t-test was used to assess the effectiveness of pyrethrin for reducing parasite intensity. Analyses of morphological measurement, growth rates and number of feeds to chicks were on mean values from each nest to avoid pseudoreplication. We examined variation in morphological measurements for six traits (body mass, naris diameter, wing length, tarsus, beak length and beak-head) using MANOVA, with treatment (parasitised or parasite-free) and chick age as fixed factors. We square root- transformed the values for (1) total food allocation to brood per feeding event (total parent beak to chick beak transfers), (2) intra-brood variance in chick mass, and (3) intra-brood variance in food allocation per feeding event to meet assumptions of normality. We used two-way between-groups ANOVAs to examine the effect of nest treatment and brood size (fixed factors) on the intra-brood variance in food allocation and intra-brood variance in chick body mass. We used a chi-squared test to examine the relationship between begging behaviour using two categories: (1) less frequent strong begging (<50% of cases) versus frequent strong begging (50% of cases) and allocation of the first feed by a parent using three categories: (1) never fed first, (2) fed first ≤ 50% of cases, and (3) fed first >50% of cases). We also used chi-square to test the effect of nest treatment and brood size on begging behaviour using two categories: (1) begs strongly <50% of cases, and (2) begs

strongly ≥50% of cases). We used two-way between-groups ANOVAs to examine the effect of nest treatment and brood size (fixed factors) on: (1) the number of feeds allocated to the whole brood per feeding event, (2) mean length of feeding visits (seconds), and (3) length of food transfers. We used a two-way ANOVA to examine the relationship between the percentage of events that a chick begged strongly (dependent variable) and survival (fixed factor) 2 categories, chick died: (1) <5 days from hatching, or (2) 5-9 days from hatching) with nest number entered as a covariate.

6.4 Results

Chick begging intensity and parental care in videoed nests

P. downsi was virtually eliminated from parasite-free nests, which contained significantly fewer parasites (mean 0.2 ± s.e. 0.2) than naturally parasitised nests (mean 27 ± s.e. 26.38) (t_{17}=3.9, p=0.001)(Table 6.2). There was no significant difference in begging intensity across treatments (χ^2=0.76, df=1, p=0.76): parasitised chicks exhibited strong begging behaviour in 56% of cases, and chicks from parasite-free nests begged strongly in 58% of cases. In both treatments, strong beggers were fed first more frequently (Likelihood ratio=12.09 df=5, p=0.034) and were allocated more food per feeding event (ANOVA, $F_{1, 45}$=5.95, p=0.019)(Figure 6.1). Parasitised chicks that begged strongly were more likely to survive to at least 5 days after hatching (ANOVA: nest: $F_{1,22}$=4.6, p=0.044; survival category $F_{1,22}$=9.7 p=0.005), although all parasitised chicks died by 9 days after hatching. Begging intensity was not affected by brood size (Likelihood ratio=4.1, df=3, p=0.25).

Parents visited nests to feed chicks about three times per hour in both treatments (Table 6.1), but female parents did so more often than males in both treatments (ANOVA: treatment $F_{1,25}$=0.5, p=0.82, sex $F_{1,25}$=25.43, p=<0.001, interaction effect $F_{1,25}$=0.06, p=0.81). From the

video recordings, we observed that parent birds inserted their beaks into those of the chicks and regurgitated food into the chicks' beaks nearly twice as often for parasitised compared to chicks from parasite-free nests; parasitised chicks therefore received more feeds per feeding event (ANOVA: nest treatment $F_{2,13}$=7.60, p=0.028, brood size $F_{3,13}$=0.68, p=0.59, interaction effect $F_{2,13}$=0.09, p=0.92) (Figure 6.1; Table 6.1). Neither the mean length of feeding visits, mean number of feeds to the entire brood per feeding visit, nor mean length of food transfers were significantly different across treatments or brood sizes (all p>0.05). Intra-brood variation in food allocation (with nest as a covariate) was not significantly different across treatments (ANOVA treatment:$F_{1,13}$=0.95, p=0.36; brood size: $F_{2,13}$=0.59, p=0.64; interaction $F_{2,13}$=3.1, p=0.11). Only female parents visited the nest solely for cleaning or grooming chicks, and did so 5 times more often in parasitised nests (ANOVA: $F_{1,12}$=6.29, p=0.028) (Table 6.1).

Chick condition and fledging success in videoed nests

Multivariate analysis of variance (MANOVA) revealed a significant effect of chick age and nest treatment on variation in morphological traits (chick age: $F_{5,18}$= 2.29, p=<0.001; Wilk's Lambda = 0.004; partial eta^2= 0.59, nest treatment: $F_{6,18}$=4.11, p=0.009; Wilk's Lambda = 4.12; partial eta^2= 0.58). The interaction effect was not significant (p=0.82). However, when the results for the dependent variables were considered separately, naris diameter was the only morphological trait to show significant variation between treatments ($F_{1,45}$= 10.37, p=0.004, partial eta^2=0.31; all other traits all p>0.05)(Figures 6.2 and 6.3). There was a significant effect of chick age on variation for all traits (all p<0.001, except naris diameter p=0.04). A two-way ANOVA showed that chick age also had a significant effect on growth rate ($F_{1,56}$= 4.29, p=<0.001), although the effect of nest treatment and the interaction effect were not significant (treatment: $F_{1,56}$= 0.02, p=.88, interaction: $F_{3,56}$= 0.94, p=.94). Intra-brood variance

in chick mass was not significantly different between treatments (ANOVA treatment: $F_{1,6}$= 0.02, p=0.89; brood size: $F_{3,6}$= 1.05, p=0.44; interaction effect $F_{1,6}$= 0.07, p=0.80).

Chick mortality and nesting outcome in all nests

We have nesting outcome data for 23 nests with chicks (14 parasitised, six parasite-free) in 2010 (three nests were still active at the end of our field-trip). In parasitised nests, all chicks showed signs of *P. downsi* larval feeding (dark, enlarged nares and body wounds). No parasitised chicks fledged, and chicks died after a mean 3.2 days from hatching (± s.e. 0.215, *N*=42 chicks). Chicks that had died due to *P. downsi* were either: (1) found dead in the nest with signs of parasitism (N=15 chicks) or (2) went missing one-by-one from the nest (N=24 chicks): video footage confirmed that parents remove dead, parasitised chicks from the nest. Other causes for chick mortality were owl predation (N= 2 chicks: the entire brood from one nest) and ant predation (N=1 chick). There was much lower mortality in the parasite-free nests with one nest depredated and two nests abandoned (Table 6.2). Overall nest depredation was low (5-27%) (Table 6.2), and depredated nests were recognised as either: (1) ripped or completely missing, indicating owl depredation; or (2) intact, but chicks were found dead inside the nest and covered with fireants (Table 6.2). Ant predation only occurred in the untreated parasitised nests.

6.5 Discussion

Our results experimentally showed that *P. downsi* parasitism increases the cost of raising a brood for Darwin finch parents. Parasitised chicks that begged strongly were allocated more food by parents, and did not suffer reduced body size or growth rates. These findings are consistent with the hypothesis that parents can compensate for parasitism through increased

feeding when they have sufficient resources (Johnson and Albrecht, 1993, Tripet and Richner, 1997). Despite a higher parental effort to maintain parasitised chicks, parental feeding could not fully compensate for the effects of *P. downsi* parasitism, which caused 80% of mortality in untreated nests. Chicks died after a mean 3.2 days after hatching which is the youngest mean age of death recorded for parasitised Darwin finch chicks (see Fessl et al., 2006b). Chick mortality probably resulted from overnight blood loss as the growing larvae consumed more chick resources in a single feeding session than parent birds could replenish during the daytime feeding.

Parental nest visitation

Contrary to our predictions, parental feeding visits were relatively infrequent (2-4 visits per hour) and did not increase for parasitised nests or strong chick begging behaviour. This contrasts with studies of great and blue tits (*Parus major, Cyanistes caeruleus*), which found that parents increased feeding visits to nests infested with blowfly larvae by 24%-65%, and that total feeding rates could exceed 30 visits per hour (Christe et al., 1996, Tripet and Richner, 1997, Hurtrez-Boussès et al., 1998). Interestingly, Kleindorfer (2007a) also reported comparably low levels of parental care (2.6-3.6 visits per hour) in small ground finches on Santa Cruz Island (however the 2.6 is mistakenly reported as 82.6 in Table 4). However, unlike our study, the Santa Cruz data were obtained in dry years (2000, 2004) when chick mortality due to *P. downsi* was low (8-30% nests). Comparing the two study sites and study years, we can conclude that the frequency of parental feeding visits is not directly related to inter-sibling competition (begging behaviour), presence or intensity of *P. downsi* larvae in nests (see O'Connor et al., 2010b), or rainfall (linked with food availability). The use of within-nest video surveillance allowed us to determine that parents allocated more food to

chicks in parasitised nests without increasing the frequency of nest visitation, as has been observed in nest-box studies of great tits (Christe et al., 1996). Perhaps parents bring more and/or better quality food to parasitised nests to eliminate the need for increased nest visitation. For example, parasitised corsican blue tit (*Parus caeruleus*) chicks are fed a significantly higher proportion of protein-rich caterpillars (Bańbura et al., 2004), and in house sparrows (*Passer domesticus*), prey size but not parental delivery rate predicted chick mass and recruitment (Schwagermeyer and Mock, 2007). Further studies are required to determine the composition of feeds allocated to parasitised Darwin's finch chicks.

Inter-sibling competition

Strongly begging parasitised chicks were fed more than their weaker siblings and were also more likely to survive to at least 5 days after hatching. Nevertheless, within-brood differences in begging behaviour, food allocation, and chick mass were similarly high between treatments. Within-brood differences in mass may be explained by different age and size of chicks (in both treatments) due to asynchronous hatching. It is likely that there are different proximate causes for intra-brood variation in begging intensity and food allocation in the parasitised and the parasite-free nests. In parasitised nests, weak or absent begging behaviour might be due to energy loss from lack of sleep (O'Connor et al., 2010b) or blood loss (Fessl et al., 2006b). In contrast, non-parasitised chicks have lower feeding requirements and hence weak begging behaviour might be due to satiation after a recent meal. For example, experimental studies have found that recently fed pigeon, *Columba livia,* barn swallow, *Hirundo rustica,* and magpie, *Pica pica,* chicks beg less intensely than food deprived siblings (Redondo and Castro, 1992, Mondloch, 1995, Saino et al., 2000). Thus heavily parasitised chicks that were too weak to beg may have been misinterpreted by parents as "recently fed", and we found that parents only allocated food to actively begging chicks. There can be intense

inter-sibling competition in parasitised nests, with older chicks competing to stand on top of other chicks to avoid *P. downsi* larvae reach up from the nest base to feed (O'Connor et al., 2010b).

Chick condition and growth

Parasitised chicks had significantly larger nares than chicks from parasite-free nests due to damage caused by 1st instar larvae feeding in the nasal cavities and 2nd and 3rd instar moving through the nares to feed internally (see Fessl et al., 2006b, O'Connor et al., 2010b). Enlarged nares have also been observed in adult Darwin's finches that were presumably parasitised as chicks (Galligan and Kleindorfer, 2009). During the first 6 days after hatching, we found that nests contained less than half as many parasites than those with 7-14 day old chicks, and during that time chicks from parasitised and parasite-free nests had similar body condition and growth rates. This could be explained by the effects of increased parental food allocation to parasitised chicks, which could compensate for the effects of larval feeding. Parasitised chicks often have lower mass than unparasitised chicks at later stages of development (Johnson and Albrecht, 1993, Richner et al., 1993, Norris et al., 2010), but we could not make this comparison because all parasitised chicks died by day 9 of the usual 14 day nestling period. Although the interpretation of our results is limited by small sample sizes, studies of Darwin's medium ground finch (*G. fortis*) have similarly found that *P. downsi* intensity had no significant effect on chick size or growth rates in parasitised versus parasite-free (Huber, 2008) or parasite-reduced nests (Koop et al., 2011). However, Koop et al. (2011) did find that chicks from parasite-reduced nests (mean 22 parasites per nest) had significantly longer wing feathers than control nests (mean 38 parasites per nest) at time of fledging.

Male and female contributions to compensatory feeds

Neither male nor female parents increased their nest visitation to parasitised nests; but females visited nests for feeding nearly 3 times more often than males in both treatments. Both sexes fed parasitised chicks more often per visit, but as they are multiple prey loaders, we cannot draw any conclusions about sexual differences in foraging location or prey quality (see for example Colombelli-Négrel and Kleindorfer, 2010). This finding of equal male and female nest visitation contrasts with a study of great tits (Christe et al., 1996), which found that males but not females increased their feeding contribution to parasitised nests by up to 50%. Female Darwin's finches incurred further costs in our study by providing all grooming visits, which were five times more frequent for parasitised nests. Other studies of parental care in Darwin's finches have also found that males provide only one quarter of feeding visits (Kleindorfer, 2007a), while female finches are the sole providers of antiparasite behaviours such as chick preening, larvae removal from chicks and nests, and removal of dead chicks from the nest (O'Connor et al., 2010b). Female Darwin's finches are contributing more to parental care by investing heavily in current broods, which may lead to trade-offs for lifespan and future reproductive success (Owens and Bennett, 1994, Richner and Tripet, 1999, Wesolowski, 2001). Low paternal investment in parental care may also be explained by reduced relatedness to their current brood. In Darwin's medium ground finch (*G. fortis*), 20% of offspring are from extra-pair copulations (Keller et al.). Parental care can thus be viewed as a limiting resource for females, whereas by investing less in parental care, males can compete to breed with several females that have high parental care (Trivers, 1972).

6.6 Conclusion

Our study demonstrates that *P. downsi* parasitism is costly and prompts adaptive responses in parent Darwin finches to reduce the negative impacts on chicks. Chicks suffer the direct costs

of increased mortality due to larval feeding, while parents provide more food per visit, and females expend extra energy in grooming visits to parasitised chicks. As a result of increased parental care, adult birds may suffer an indirect cost of reduced future reproduction and survival. Since Darwin's finches are unable to fully compensate for the negative effects of parasitism, and some species are already experiencing significant population declines (Grant et al., 2005, Fessl et al., 2010, O'Connor et al., 2010c, O'Connor et al., 2010d, Dvorak et al., in press), our results emphasise the need for immediate research on the biological control of *P. downsi*.

6.7 Acknowledgements

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Table 6.1 Overview of nesting outcome and parental care in the subset of videoed small ground finch nests in 2010. Values given as means ± standard error with N in parentheses.

	Parasitised nests	**Parasite-free nests**
No. nests	7	9*
No. chicks	25	30
No. chicks known fate	25	20
% Chick mortality due to parasitism	92% (23)	0%
% Chicks depredated**	8% (2)	20% (4)
% Chicks fell out of nest	0%	7% (2)
% Chicks fledge	0%	40% (8)
% Chicks abandoned***	0%	30% (6)
Frequency of feeding visits per hour	2.17-3.76	2-4
Mean feeding visits to nest per hour	3.05 ± 0.27	2.80 ± 0.28
Mean female feeding visits to nest per hour	2.45 ± 0.30	2.15 ± 0.41
Mean male feeding visits to nest per hour	0.66 ± 0.22	0.63 ± 0.22
Mean female nest visits to groom chicks (per hour)	1.12 ± 0.29	0.25 ± 0.19
Mean feeds to chick per feeding visit	10.97 ± 1.33	6.09 ± 0.58
Range in number of feeds to chick per feeding event	0-51	0-45

***** Three nests had unknown outcome (10 chicks were still alive at day 7)
** Whole nest depredated by a short-eared owl (*Asio flammeus*)
*** Two nests were abandoned before we could assess parental care

Table 6.2 Summary of nesting success: all small ground finch nests containing chicks (only nests with known outcome included). Values given as means ± standard error with N in parentheses.

	Parasitised	**Parasite-free**
Nests with chicks	14	6
Mean brood size	3.1 ± 0.27	3.3 ± 0.33
% nests fledged some or all young	0%	50% (3)
% nests with total brood loss (parasitism)	80% (12)	0% (0)
% nests with partial brood loss (parasitism)	0%	0%
% nests abandoned	0%	33% (2)
% nests depredated (owl)	7% (1)	17% (1)
% nests depredated (fireants)*	7% (1)	0%
Mean parasites per nest with >6 day old chicks	53 ± 7.0 (2)	0.5 ± 0.34 (6)
Mean parasites per nest with <6 day old chicks	22.73 ± 3.9 (11)	
Mean parasites per chick (>6 day old)	26.5 ± 3.5 (2)	0.17 ± 0.01 (6)
Range in parasite intensity	3-60 (13)	0-2 (6)

* Parasite intensity could not be determined from nests with ant depredation (ants consume *P. downsi* larvae)

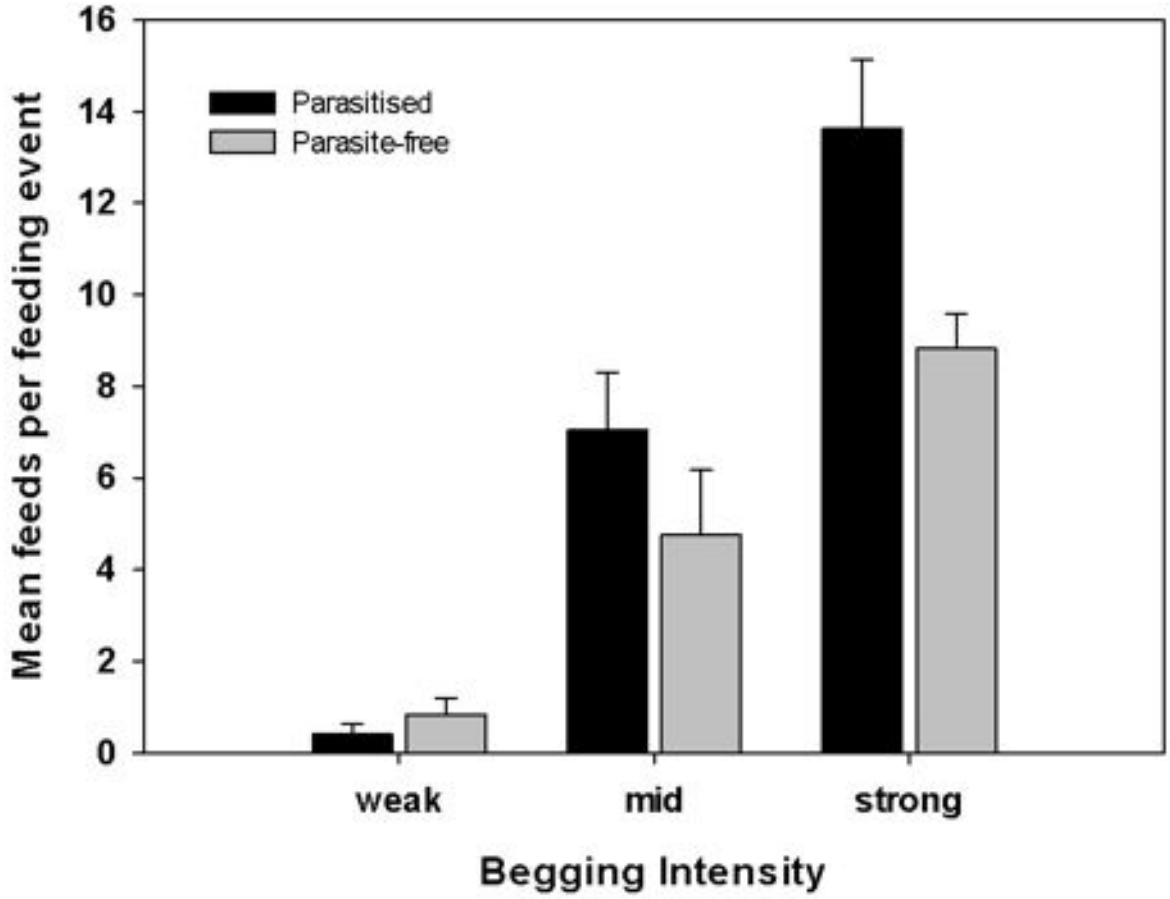


Figure 6.1 The positive association between strength of chick begging behaviour and the number of feeds received per feeding event in parasitised and parasite-free nests. Data is from 13 videoed nests with a total of 42 chicks. Mean feeds per event is shown as mean ± s.e (from 5 feeding events per nest).

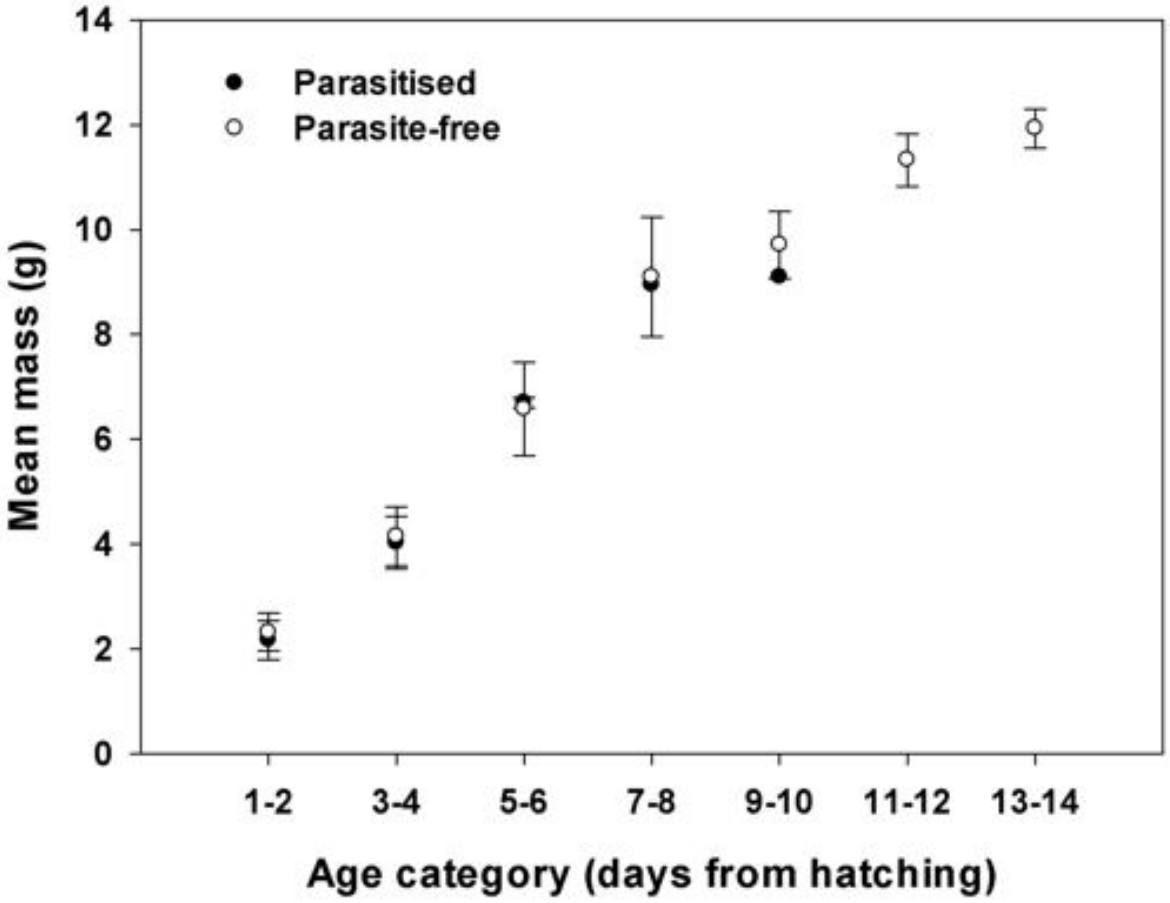


Figure 6.2 Mean body mass (± s.e) of chicks in parasitised and parasite-free nests. Age categories represent grouped values for each 2-day range in chick age. Data is from 19 chicks in 6 parasitised nests and 23 chicks in 7 parasite-free nests.

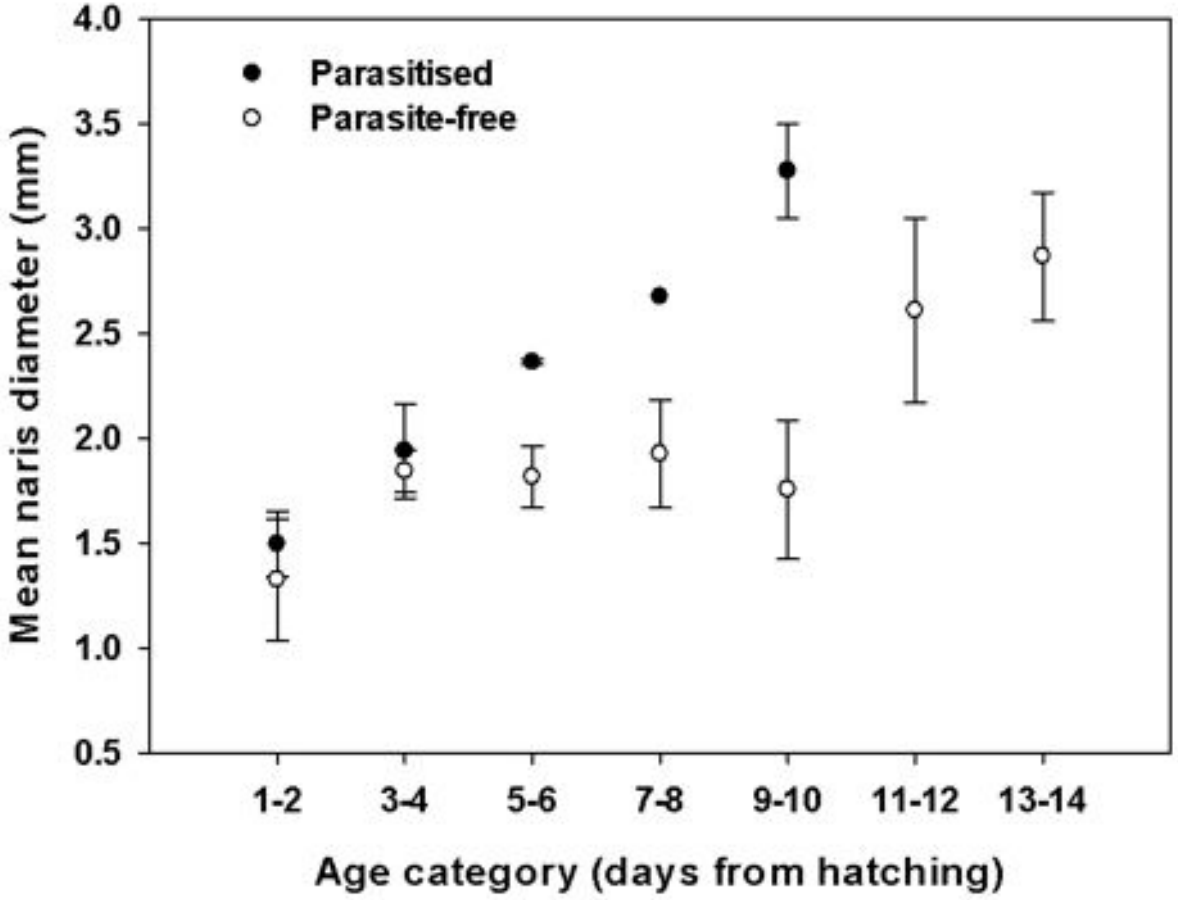


Figure 6.3 Mean naris diameter (± s.e) of chicks from parasitised and parasite-free nests. Data is from 19 chicks in 6 parasitised nests and 23 chicks in 7 parasite-free nests.

Are Darwin's tree finches a hybrid swarm? The difficulty of assessing speciation and extinction in sympatry.

Sonia Kleindorfer, Jody A. O'Connor, Rachael Y. Dudaniec, Steven A. Myers, Frank J. Sulloway

7.1 Abstract

How and why do species form? This question goes to the heart of evolutionary biology, and is underpinned by the contentious concepts of how to define species. We use Darwin's tree finches on Floreana Island to examine gene flow between sympatric species. We test the idea that each tree finch species (*Camarhynchus parvulus, C. pauper, C. psittacula*) is morphologically distinct with low gene flow between species. We compare patterns during two years that differed in rainfall: 2005 (low rainfall) and 2010 (high rainfall). The results are as follows: (1) as ornithologists, we perceived three clusters of morphological data in 2005 and 2010 – but a model-based, unsupervised clustering method (MCLUST) identified two morphological clusters, (2) David Lack's data showed three morphological clusters in 1938/39 (the large tree finches we encountered were smaller than those measured by Lack), (3) there were two genetic clusters in 2005 and 2010, but there was a morphological shift across years in species with mixed genetic assignment, (4) we found stronger size assortative pairing within species in 2005 than 2010. Birds with small beak and body size were assigned to one genetic population whereas birds with large beak and body size were assigned to the other. Individuals with mixed genetic assignment were nestled primarily within the smaller birds in 2005, but in 2010, birds with mixed genetic assignment primarily occupied new intermediate morphological space. We discuss the possibility that the large tree finch (*C. psittacula*) is locally extinct on Floreana Island as well as the role of hybridisation for species persistence. Relaxed size-assortative pairing is a possible mechanism for hybridisation, and novel genetic variance to outcompete parasites, or create new evolutionary trajectories in changing environments, is a possible function.

7.2 Introduction

The persistence of closely-related species in sympatry infers that natural and/or sexual selection can minimise interspecific gene flow to maintain species barriers. But interbreeding between sympatric populations of recently diverged species is common, especially in birds (Grant and Grant, 1992). Hybridisation can drive the formation of new species (Brelsford et al., 2011) but can also result in the extinction of a parental species when selection favours the persistence of hybrids – often due to changing environmental conditions (Taylor et al., 2006). Speciation can thus occur "in reverse" when selection for extreme phenotypes is removed and interspecific hybrids maintain or increase fitness of parental types (Gow et al., 2006, Seehausen, 2006, Taylor et al., 2006). Human-induced landscape changes such as the introduction of new food resources, predators and pathogens can alter the evolutionary trajectory of closely-related taxa, and should be considered when evaluating drivers of hybridisation, speciation and extinction (Hendry et al., 2006, Taylor et al., 2006, De León et al., 2011)

The 14 recently diverged species of Darwin's finches in the Galápagos Islands are classic examples of allopatric speciation (Lack, 1947, Grant and Grant, 1997a, Grant, 1999, Grant et al., 2000, Grant and Grant, 2008), but recent studies have also found evidence for restricted gene flow that may promote speciation in sympatry. For example, disruptive selection explains the finding of genetically distinct and morphologically bimodal populations of the medium ground finch (*Geospiza fortis*) on Santa Cruz Island (De León et al., 2011). In contrast, Kleindorfer et al (2006) found divergent morphological populations in small ground finch (*G. fuliginosa*) at the extremes of a clinal population on Santa Cruz Island – under conditions of high gene flow in both high and low rainfall years (Galligan et al., in review). Traditionally, the maintenance of species barriers in Darwin's finches studied on low and flat islands of the Galápagos Archipelago has been related to ecological conditions

(Grant and Grant, 2008). Specifically, the work of Peter and Rosemary Grant and their colleagues has shown that long periods of drought favour niche competition, strong character divergence, and low gene flow between sympatric finch species. Conversely, during high rainfall years (associated with higher resource diversity), there was evidence for increased gene flow between congeneric species – that is hybridisation – and hence relaxed selection for assortative mating and character divergence (Grant and Grant, 1992, Grant et al., 1996). Notably, during boom years, the hybrid offspring between Darwin finch species had comparable or higher fitness than the parental species (Grant and Grant, 1992). Therefore, based on this precedent, we predict that high rainfall years can promote increased gene flow and hybridisation between sympatric congeneric finch species. We address this question in the tree finch group (*Camarhynchus* spp.).

Most field research to date about Darwin's finches has come from long term studies of the ground finches *(Geospizinae)*, while remarkably little is known about any aspect of the tree finches (*Camarhynchus* spp), including speciation scenarios, temporal and spatial patterns of hybridisation, and population genetic structure. Because this is the first study to address these fundamental questions, we begin with a descriptive approach and describe the morphology and population genetic structure of three sympatric congeneric tree finch species that live in the same *Scalesia* forest habitat on Floreana Island (O'Connor et al., 2010c, O'Connor et al., 2010d). The three focal species are: small, medium, and large tree finch (*C. parvulus*, *pauper,* and *psittacula*). The medium tree finch is locally endemic to Floreana Island, whereas the small and large tree finches are found on nine and eight other islands respectively (Grant, 1999). We do not attempt to address why the medium tree finch only occurs on Floreana Island, however it has been hypothesized that this species fills a niche that is similar to that of the woodpecker finch, which is absent from the island (Grant, 1999, Christensen and Kleindorfer, 2009).

Although a number of field studies have provided snapshots of morphological data for the Floreana Island finches since the 1880s, no study has examined genetic differences between the small, medium and large tree finches. For example, recent microsatellite and mitochondrial DNA phylogenies of Darwin's finches (Petren et al., 1999, Sato et al., 1999) include small and medium tree finches from Floreana Island, but large tree finches were only sampled from other islands. Although Floreana's tree finches are consistently described as separate species in collections made by the Californian Academy of Sciences (1905-1906), David Lack (1947) and Robert Bowman (1961), recent surveys have found many intermediate individuals (Kleindorfer and O'Connor unpublished data). We suspect that the large tree finch today has a smaller beak and body size than the measurements reported by Lack (1947), for example. Therefore, we aim to provide a current morphological overview of the three congeneric finch species and test two ideas about their population genetic structure. We collect morphological and genetic data during two sampling periods: 2005 (a dry year within a drought period that extended from 2000 to 2007) and 2010 (an El Niño high rainfall year following on from a high rainfall 2008) (also discussed in Snell et al., 1996, Dudaniec et al., 2007, Galligan et al., in review). We test the predictions that: (1) the three species will have distinct morphological and population genetic structure; (2) during the high rainfall year, we will find more evidence for hybridisation between the three species (tested using genetic data), and (3) we will find a shift in size assortative pairing during the high rainfall year (tested using observational data). To test the idea that the large tree finch has become smaller since David Lack sampled the birds, we compare historical (1938-39) and recent (2005 and 2010) morphological data to assess changes in mean morphological measurement per species over time.

7.2 Methods

Study species and site

The three focal species for this study were Darwin's small tree finch (*Camarhynchus parvulus*), medium tree finch (*C. pauper*), and large tree finch (*C. psittacula*) (Figures 7.1a-c), which co-occur in sympatry in the highland *Scalesia* forest of Floreana Island, Galápagos Archipelago. We collected data during the onset of the breeding season from Jan-April during the years 2005 and 2010. We mist-netted and sampled birds along the main walking trail through native *Scalesia* forest at the base of Cerro Pajas Volcano (1°17'43.S, 90°27'23.W) between 300-400m elevation. Sampling effort and location within the 2.4km^2 site was comparable across years; we placed six 12m mist-nets along the track every morning; sampled the location once, and moved all nets further up the track at the end of the day to be ready for mist-netting the next morning. The study site characteristics are described in O'Connor et al. (2010c). The population status for the three focal species on Floreana Island is as follows: the small tree finch is most common (~3700 individuals), the medium tree finch is locally endemic and IUCN red-listed (<1700 individuals), and the large tree finch is rare (<500 individuals) (O'Connor et al., 2010c). We assigned individuals to a species classification using morphological data that we collected in 2004 (Kleindorfer et al. in review), and in consultation with data tables in Lack (1947). We analyse the morphological data in two ways: (1) based on our subjective classification (Table 7.1), and (2) using MCLUST software, an unsupervised clustering method that removes possible observer bias.

Sample collection

Finches were captured in mist-nets and subsequently banded with a unique aluminium identification band and colour band combination. We collected blood samples in 2005 and 2010 from 94 and 107 tree finches respectively. The sample size for each species and sex (M=male, F=female, U=unknown sex) per year is: 2005: *C. parvulus* N=62 (M=44, F=18), *C. pauper* N=24 (M=19, F=4, U=1), *C. psittacula* N=8 (M=5, F=3), and 2010: *C. parvulus* N=46 (M=32, F=12, U=2), *C. pauper* N=32 (M=27, F=3, U=2), *C. psittacula* N=29 (M=24, F=3, U=2). We stored the blood sample on FTA® databasing paper and excluded juvenile finch samples, to minimize the use of genetically related individuals (such as parents and offspring).

Morphology

We measured the following morphological traits per bird: (1) beak-head (beak tip to back of head); (2) beak-naris (beak tip to naris opening); (3) beak-feather (tip of beak to feather line); (4) beak depth (measured at the base of the beak) (5) beak width (measured at the base of the bill); (6) tarsus length; (7) naris length (taken from extremes of naris opening) (8) wing length, and (9) body mass. Morphological measurements were taken to the nearest 0.01mm using calipers. Mass was measured to the nearest 0.01g using scientific scales. All measurements were taken by SK in 2005 (N=94) and by both SK (N=23) and JO'C (N=84) in 2010. SK and JO'C had high calibration between morphological measurements, and agreed on species classifications in the field. Specifically, the morphological measurements (per species) between SK and JO'C in 2010 was not significant (t-test all traits; $P>0.05$). Inter-measurer reliability tested in the field for 15 birds was extremely high: SK and JO'C had measurement differences that were always less than 0.2mm for each trait. Historical

morphological data (David Lack, 1938-9) was obtained from the "Beagle Investigations Return with Darwinian Data (BIRDD)" website: http://www.bioquest.org/birdd/morph.php.

We used two methods to analyse the modern (O'Connor and Kleindorfer, 2005/10) morphological data: (1) a model-based unsupervised clustering method using MCLUST software (implemented as an "R" library), and (2) multivariate analyses (MANOVA) of mean morphological measurements between the three putative species (using SPSS for Mac v. 17.0). For the historical data, we used MCLUST only, but compared the three morphological traits (beak depth, beak-naris and wing length) given in this dataset to that of modern birds using one-way ANOVAs. The measurement "mass" was excluded from all morphological analyses due to small sample sizes in 2005. Females were excluded from morphological analyses because: (1) sample sizes were small and variable (10-37% of N per species per year), and (2) their measurements are significantly smaller compared to males, and may therefore skew results. We used MCLUST software to identify morphological clusters that were present on Floreana in each year (2005 and 2010) using principal components scores for beak size (derived from the following variables: beak-head, beak-feather, beak-naris, beak depth, beak width) and body size (derived from wing length and tarsus variables) The program fits the observed frequency distribution to ten alternative models, and the 'best' model is taken to be the one with the highest Bayesian Information Criterion (BIC). Using MANOVA, we examined differences in mean morphological measurements of the remaining seven measured traits with the fixed factors (1) putative species and year, or (2) genetic population (STRUCTURE assigned) and year.

Historical morphology

We compared historical and contemporary morphological measurements of the tree finches. To this end, we compared our measurements (2005, 2010) with those of David Lack (sampled between 1938/39) using one-way ANOVAs (split file by putative species). The sample size of males measured by David Lack in 1938-39 are as follows: small tree finch: N=87, medium tree finch: N=80, large tree finch: N=4.

Size assortative pairing

We analysed data on size assortative pairing (beak shape, tarsus length) during two sampling periods (2004/2005 and 2010) (see also Kleindorfer et al., in review). Rainfall on the Galápagos Islands tends to change between prolonged *La Niña* periods of low rainfall (2-11 years) and brief *El Niño* periods of high rainfall (1-2 years: Snell and Rae 1999). In our study, the mean annual rainfall was ~170 mm in the highlands in 2004 and 2005 (low rainfall years), and ~600 mm in 2010 (high rainfall year). The sample size for size assortative pairing per sampling year and species is as follows. During 2004/2005, we have data for pairs of 16 small tree finch, 7 medium tree finch, and 2 large tree finch. During 2010, we have data for pairs of 11 small tree finch, 5 medium tree finch, and 1 large tree finch. Pairs were identified if both male and female birds at a nest had colour bands; we cross-referenced the morphology measurements for the colour-banded birds. We analysed the size assortative pairing data using regression analysis within each year, and in a MANOVA with species and year as fixed factors. We used principal components analysis to calculate a beak size factor score derived from the variables bill length, depth, and width (different from the principal component scores used for MCLUST analyses). In males, the derived PC size explained 86.6% of the variance and had high factor loadings for bill length (0.92), bill depth (0.95), and bill

width (0.93). In females, the derived PC beak shape explained 79.3% of the variance and had high factor loadings for bill length (0.89), bill depth (0.95), and bill width (0.83).

DNA extraction and PCR amplification

Squares of $2mm^2$ were cut from blood-stained sections of FTA paper, washed for 30 minutes in 200µl of FTA lysis buffer (100mM Tris, 0.1% SDS), then washed twice for 10 minutes in 200µl of DNAzol®. FTA squares were washed twice with 200µl of water for 10 minutes, and then mixed with 200µl of 95% ethanol to denature the DNA. Samples were dried and eluted in TE buffer (by incubating at 90° for 5 minutes). We performed PCR amplification (in 15 µL volumes) with: 1 mM dNTP; 0.8 x PCR Gold Buffer (Applied Biosystems); 4 mM $MgCL_2$; 0.02 U/µL Ampli*taq* Gold® DNA polymerase (Applied Biosystems); 0.3 uM of each primer; and 10-30 ng/µL DNA. PCR conditions were: 9 minutes at 94^oC, followed by 40 cycles of 94^oC for 45 seconds, annealing at 54^oC for 45 seconds and extension at 72^oC for 1 minute, with a final extension temperature of 72^C for 30 minutes and 25^oC for 30 seconds.

Microsatellite analysis

We genotyped 201 individuals at 10 microsatellite loci: Gf01, Gf03, Gf04, Gf05, Gf06, Gf07, Gf08, Gf09, Gf11, Gf12, Gf13 (Petren, 1998). Genotypes were analysed on an ABI 3770 (Applied Biosystems) automated sequencer and scored using Genemapper version 4.0 (Applied Biosystems).

We carried out tests of linkage disequilibrium for each locus by putative population using GENEPOP v4.0.10. After Bonferroni correction (Rice, 1989), significant departure from linkage disequilibrium ($P < 0.01$) was detected for one locus pair, Gf09 with Gf11, although it was only detected in a single putative species in a single sampling period. For this

locus pair we used Linkdos software (Garnier-Gere and Dillman, 1992, http://genepop.curtin.edu.au/linkdos.html) to estimate the correlation co-efficient: r_{LD} (Black and Krafsur, 1985), which has been shown to be correlated with distance between loci (Kaeuffer et al., 2007). The r_{LD} for this locus pair was < 0.3 ($P < 0.05$), indicating a probable distance of greater than 3 cM between loci, which is sufficient distance that any linkage effect does not bias clustering analyses (Pritchard and Wen, 2004). Therefore, we chose to retain these loci in further analyses.

The number of alleles (N_A), expected and observed heterozygosities (H_E, H_O), and the pairwise FST (Weir and Cockerham, 1984) were calculated for each locus by putative population (Appendix 7A) and globally for each locus (Appendix 7B) using GENEPOP v4.0.10 (Raymond and Rousset, 1995, Rousset, 2008) and GenAlex v6.1 (Peakall and Smouse, 2006).

Population genetic structure

We determined population structure using a Bayesian model-based clustering method in the program STRUCTURE v2.3.2 (Pritchard et al., 2000, Falush et al., 2007, Hubisz et al., 2009). In STRUCTURE, the user defines the number of clusters, *K*, and the model probabilistically assigns individuals to a cluster in a way that minimises departure from Hardy-Weinberg equilibrium at each locus while conforming to the set value of *K*. The model assumes that loci *within* clusters are in Hardy-Weinberg equilibrium and linkage equilibrium. Because we expect potential hybridisation, we used the admixture model, which allows individuals to have partial ancestry in each cluster (Pritchard et al., 2000). For the same reason, we used the option that takes into account the likelihood that allele frequencies are correlated across clusters. We set allele frequency priors according to the data; mean = 0.15, standard deviation = 0.05, Lambda=1 and ran 10 MCMC replicates for K=1-10. We expect the degree

of admixture to be relatively low, so we set Alpha at 0.5. We tested the suitability of these priors, with attention to Alpha and Lambda, by comparing convergence dynamics of multiple MCMC chains for a range of priors that included our custom priors and the default STRUCTURE priors. Pritchard et al. (2000) suggest that chains should converge within $1x10^4$ and $1x10^5$ MCMC iterations, so we investigated convergence dynamics with chains of $1x10^5$ MCMC iterations for K = 1-6. The results supported the use of our custom priors. Chains run using our custom priors appeared to converge with mixing within $8x10^4$ MCMC iterations; therefore, we chose a relatively conservative burn-in of $1x10^5$ MCMC iterations, which we fixed for all further runs. Exploration of the data for consistency across longer and shorter chains for a range of K indicated that a chain length of $5x10^5$ MCMC iterations was most appropriate. Using our optimised burn-in length ($1x10^5$ iterations) and MCMC length ($5x10^5$ iterations), we ran 10 MCMC replicates for K = 1-6. Because we are unsure of the dynamics of our data we chose to use two methods for inferring clusters: (1) the method published in the original structure paper which involves comparing mean log likelihoods penalized by one-half of their variance (Pritchard et al., 2000); and (2) the method published in Evanno et al. (2005), which involves calculating delta K, a quantity based on the second order rate of change of the likelihood function with respect to K. As we have relatively few loci we were concerned that differentiation between populations may be more difficult to detect, so we also implemented the LOCPRIOR model – a STRUCTURE model that incorporates putative population information in the inference by using a modified prior distribution for clustering that allows the distribution of cluster assignments to vary by putative population (Hubisz et al., 2009).

7.3 Results

Morphology

Our subjective classification of species' morphology showed highly significant differences between the three tree finch species, with a significant interaction term species x year (MANOVA: $F_{16, 236} = 3.93$, P =<0.001; Wilk's Lambda=0.62) (Table 7.2). Therefore, we compared the species' morphology separately within each year, and found significant differences between species in 2005 (MANOVA results) and 2010 (MANOVA results). Our test of species differences across years showed no change for any species (t-test all traits; P>0.05).

In contrast to our subjective analysis of morphological classification, which identified three species, MCLUST analyses only found evidence for two distinct morphological clusters in both 2005 and 2010 (Figure 7.2). Cluster one contained individuals with smaller beak and body size compared with cluster two. The difference between the cluster means was highest in 2005 (Table 7.3 and Figure 7.2), when clusters were discrete and had few individuals in between (there was a lot of empty morphological space between cluster one and cluster two). In 2010, the two clusters were closer together, and had many "intermediate" individuals in between the two cluster centroids (Table 7.3 and Figure 7.2). There were fewer very small or very large birds in 2010.

Historical comparison of morphological data

A comparison of our modern finch morphological data with that of David Lack (1938-39) is shown in Table 7.4. Both medium and large tree finches measured by Lack were larger than those that we measured in 2005 and 2010 (Table 7.4). Notably, despite larger sample sizes in the 2000s, we never caught a large tree finch on Floreana Island with measurements as large

as those reported by Lack (1947). MCLUST analyses found evidence for three distinct morphological clusters in Lack's data from 1938-39 (Figure 7.3). Individuals with small morphology (i.e. small tree finches) form a very distinct cluster, whereas the distance between cluster means was less for the two clusters with larger morphology (ie medium and large tree finches)(Table 7.3).

Size assortative pairing

There was significant size assortative pairing for beak shape in small tree finch ($r=0.73$, $P<0.001$, $N=26$), but not medium tree finch ($r=0.47$, $P=0.116$, $N=11$); data were insufficient to statistically test the large tree finch ($N=3$). We used MANOVA with two dependent variables: (1) the difference in pair male and female beak shape, and (2) the difference in pair male and female tarsus length, against the fixed factors: (1) species, (2) year (2004/05 versus 2010), and (3) species x year interaction term. We found a significant effect of species on assortative pairing for beak shape ($F_{2,41}=7.21$, $P=0.002$), but not for tarsus length ($F_{2,41}=0.65$, $P=0.526$). There was no significant effect of year or the interaction term (all $P>0.3$). Post-hoc tests showed significant differences between pair male and female beak shape in small tree finch versus medium tree finch ($P=0.050$) and large tree finch ($P=0.037$), as well as medium tree finch versus large tree finch ($P=0.002$). Inspection of Figure 7.4 shows a shift in species' overlap in the difference between pair male and female beak shape between the sampling periods (2004/05 versus 2010). In 2004/05, small tree finch pairs were size assortatively paired with no overlap between small versus medium and large tree finch pairs. In 2010, the difference between pair male and female beak shape had an overlapping distribution in small and medium tree finch (Figure 7.5).

Locus characteristics and genetic diversity

We carried out tests of Hardy-Weinberg Equilibrium (HWE) for each locus by putative population using GENEPOP v4.0.10. After Bonferroni correction, six loci (Gf01, Gf03, Gf04, Gf07, Gf09, Gf11) showed significant departure from HWE ($P < 0.01$), although three of these loci departed from HWE in only a single putative species (Appendix 7A). All six loci showed heterozygote deficiency (Appendix 7A and 7B). As we expect our data to potentially contain hybrids that will influence HW dynamics, we were careful in our interpretation of these results and subsequent treatment of the data. The loci Gf09 and Gf11 showed deviation from HWE across three and two of the putative species respectively (although only in 2010) with large heterozygote deficit (Appendix7A); therefore, we removed these loci from further use. We examined the influence of Gf09 and Gf11 on the results by comparing analyses with and without their inclusion. Results were consistent in all cases and we concluded that the observed departures from Hardy-Weinberg equilibrium for these loci were most likely not strong enough to significantly bias results; therefore, we consider results obtained with the inclusion of these loci.

Missing data across loci was 1-8%. Across all individuals, the number of alleles per locus ranged from 3 to 20 (mean 10.1 ± SE 1.6), expected heterozygosity ranged from 0.08 to 0.9 (mean 0.56 ± SE 0.09).

Population genetic structure

Estimates of the logarithm of probability of the data averaged over the 10 MCMC replicates for K = 1-6 and K = 0-5 were maximal for K = 2 under the standard admixture model (Appendix 1C and 1D). Applying the LOCPRIOR model to our data, estimates of the logarithm of probability of the data averaged over the 10 MCMC replicates for K = 1-6 and K = 0-5

were also maximal for $K = 2$ (Fst between clusters = 0.09, P < 0.02)(Appendix 7E and 7F) As the standard and LOCPRIOR models both estimated the logarithm of probability of the data as maximal for $K = 2$, we conclude that the inclusion of putative population information in the model did not bias clustering. We chose to interpret the individual ancestry assignments provided by the LOCPRIOR model, which can often provide more accurate inference of individual ancestry in data sets where the signal of structure is weak (Pritchard et al. 2009). Mean individual cluster memberships across the 10 MCMC replicates for $K = 2$ using the LOCPRIOR model are shown in Figure 7.5. As *Camarhynchus* spp. occur completely in sympatry on Floreana we were unable to use pure samples to determine an appropriate threshold to distinguish between pure and hybrid individuals, so we selected a tentative value (0.75) based on values from hybridisation studies to help investigate the data. Clusters were generally representative of groups of putative populations; one cluster (Population 2) contained the majority of individuals from putative *C. parvulus* from 2005 and 2010 and the other cluster (Population 1) contained the majority of individuals from putative *C. pauper* from 2005 and putative *C. psittacula* from 2005 and 2010, while the majority of individuals from putative *C. pauper* from 2010 showed intermediate memberships (assigned to the category "Mixed")(Figure 7.6, Table 7.5). There was a significant association between putative species and genetic population (χ^2=155.3, df=4, P=<0.001).

The association between morphology and population genetic structure

Mean morphological measurements were significantly different between genetic populations (MANOVA $F_{16, 352} = 20.78$, P =<0.001; Wilk's Lambda=0.26, Partial $Eta^2 = 0.49$) and years ($F_{8, 176} = 7.08$, P =<0.001; Wilk's Lambda=0.76, Partial $Eta^2 = 0.24$)(Figure 7.6). The interaction effect was not significant ($F_{16, 352} = 1.37$, P =0.15; Wilk's Lambda=0.89, Partial $Eta^2 = 0.06$). All traits were found to have a significant effect on variation across species, and

across years. Population 1 predominantly contained individuals with large morphology (i.e. beak size), whereas small individuals were mostly assigned to Population 2 (Figure 7.6 & 7.6, Table 7.6). Individuals with mixed assignments between the two populations had intermediate morphology (Figure 7.6 and Table 7.5).

7.4 Discussion

The results presented here go to the heart of evolutionary biology: by what criteria do we denote species, and by what criteria do new species form or collapse? Our data on morphological differences between the species show two things: (1) as ornithologists, we perceived three clusters of morphological data – but the blind software program identified two morphological clusters, and (2) the mean morphological trait values changed across historical time for the large tree finch (1938/39 versus 2005/10), and in contemporary time (2005 versus 2010) for the medium tree finch. As we know, there are many examples of bird species that are indistinguishable by morphology, and only differ in song. In these cases, song is an effecting pre-mating barrier (reviewed in: Kroodsma, 2005, Price, 2008, Toews and Irwin, 2008). Therefore, we asked: is there gene flow between the three *Camarhynchus* species on Floreana Island? It turns out that the answer to this question is more complex than we imagined. To summarise: we found two genetic clusters in both study years (2005, 2010), as well as individuals with mixed genetic assignment. Intriguingly, birds with small beak and body size were assigned to one genetic population whereas birds with large beak and body size were assigned to the other. Individuals with mixed genetic assignment were nestled primarily within the smaller birds in 2005, but in 2010, birds with mixed genetic assignment primarily occupied the new morphological space in between the smaller and larger birds.

The historical data collected by David Lack and published in 1947 further informs our interpretation of the tree finches on Floreana Island. Compared with Lack, our largest large tree finch birds were 18% smaller. In the 2000s, mean beak length in large tree finches was 9.1 mm compared with 11.0 mm in 1938-39 (Lack, 1947). In the 2000s, the large tree finch beak size (9.1 mm) was comparable to Lacks' medium tree finch (9.0 mm), the medium tree finch (8.3 mm) was smaller than Lack's medium tree finch (9.0 mm), but the small tree finch

beak length remained similar (7.3 and 7.4 mm respectively). Furthermore, cluster analysis of morphological data from 1938-39 shows three distinct clusters compared to the two clusters found in 2005 and 2010 data.

This throws light on our observations, and raises several plausible scenarios. Scenario 1: the large tree finch is so rare that we did not catch any. Scenario 2: the large tree finch is extinct (and we were mistaken to believe that we had mist-netted large tree finches). Scenario 3: the large tree finch and medium tree finch have both experienced directional selection for smaller body size, and still persist today.

We argue that scenario 2 is most plausible – namely, that the large tree finch is extinct on Floreana Island. We also argue that our modern "large" tree finch is the extant medium tree finch and that the medium tree finch is now bimodal (similar to the situation on Santa Cruz Island with the bimodal medium ground finch)(Hendry et al., 2006). We have previously shown that the small, medium, and large tree finches forage differently (Christensen and Kleindorfer, 2009). This does not contradict our new interpretation that the large tree finch is extinct: rather, we were able to distinguish the bimodal morphs of the medium tree finch in the field. We analyse the song data in a separate manuscript (Kleindorfer et al., in review). The song data paint a similar picture: each "species" is clearly distinguishable by song, but only the small and large tree finch males respond more strongly to their species' song – the medium tree finch males did not show a differentiated response, and responded to all playback of song (small, medium, large tree finch)(Kleindorfer et al., in review). Intriguingly, all three "species" were morphologically distinguishable. This finding contradicts the many examples of cryptic species, which are morphologically indistinguishable, but which use song as an effective pre-mating barrier (Toews and Irwin, 2008)

Darwin's finches have always been a puzzle to evolutionary biologists. The Floreana tree finches pose a particular challenge. Ornithologists can distinguish three species based on appearance and song, but the birds only show behavioural response to two species, and the genetic data confirm two genetic populations with so much gene flow as to justifiably refer to the population structure in 2010 as that of a hybrid swarm.

We cannot resolve the species dilemma for the three tree finch species here. Clearly much work is needed to understand the *Camarhynchus* relationships in the light of different species concepts. But this study does offer an excellent opportunity to evaluate the role of hybridisation for gene flow patterns under conditions of high rainfall.

Relaxed selection for mate choice: the mechanism for hybridisation

Our data for small and medium tree finch (there were too few data for large tree finch) show that birds had assortative pairing for body size in 2004/2005 (low rainfall years) – but not in 2010 (an extremely high rainfall year). Specifically, in 2004/05, the three species had size assortative pairing for the three species clusters; but by 2010, we found that size assortative pairing occurred in two clusters over a narrower size range, with overlap. This finding has similarity with results found by Galligan and Kleindorfer (in review) in small ground finch *G. fuliginosa* on Santa Cruz Island. Lowland birds (under conditions of low rainfall) had strong assortative pairing for body size, but highland birds (under conditions of high rainfall) did not. Galligan and Kleindorfer (in review) go on to interpret this pattern as evidence for relaxed selection in benign environments, and also as a mechanism to facilitate morphological and genetic variance (under benign conditions) to cope with unpredictable novel habitats. In the Galápagos Islands, high rainfall years recalibrate the plant community because seed banks change dramatically (reviewed in Grant 1999). In the face of this

unpredictability, many species – famously including Darwin's finches – increase their genetic variance by introgression of genes during boom years (Grant and Grant, 1992). There is no immediate fitness penalty for relaxed size assortative pairing during conditions of high and variable resource abundance, plus there are possible future benefits of having the right phenotype for an unknown future resource distribution. Therefore, across years that differ in rainfall, the prediction is that we find different selection for size assortative mate choice, with high selection for size assortative pairing during drought years (because selection favours phenotype specialization that maximize dwindling resources) and relaxed selection for size-assortative pairing during high rainfall years (to exploit a range of resources with the benefit of variance to cope with future change). Our data support this interpretation.

Outcompeting parasites: the function of hybridisation

The Red Queen hypothesis predicts that genetic variance keeps you just one step ahead of doom, in a never-ending cycle of competition. Darwin's finches of the Galápagos Islands are being massively impacted by parasitic larvae of an introduced fly, *Philornis downsi* (Fessl et al., 2001, Fessl and Tebbich, 2002, Dudaniec and Kleindorfer, 2006, O'Connor et al., 2010d). Nestling mortality due to *P. downsi* parasitism varies between 19-100% across years (Fessl and Tebbich, 2002, Fessl et al., 2006b, Dudaniec et al., 2007, Huber, 2008, O'Connor et al., 2010a, O'Connor et al., 2010d). Long-term study has shown that the prevalence and intensity of *P. downsi* infestation is higher during high rainfall years (Dudaniec et al., 2007), which is a common pattern for many parasites, especially when the adult life stage is dependent on fruit. Therefore, it is plausible to suggest that extreme selection from a parasite that is favoured by rainfall can also select for introgression of genes in host populations via hybridisation to create novel genetic combinations that are incompatible to the parasite.

We noticed a marked decrease in body size in the 2010 tree finches compared with

2005, and also compared with the historical data. We know that larger tree finches build larger nests, and that larger nests harbour more *P. downsi* parasites (Kleindorfer and Dudaniec, 2009). Therefore, it is possible that *P. downsi* is creating strong selective pressure for smaller host size, and smaller nest size. Future research can test the role of *P. downsi* for host phenotype (body size) and extended phenotype (nest size) that may predict parasite intensity. Notably, the two species of Darwin's finches that have shown alarming population decline over the last decade on Santa Cruz Island, the woodpecker (*C. pallidus*) and warbler (*Certhidea olivaceae*) finch (Dvorak et al., in press), have the highest *P. downsi* intensity (Dudaniec et al., 2007).

Conclusion

This study adds to a growing body of evidence that hybridisation is common in birds. Here we show a possible mechanism for hybridisation, namely relaxed size-assortative pairing across species during a high rainfall year. We also discuss a possible function of hybridisation, which we suggest could be to outcompete a lethal and newly introduced parasite. Darwin's finches are renowned for their behavioural plasticity, and rapid evolution under extreme selective pressures. Here we show that the sympatric tree finch species form what may best be described as a hybrid swarm during a high rainfall period.

a)

b)

c)

Figure 7.1 Tree finches from Floreana Island: a) small tree finch (*C. parvulus*) male (~2 year old), b) medium tree finch (*C. pauper*) male (~4 year old), c) large tree finch (*C. psittacula*) male (~3 year old).

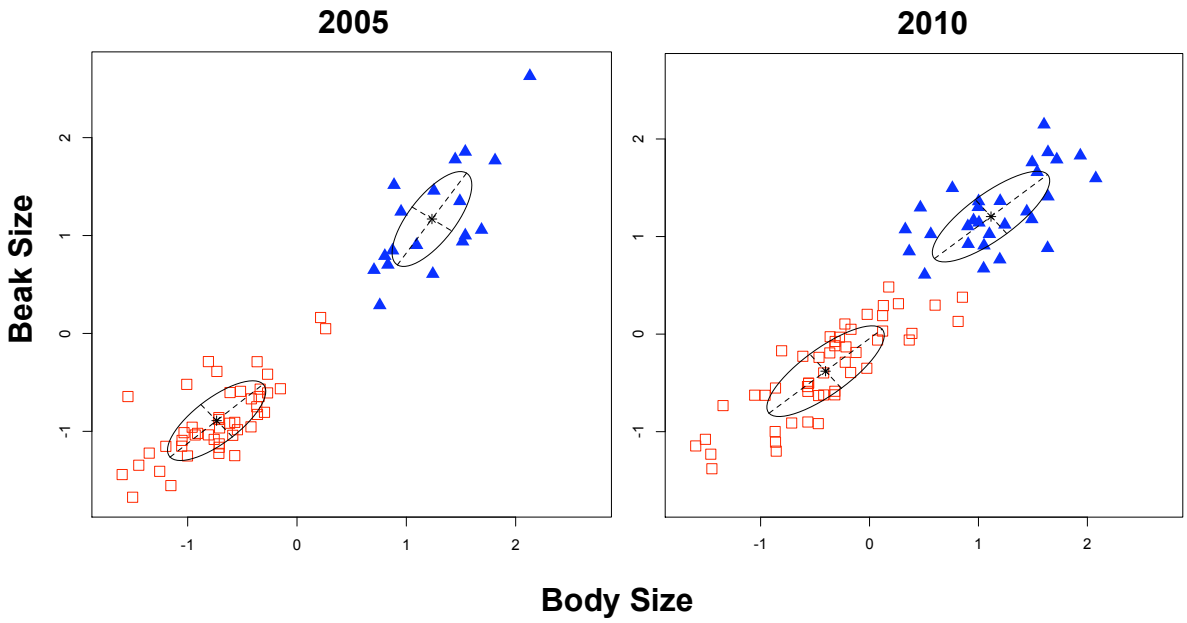


Figure 7.2 A projection of the Floreana tree finch morphological data (male only) collected by Kleindorfer and O'Connor in 2005 & 2010. Analysis was performed on principal components scores for body size and beak size. The method distinguished two clusters, which are indicated with different symbols. For each cluster, component means are marked by an asterisk, and ellipses with axes indicate covariances.

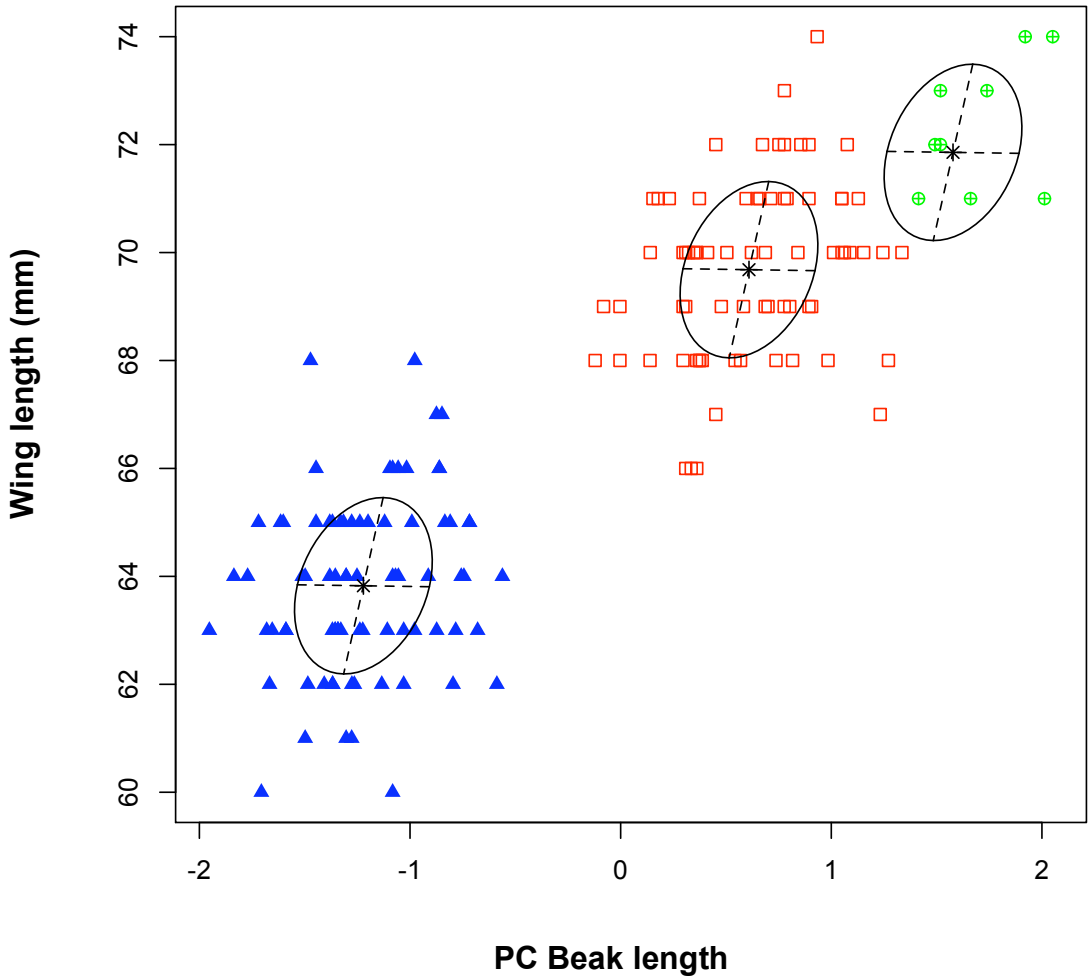


Figure 7.3 A projection of the Floreana tree finch morphological data (male only) collected by David Lack in 1938-39. The method distinguished three clusters, which are indicated with different symbols. For each cluster, component means are marked by an asterisk, and ellipses with axes indicate covariances.

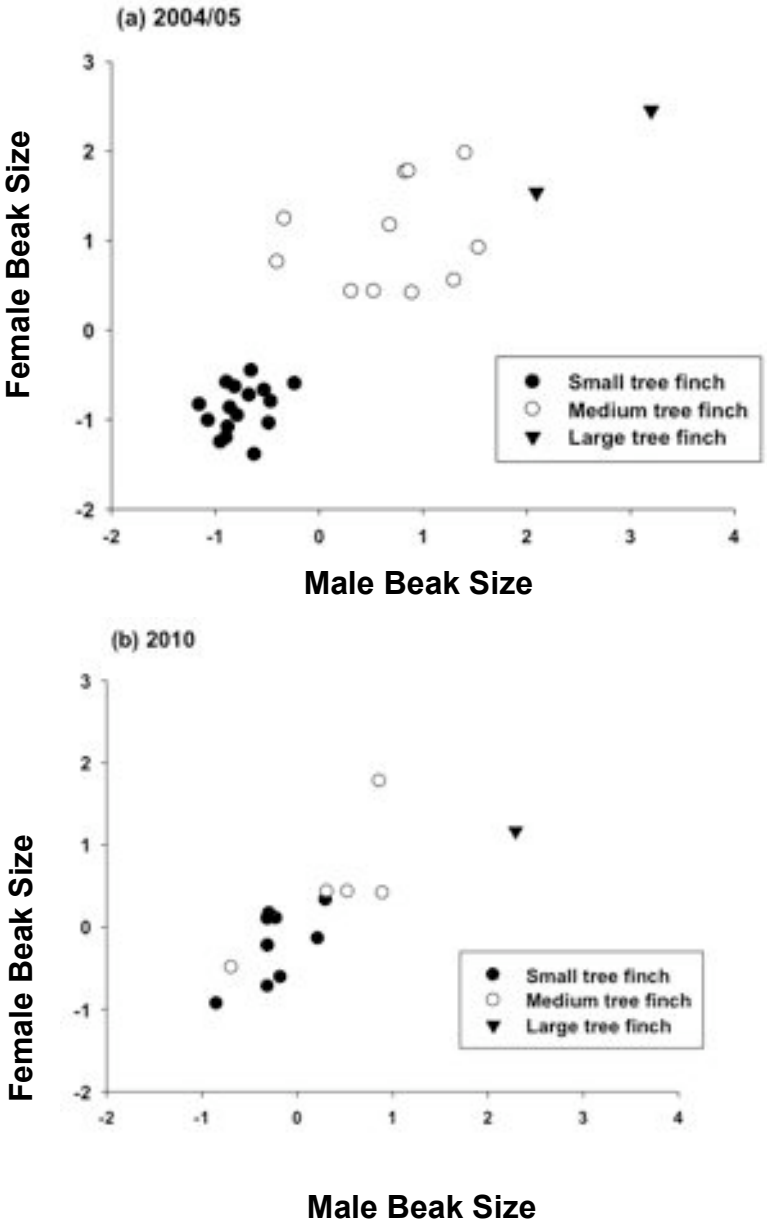


Figure 7.4 The relationship between pair male and female beak shape. Evidence for size assortative pairing within species in 2004/05 (a low rainfall year), whereas size assortative pairing in 2010 (a high rainfall year) occurred across species, but not within species

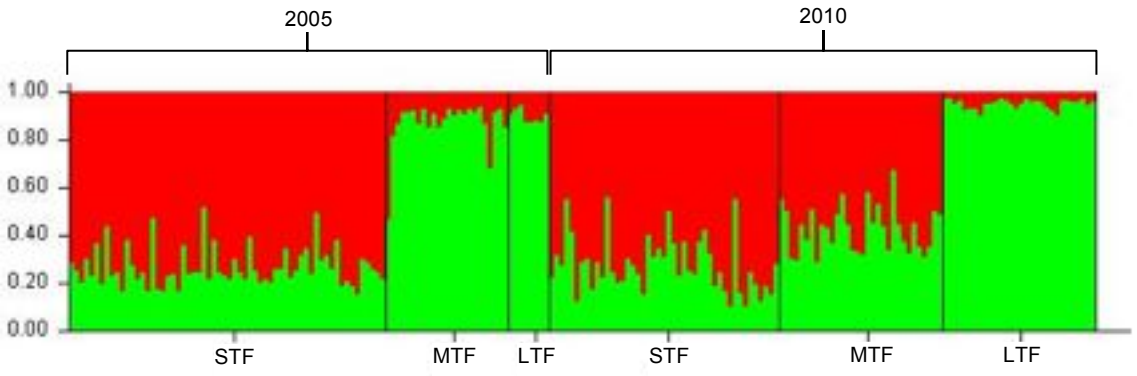


Figure 7.5 Mean individual cluster memberships across the two clusters detected by the LOCPRIOR model in STRUCTURE. Data is shown for individuals classified as a small tree finch (STF), medium tree finch (MTF) or large tree finch (LTF) in 2005 and 2010.

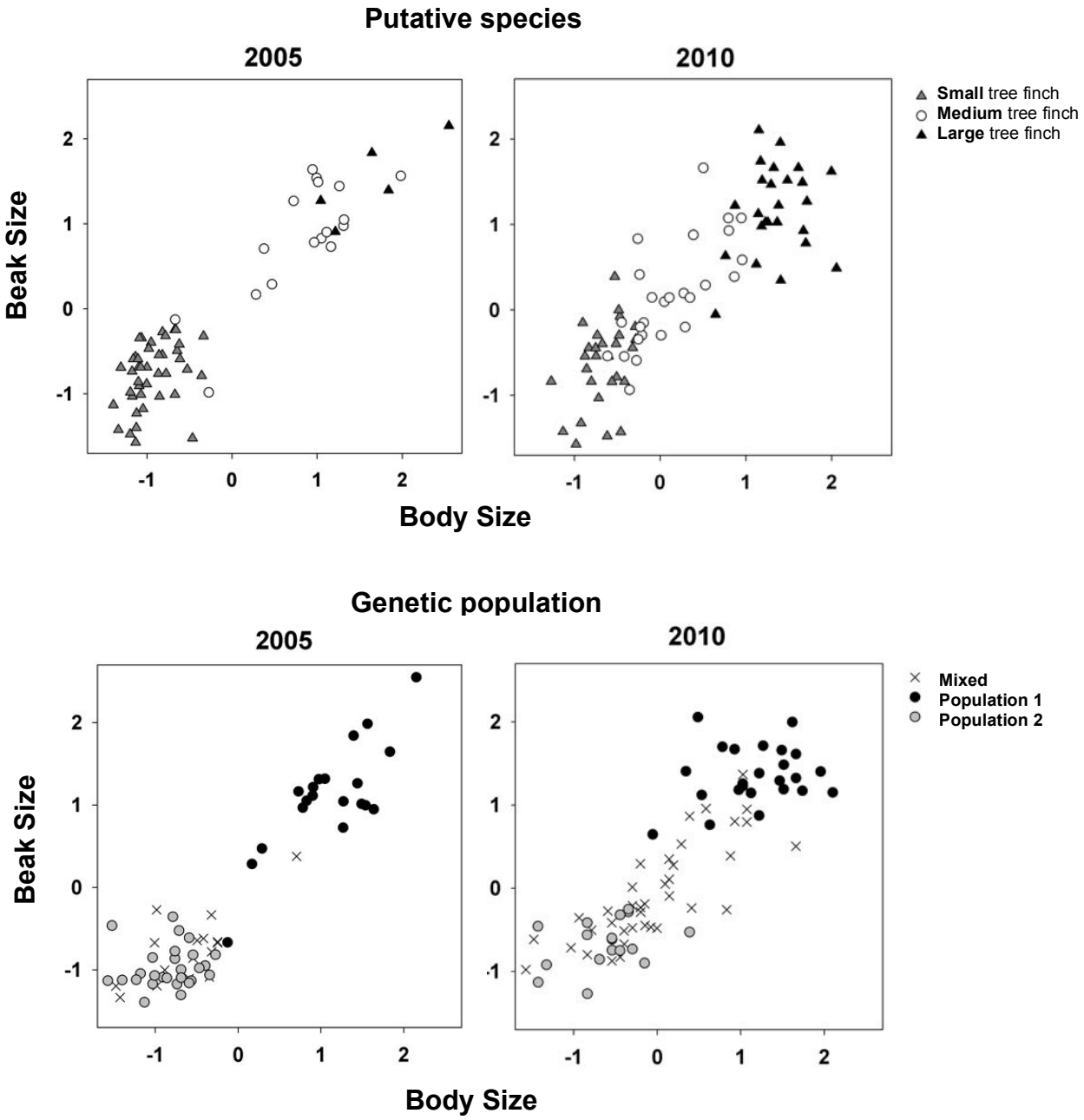


Figure 7.6 Interannual variation in beak size and body size (shown as principal component scores) for putative species and genetic populations of tree finches on Floreana. Data are for males only and separated by year (2005, 2010). Genetic populations are represented by three categories: 1) Population 1 (Pop 1); 2) Population 2 (Pop 2), and 3) mixed assignment (Mixed).

Table 7.1 Morphological variation between the small, medium and large tree finch on Floreana. Shown are range in measurements (minimum and maximum in mm), means (mm) with standard deviation for males from 2005 and 2010 combined.

		Small tree finch	Medium tree finch	Large tree finch
Beak-head	**Range**	25 - 27.7	26.3 - 30	28.1 – 30.8
	Mean SD	26.3 ± 0.5	28.1 ± 1.2	29.5 ± 0.7
	N	72	44	29
Beak-feather	**Range**	12.5 - 14.1	13.3 - 16	14.4 – 16.9
	Mean SD	13.3 ± 0.4	14.7 ± 0.7	15.7 ± 0.6
	N	71	44	29
Beak-naris	**Range**	6.8 - 8	7.6 - 9	8.4 – 9.5
	Mean SD	7.4 ± 0.3	8.3 ± 0.4	9.0 ± 0.3
	N	72	44	29
Beak depth	**Range**	6.7 - 8.1	6.8 - 9.2	8.1 – 9.4
	Mean SD	7.3 ± 0.3	8.0 ± 0.5	8.6 ± 0.3
	N	72	44	29
Beak width	**Range**	6 - 7	6.2 - 8.1	6.8 – 8.4
	Mean SD	6.4 ± 0.3	7.0 ± 0.4	7.4 ± 0.3
	N	70	44	29
Tarsus	**Range**	18.6 – 21.6	19.9 - 23	21.1 – 24
	Mean SD	20.4 ± 0.7	21.6 ± 0.7	22.6 ± 0.7
	N	71	44	29
Wing length	**Range**	59 - 64	61 - 71	65 – 72
	Mean SD	62.2 ± 1.3	66 ± 2.8	68.4 ± 1.7
	N	70	44	28
Mass	**Range**	11 - 15	12 - 20	16 – 19
	Mean SD	12.8 ± 0.8	14.4 ± 2.3	17.9 ± 0.9
	N	38	27	25

Table 7.2 Variation in mean morphological traits between putative species and year. Shown are mean values and standard deviations for males (females excluded). Results of MANOVA show the effect of dependent variables on variation in male morphology (for putative species). F-values (F), and P-values are shown. Bold indicates significant values.

	Small tree finch		**Medium tree finch**		**Large tree finch**				
	Mean±SD (N)		**Mean±SD (N)**		**Mean±SD (N)**		**Year F P-value**	**Species F P- value**	**Interaction effect F P-value**
	2005	**2010**	**2005**	**2010**	**2005**	**2010**			
Beak-head	**26.2**±0.5 42	**26.4**±0.5 30	**29.0**±1.1 19	**27.5**±1.0 26	**29.8**±0.7 5	**29.5**±0.6 24	11.28 **0.001**	177.66 <**0.001**	14.47 **<0.001**
Beak-feather	**13.3**±0.4 42	**13.4**±0.4 30	**15.2**±0.6 19	**14.4**±0.5 26	**15.9**±0.9 5	**15.7**±0.5 24	6.45 **0.012**	196.12 **<0.001**	9.80 **<0.001**
Beak-naris	**7.4**±0.3 42	**7.5**±0.3 31	**8.6**±0.3 19	**8.0**±0.3 26	**9.3**±0.2 5	**8.9**±0.3 24	16.90 **<0.001**	196.23 **<0.001**	10.60 **<0.001**
Naris length	**1.6**±0.3 42	**1.8**±0.3 28	**1.7**±0.3 19	**2.1**±0.4 24	**1.8**±0.3 5	**2.2**±0.3 23	22.68 **<0.001**	6.30 **0.002**	1.01 **0.37**
Beak depth	**7.2**±0.2 42	**7.4**±0.3 31	**8.3**±0.5 19	**7.8**±0.5 26	**8.7**±0.5 5	**8.6**±0.3 24	0.61 0.44	129.14 **<0.001**	9.60 **<0.001**
Beak width	**6.3**±0.2 42	**6.7**±0.3 31	**7.2**±0.5 19	**6.9**±0.4 26	**7.6**±0.5 5	**7.4**±0.3 24	0.14 0.70	94.73	6.80
Tarsus	**20.4**±0.6 42	**20.5**±0.8 30	**21.9**±0.8 19	**21.5**±0.7 26	**23.2**±0.3 5	**22.5**±0.7 24	5.58 **0.02**	104.43 **<0.001**	13.46 0.15
Mass	**13.3**±0.9 9	**12.7**±0.8 30	**18.7**±5.2 4	**14.1**±2.0 24	**19.4** 5	**17.8**±0.9 24			

Table 7.3 Mean and variance of: (1) beak size (principal components) and wing length for tree finches assigned to each of the three clusters in 1938-39, and (2) beak and body size (principal components) for tree finches assigned to each of the two clusters in each 2005 and 2010. Distance between cluster means is given between clusters one and two (1&2) and two and three (2&3) for historical data and clusters one and two for modern data.

Year	Component	Cluster	Mean Scores	Variance	Distance between cluster means
1938-39	Beak size	1	-1.22	0.97	1.84 (1&2)
		2	0.62	0.17	
		3	1.70	0.40	1.08 (2&3)
	Wing length	1	63.82	2.72	5.86 (1&2)
		2	69.68	2.92	
		3	72.33	3.28	2.65 (2&3)
2005	Beak size	1	-0.88	0.15	2.0
		2	1.12	0.33	
	Body size	1	-0.73	0.18	1.98
		2	1.25	0.17	
2010	Beak size	1	-0.37	0.23	1.63
		2	1.26	0.15	
	Body size	1	-0.39	0.32	1.56
		2	1.17	0.22	

Table 7.4 Comparison of Floreana tree finch morphological measurements taken by David Lack (1938-39) and Sonia Kleindorfer and Jody O'Connor (2005 & 2010). All data is for male finches. Results of one-way ANOVA show the effect of dependent variables on variation in male morphology. F-values (F), and P-values are shown.

		David Lack		**Kleindorfer & O'Connor**		
	Species	**N**	**Mean**±SD (Range)	**N**	**Mean**±SD (Range)	**F** **P-value**
Beak depth	**small tree finch**	55	7.5±0.3 (6.7-8.7)	73	7.3±0.3 (6.3-8.3)	22.70 **<0.001**
	medium tree finch	66	8.8±0.4 (8.1-9.9)	44	8.0±0.5 (6.8-9.2)	83.87 **<0.001**
	large tree finch	3	10.7±0.6 (10-11.1)	29	8.6±0.3 (7.7-9.4)	88.86 **<0.001**
Beak-naris	**small tree finch**	87	7.3±0.3 (6.8-8.1)	73	7.4±0.3 (6.5-8.4)	1.86 0.17
	medium tree finch	80	9.0±0.4 (8.0-10.2)	44	8.3±0.4 (7.6-9.1)	101.33 **<0.001**
	large tree finch	4	9.9±0.3 (9.5-10.2)	29	9.0±0.3 (8.3-9.5)	31.53 **<0.001**
Wing	**small tree finch**	80	63.8±1.7 (60-68)	72	62.2±1.4 (56-70)	40.80 **<0.001**
	medium tree finch	82	69.9±1.7 (66-74)	43	65.3±5.0 (60-71)	55.63 **<0.001**
	large tree finch	4	72.3±2.1 (70-74)	29	68.2±2.0 (62-72)	13.40 **<0.001**

Table 7.5 Percentage membership of putative species (small, medium and large tree finch) to a genetic population based on STRUCTURE assignment (Population 1 (Pop 1), Population 2 (Pop 2), or mixed assignment (probability of assignment (<0.75)). Data is for both males and females.

		Small tree finch	Medium tree finch	Large tree finch
2005	**% Pop 1**	0%	92% (22)	100% (8)
	% Pop 2	61% (38)	0%	0%
	% Mixed	39% (24)	8% (2)	0%
2010	**% Pop 1**	2% (1)	3% (1)	97% (28)
	% Pop 2	48% (22)	3% (1)	0%
	% Mixed	50% (23)	94% (30)	3% (1)

Table 7.6 Variation in mean morphological traits between genetic populations and year. Shown are mean values and standard deviations for males (females excluded). Results of MANOVA show the effect of dependent variables on variation in male morphology. F-values (F), and P-values are shown. Bold indicates significant values.

	Population 1		**Population 2**		**"Mixed"**				
	Mean±SD (N)		**Mean±SD (N)**		**Mean±SD (N)**		**Year** F **P-value**	**Population** F **P- value**	**Interaction effect** F **P-value**
	2005	**2010**	**2005**	**2010**	**2005**	**2010**			
Beak-head	**29.3**±0.9 30	**29.2**±1.0 30	**26.1**±0.4 38	**26.3**±0.5 23	**26.2**±0.8 26	**26.9**±1.1 56	3.81 **0.05**	208.55 **<0.001**	4.83 **0.01**
Beak-feather	**15.4**±0.7 30	**15.5**±0.7 30	**13.2**±0.4 38	**13.4**±0.4 23	**13.3**±0.7 **26**	**13.9**±0.8 53	11.58 **<0.001**	188.91 **<0.001**	3.65 **0.03**
Beak-naris	**8.8**±0.4 30	**8.8**±0.6 30	**7.4**±0.3 38	**7.4**±0.3 23	**7.4**±0.4 26	**7.8**±0.5 54	4.67 **0.03**	181.15 **<0.001**	3.25 **0.04**
Naris length	**1.7**±0.3 30	**2.1**±0.4 29	**1.6**±0.2 38	**1.8**±0.3 20	**1.6**±0.4 26	**2.0**±0.4 50	31.70 **<0.001**	5.02 **0.01**	2.16 0.12
Beak depth	**8.3**±0.5 30	**8.5**±0.5 30	**7.1**±0.2 38	**7.4**±0.3 23	**7.2**±0.4 26	**7.7**±0.5 54	19.80 **<0.001**	125.36 **<0.001**	3.53 **0.03**
Beak width	**7.2**±0.5 30	**7.3**±0.4 30	**6.3**±0.2 38	**6.5**±0.3 23	**6.3**±0.3 26	**6.8**±0.4 54	24.26 **<0.001**	89.49 **<0.001**	7.05 **<0.001**
Tarsus	**22.1**±0.8 30	**22.2**±1.0 30	**20.3**±0.5 38	**20.4**±0.8 22	**20.3**±0.8 26	**20.9**±1.0 54	4.54 **0.04**	77.30 **<0.001**	2.27 0.11
Wing	**66.7**±0.9	**67.3**±2.9	**61.54**±1.2	**61.73**±2.0	**61.8**±2.0	**63.5**±2.5	4.44 **0.04**	50.56 **<0.001**	0.89 0.41
Mass	**17.3**±3.4	**17.4**±1.6	**12.7**±1.1	**13.1**±1.0	**13.0**±1.1	**13.5**±1.8			

8 General Discussion

This thesis identifies the trends and causal mechanisms behind population declines for Darwin's finches in the Galápagos Islands. Ecological and molecular genetic data give the first comprehensive assessment of conservation issues for Darwin's tree finches. Here, I show that *P. downsi* parasitism is the number one threat to Darwin's finches due to its severe and unprecedented impacts on nestling mortality (Chapters 3-6). In response to *P. downsi*, Darwin's finches can alter life history traits such as clutch size and parental care (Chapters 3-6) and produce *P. downsi*-specific antibodies (Huber et al., 2010), however none of these defences are sufficient to mitigate the negative effects of this parasite. Darwin's finches must also contend with nest predation (Chapters 3&4) and habitat clearance (Chapter 2). The combined impacts of introduced parasites, introduced predators, and habitat loss causes population decline in many endemic finch populations. As a result of this research, the IUCN RedList status of Darwin's medium tree finch has been reassessed from "vulnerable" but "data deficient" to "critically endangered". Declining population sizes and hybridisation between closely-related tree finch species has resulted in a loss of genetic diversity, and the possible local extinction of Darwin's large tree finch on Floreana Island. This finding demonstrates the necessity for enhanced ecological monitoring of Darwin's finches across the archipelago, especially for species that have small populations and unknown population trends. It is now time for the development and implementation of intensive conservation management plans to protect the Galápagos avifauna. Archipelago-wide *P. downsi* and rodent control plans will be crucial for the persistence of Galápagos land birds and the reintroduction of other critically endangered bird species such as the Floreana mockingbird, *M. trifasciatus,* and Darwin's mangrove finch, *C. heliobates,* back into habitats from which they had been extirpated (Fessl et al., 2010, Deem et al., in press).

8.1 Darwin's tree finches are at high risk of future population decline

Habitat loss and high parasite intensity in native highland forest

This thesis identifies Darwin's tree finches (*Camarhynchus* spp.) as being at a high risk of future population decline, with significant declines already reported for the medium tree finch (Chapters 2&3), and a loss of genetic diversity within the Floreana tree finch group (Chapter 7). Compared to ground finches (*Geospizinae*), tree finches have more specialised habitat requirements and are largely restricted to elevated forest habitat where their preferred nesting tree – *Scalesia*– is critically endangered. Elevated forest habitats harbour the highest abundance of *P. downsi* parasites, and since tree finches have small brood sizes, the impacts of *P. downsi* larvae are intensified on fewer nestlings (Chapter 3). A recent survey of nine bird species on Santa Cruz Island (Dvorak et al., in press) found population declines in six species, with the most significant declines occurring at higher elevations in humid native forest and agricultural areas. The two Santa Cruz species that have the most *P. downsi* parasites per nest (Dudaniec et al., 2007), showed the strongest declines (Dvorak et al., in press), which is comparable to our results of high parasite intensity and population decline in the medium tree finch (Chapter 3). Large tree finches show strong declines across the archipelago (Dvorak et al., in press), and we provide evidence for the possible loss of this species via hybridisation with smaller species (i.e. the medium tree finch)(Chapter 7). Although the native highland forest of Floreana Island is partially cleared and degraded, it appears to contain the largest and best-quality *Scalesia* habitat (albeit only 4km^2) left on the entire archipelago (O'Connor and Kleindorfer personal observation, and M. Dvorak personal communication). It is imperative that these last stands of quality habitat are conserved for the persistence of Floreana's tree finches, including the only population of Darwin's medium tree finch (Chapter 2).

Low recruitment

A significant finding of this thesis is a quantification of low recruitment into the host breeding population as the result of *P. downsi* parasitism (Chapters 2-6). Currently, it is possible to observe many Darwin finch species, including numerous small and medium ground finches and small tree finches (Dvorak et al., in press, Chapter 2). But the chapters of this thesis show extremely low recruitment into the breeding population over the last five years (Chapters 3-6). The implication of this observation is that we will observe significant population crashes in the near future. Finches show prolific nesting activity in very wet years (often after many dry years with little breeding), which we found in 2008 (Chapters 3&5). But *P. downsi* intensity also increases with rainfall, and causes high nestling mortality (Dudaniec et al., 2007, Chapters 3,5&6). In fact, recent surveys have confirmed that finch population numbers did not increase after the heavy rains of 2008 as would be expected according to the high level of nesting activity in that year (Dvorak et al., in press, Chapters 3,5&6). It must also be noted that, in this thesis, nestling mortality due to parasitism was probably underestimated, and nest predation rates were overestimated in Chapters 3 & 4 (data from 2004-2006). After installing video cameras in nests (in 2008 & 2010), I found that dead, parasitised nestlings were frequently removed from the nest by parents; the video images did not show any nestlings being taken from the nest by predators (Chapters 5 & 6). Factors affecting population recruitment must therefore be considered in the ongoing conservation management of Galápagos birds.

Loss of genetic diversity via hybridisation

Hybridisation is common between closely-related species that occur in sympatry (Grant and Grant, 1992). This natural process can, however, be a conservation concern for species with small and/or declining populations if it is swamped by introgression from closely-related taxa (Dabrowski and Fraser, 2005, Taylor et al., 2006). Human induced ecological change has been shown to increase rates of hybridisation leading to phenotypic changes within closely-related populations (Dabrowski and Fraser, 2005, Taylor et al., 2006). We propose a similar mechanism to explain the decline in large tree finches on Floreana Island (Chapter 7). Large tree finches have always been rare on Floreana Island (Steadman, 1986, Grant et al., 2005), but despite extensive mist-netting surveys in prime *Scalesia* habitat, we have not captured any large tree finches that are as big as those found by David Lack in 1938-39 (Chapter 7). Analysis of Floreana tree finch morphological data from 1938-39 (Lack, 1947) showed three distinct morphological clusters, which correspond with Lack's classification of small, medium, and large tree finches respectively. Analysis of modern morphological data (2005 & 2010) detected only two genetic and morphological clusters within the Floreana tree finch group. This suggests that only two species may currently exist: one with small morphology (small tree finch) and one with larger morphology (which corresponds to Lack's classification of a medium tree finch). The high proportion of hybrid individuals detected in our modern dataset suggests that the large tree finch may have disappeared via hybridisation with smaller individuals (e.g. medium tree finches). Hybridisation is traditionally identified as a mechanism for speciation (Grant et al., 1996, Price, 2008, Brelsford et al., 2011), but our findings add to a new body of evidence that demonstrates the role of hybridisation for the *loss* of biodiversity; that is, "speciation in reverse" (Seehausen, 2006, Taylor et al., 2006, De León et al., 2011, Webb et al., 2011)

8.2 *Philornis downsi* control programs- an immediate priority

Since the discovery of *P. downsi* larvae in Darwin's finch nests in 1997, we have assessed the interspecific, temporal, and spatial variation in its prevalence, impacts, and invasiveness (Fessl and Tebbich, 2002, Causton et al., 2006, Dudaniec et al., 2007, Wiedenfeld et al., 2007, Kleindorfer and Dudaniec, 2009, Dudaniec et al., 2010, O'Connor et al., 2010a, O'Connor et al., 2010d), documented its life cycle (Fessl et al., 2006b, Dudaniec et al., 2010, O'Connor et al., 2010b), and identified microsatellite markers for genetic analyses (Dudaniec et al., 2008, Dudaniec et al., 2010). This thesis explicitly demonstrates the inability of Darwin's finches to adapt to the impacts of this parasite, at least since 1997. Despite using behavioural defences such as the removal of parasites from nests, removal of dead infested nestlings from nests, increased preening, and increased feeding of parasitised nestlings (Chapters 5 & 6), the majority of nestlings still died due to the severe impacts of larval feeding (Chapters 2-6).

The immediate priority for the conservation of Galápagos avifauna is to develop an effective control program for *P. downsi*. The only known effective treatment to reduce *P. downsi* infestations is to spray individual finch nests with 1% pyrethrin solution, which effectively eliminates infestation by killing larvae and deterring flies from the nest (Fessl et al., 2006a, Chapter 6, O'Connor personal observation). This method is labour–intensive, difficult to apply to very high nests (especially those in *Opuntia* cacti), may result in insecticide resistant populations, and is therefore not feasible as a long-term strategy for parasite control. An effective trapping method for *P. downsi* flies has not yet been developed: various traps and lures have been trialled by myself and scientists at the Charles Darwin Foundation with little success. The documentation of fly and larval behaviour in nests (Chapetrs 5&6) has contributed to experimental design of current research on the use of long-range *P. downsi* pheremonal attractant to lure flies into traps (pers comm, R.Collignon, Syracuse University).

For example, the lack of mating behaviour in nests (Chapters 5&6) provides excellent evidence that *P. downsi* flies may instead use a long-range pheremonal attractant to locate each other for mating. Two new observations from video footage of flies in nests have clarified other uncertainties about how flies find host nests and how the 1st instar larvae infect nestlings. Chapter 5 clarifies that flies lay eggs on the nest lining which then hatch into larvae and infect nestlings via the nares (it was previously assumed that flies laid eggs or live larvae directly on nestlings' nares (Fessl et al., 2006b)), and that flies do not enter nests when parent finches are present. These observations support the idea that flies use multiple cues to locate hosts nests (and not just CO^2 emitted from birds in nests see (Muth, 2007, Kleindorfer and Dudaniec, 2009), and will therefore assist in the development of methodology for fly control in the future.

Further research should focus on biological control agents or environmentally benign measures such as or the sterile insect technique to reduce *P. downsi* fly populations. A promising approach for biological control of *P. downsi* could come from exploiting a natural interaction between *P. downsi* and two species of wasp parasitoids (*Spalangia endius, Brachymeria podagrica)*, which have also been accidentally introduced to the Galápagos (Causton et al., 2006). *Spalangia* and *Brachymeria* larvae parasitise Dipteran species in their pupal stages (Couri et al., 2006, Oliva, 2008). Preliminary studies have found that 5% of *P. downsi* pupae are parasitised by wasp parasitoids on Santa Cruz Island (C. Causton and P. Lincago, unpublished data), but no parasitised pupae have been recorded from Floreana (O'Connor, unpublished data). Further research should aim to determine the current distribution of these parasitoids across the archipelago and conduct a cost-benefit analysis to assess the potential impacts of parasitism of native Galápagos insects. Incidentally, *Spalangia* and *Brachymeria* wasp parasitoids may also help control other invasive Dipterans that have been introduced to the Galápagos. For example, *Spalangia* and *Brachymeria* wasps are known

to parasitise *Chrysoma*, *Peckia*, *Oxysarcodexia*, *Musca* & *Stomoxys* flies (Marchiori et al., 2003, Birkemoe et al., 2004, Geden et al., 2006, Oliva, 2008), which are invasive species to the Galápagos (Causton et al., 2006). Of the 90 known Dipterans in the Galápagos, only 23 are confirmed as native, 26 introduced, and the rest of unknown origin (Causton et al., 2006). Another avenue for *P. downsi* management is the sterile insect technique: a method of pest control in which masses of sterile males of the target species are released (Krafsur, 1998, Lance and McInnis, 2005). When wild fertile females mate with these males, their reproduction is reduced such that – over a number of generations – the population shrinks to an unsustainable density and dies out (Krafsur, 1998, Lance and McInnis, 2005). This method has been used to eradicate fruit fly *Bactrocera cucurbitae* from South western islands of Japan and Tsetse flies from parts of Nigeria and Tanzania, and is in use world-wide against a number of other pest species (Hendrichs et al., 2005). However, this technique may not be successful with *P. downsi* as recent genetic evidence shows that female *P. downsi* flies frequently mate with multiple males (Dudaniec et al., 2010) and may therefore achieve mating success with fertile males amongst the sterile release (Lance and McInnis, 2005). The technique also requires intensive fly breeding programs to produce millions of male flies for sterilization. However, captive breeding programs for *P. downsi* have not successfully reared any larvae past 2nd instar stages (*P. downsi* has three larval instar stages before pupation). More research is required to close the *P. downsi* life cycle under laboratory conditions for the sterile insect technique to be considered feasible (P. Lincago and C. Causton, unpublished data). A *Philornis* workshop, involving all *P. downsi* researchers, has been planned for February 2012 with the goal to synthesise all current knowledge and ideas for control. A key outcome of this meeting will to collectively devise a strategic research and management plan to mitigate the effects of *P. downsi* parasitism on native birds.

8.3 A multi-faceted approach to conservation in the Galápagos

The chapters of this thesis provide a crucial assessment of the conservation issues affecting species declines for birds on Floreana Island, especially for the previously understudied tree finch group. The next era of bird conservation in the Galápagos must focus on the control of invasive species, restoration of critical habitats, and continued monitoring of sensitive species. Such an approach requires joint involvement from researchers and managers to develop and implement effective programs. Specifically, the current relationships between the Charles Darwin Foundation, Galápagos National Parks, visiting scientists, and NGOs (e.g. WWF, Conservation International, Durrell Wildlife Trust) must continue to foster cohesive management plans with clear conservation goals. Targeted conservation management for critically endangered species such as the mangrove finch, medium tree finch, Floreana mockingbird, and Galápagos petrel are essential for their recovery. All four species suffer from reduced recruitment due to nest predation by introduced rats (Curry, 1986, Cruz and Cruz, 1990, Fessl et al., 2010, O'Connor et al., 2010d), and would benefit from large-scale rat control programs. For example, Floreana National Parks currently bait a small area within the central cone of Cerro Pajas volcano to control rodent populations around a nesting site for Galápagos Petrels. This baiting should be extended to the adjoining tree finch breeding habitat in *Scalesia* forest where rodents also depredate finch eggs (Chapters 3,4,6). Avian disease monitoring is also a high priority, especially considering the negative impacts of Avian pox virus on Santa Cruz (Wikelski et al., 2004, Kleindorfer and Dudaniec, 2006), and the recent discovery of *Plasmodium* blood parasites (which cause avian malaria) in the Galápagos penguins (Levin, 2009). Disease transmission from introduced poultry has also been identified as a concern for Galápagos birds, with a recent survey finding pathogens such as adenovirus and paramyxovirus in wild birds on Floreana (Deem et al., in press). Broad-scale habitat restoration of degraded mangrove *Scalesia* forest and *Opuntia* habitats will have positive

effects on many Galápagos fauna (Curry, 1986, Fessl et al., 2010, O'Connor et al., 2010c, Dvorak et al., in press). This will be particularly achievable due to the eradication of habitat-destroying feral goats and donkeys from many islands (Guo, 2006, Carrion et al., 2007)

In a study of prehistoric versus modern extinction rates of Pacific Island birds, David Steadman concludes: “We expect extinction after people arrive on an island. Survival is the exception” (Steadman, 1995). There is still time for Galápagos birds to be the exception to this rule. The Galápagos archipelago retains 100% of its native avifauna species, hence we now have a small “window of opportunity” to prevent species extinctions in the future. This thesis adds to a body of evidence, which now identifies conservation issues for the most threatened avifauna of the Galápagos Islands.

Appendix

Appendix 7A. Allelic variation at 10 microsatellite loci across two years (2005 and 2010). Loci that depart significantly from Hardy-Weinberg equilibrium are indicated in bold.

		Locus	N	N_A	H_E	H_O
2005	small tree finch	Gf01	62	16	0.88	0.90
		Gf03	59	10	0.67	0.61
		Gf04	**61**	**3**	**0.24**	**0.18**
		Gf05	57	6	0.60	0.60
		Gf06	62	3	0.06	0.03
		Gf07	60	6	0.36	0.37
		Gf09	59	7	0.33	0.29
		Gf11	62	7	0.55	0.56
		Gf12	62	10	0.82	0.76
		Gf13	62	13	0.75	0.73
	medium tree finch	**Gf01**	**24**	**13**	**0.89**	**0.75**
		Gf03	23	7	0.68	0.61
		Gf04	24	3	0.16	0.08
		Gf05	22	5	0.76	0.77
		Gf06	24	3	0.16	0.13
		Gf07	24	3	0.19	0.21
		Gf09	24	5	0.35	0.33
		Gf11	24	5	0.44	0.33
		Gf12	24	9	0.75	0.75
		Gf13	24	10	0.54	0.46
	large tree finch	Gf01	8	10	0.88	1.00
		Gf03	7	5	0.61	0.43
		Gf04	8	1	0.00	0.00
		Gf05	8	4	0.68	1.00
		Gf06	7	1	0.00	0.00
		Gf07	6	2	0.15	0.17
		Gf09	8	2	0.12	0.13
		Gf11	8	4	0.65	0.75
		Gf12	8	6	0.73	0.75
		Gf13	8	3	0.40	0.50
2010	small tree finch	**Gf01**	**44**	**15**	**0.90**	**0.73**
		Gf03	42	9	0.71	0.55
		Gf04	45	2	0.16	0.13
		Gf05	42	5	0.63	0.26
		Gf06	45	2	0.10	0.07

	Gf07	43	7	0.58	0.56
	Gf09	**41**	**7**	**0.61**	**0.22**
	Gf11	44	5	0.59	0.34
	Gf12	39	9	0.84	0.87
	Gf13	42	9	0.61	0.52
medium tree finch	Gf01	32	14	0.89	0.81
	Gf03	**30**	**8**	**0.75**	**0.63**
	Gf04	32	3	0.12	0.13
	Gf05	30	5	0.71	0.63
	Gf06	31	1	0.00	0.00
	Gf07	29	7	0.41	0.48
	Gf09	**31**	**8**	**0.59**	**0.29**
	Gf11	**31**	**8**	**0.56**	**0.35**
	Gf12	32	10	0.83	0.81
	Gf13	31	9	0.69	0.52
large tree finch	**Gf01**	**28**	**15**	**0.90**	**0.64**
	Gf03	29	9	0.68	0.59
	Gf04	27	2	0.07	0.00
	Gf05	26	6	0.70	0.73
	Gf06	30	2	0.10	0.10
	Gf07	**29**	**6**	**0.51**	**0.45**
	Gf09	**30**	**6**	**0.59**	**0.20**
	Gf11	**20**	**6**	**0.61**	**0.30**
	Gf12	26	10	0.86	0.81
	Gf13	30	5	0.69	0.63

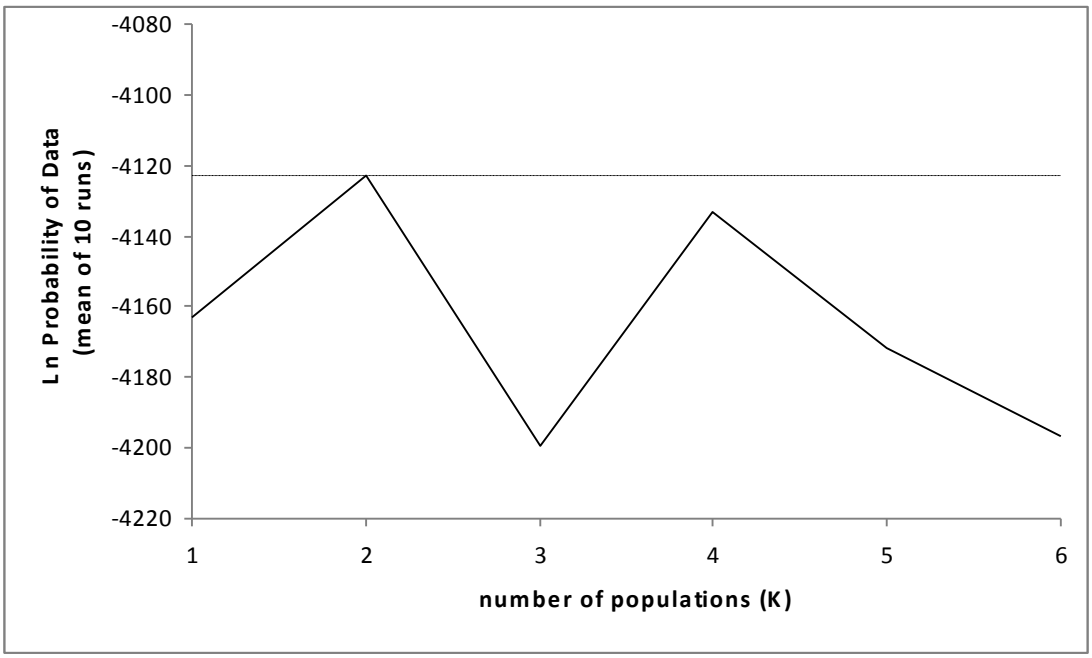
N= sample size; N_A =number of alleles; H_E =observed heterozygosity; H_O =expected heterozygosity (GenAlex; v 6.4.1)(GENEPOP v 4.0.10).

Appendix 7B**.** Global allelic variation at 10 microsatellite loci across two years (2005 and 2010). Loci that depart significantly from Hardy-Weinberg equilibrium are indicated in bold.

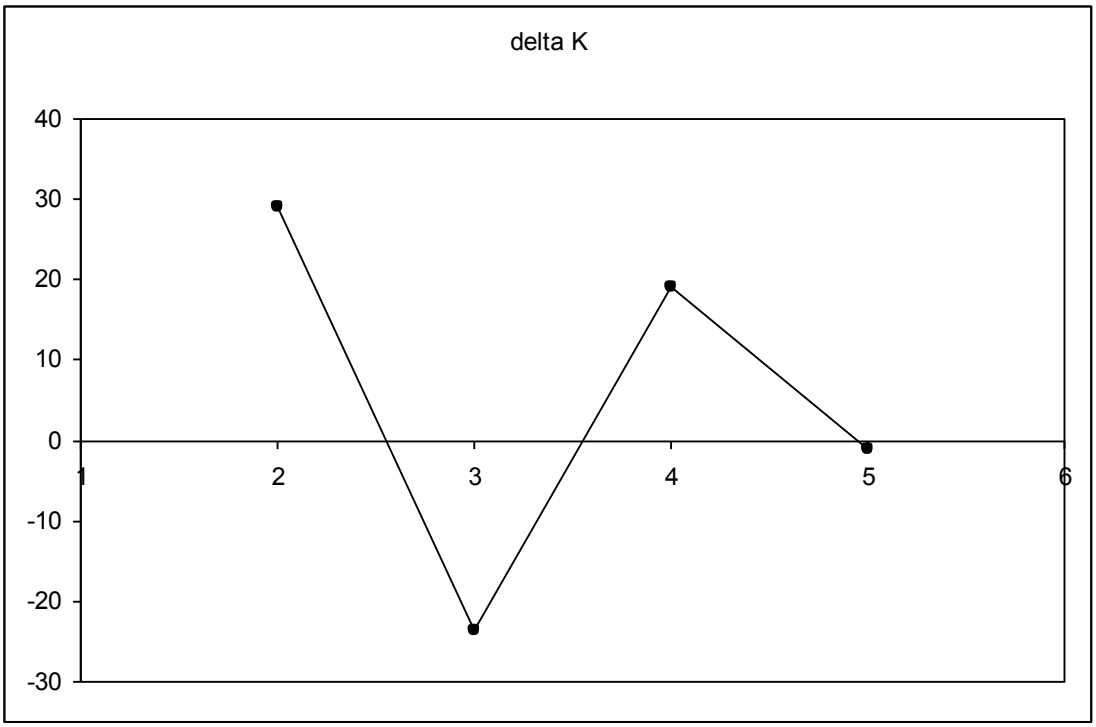
Locus	*N*	N_A	H_E	H_O	F_{IS}
Gf01	198	20	0.90	0.80	0.12
Gf03	190	11	0.71	0.59	0.17
Gf04	197	4	0.16	0.12	0.29
Gf05	185	7	0.68	0.58	0.15
Gf06	199	3	0.08	0.06	0.29
Gf07	191	8	0.43	0.41	0.03
Gf09	**193**	**12**	**0.57**	**0.26**	**0.55**
Gf11	**189**	**10**	**0.57**	**0.43**	**0.25**
Gf12	191	12	0.84	0.80	0.06
Gf13	197	14	0.69	0.59	0.14

N= sample size; N_A =number of alleles; H_E =observed heterozygosity; H_O =expected heterozygosity (GenAlex; v 6.4.1); F_{IS} =inbreeding co-efficient (Weir and Cockerham estimate)(GENEPOP v 4.0.10).

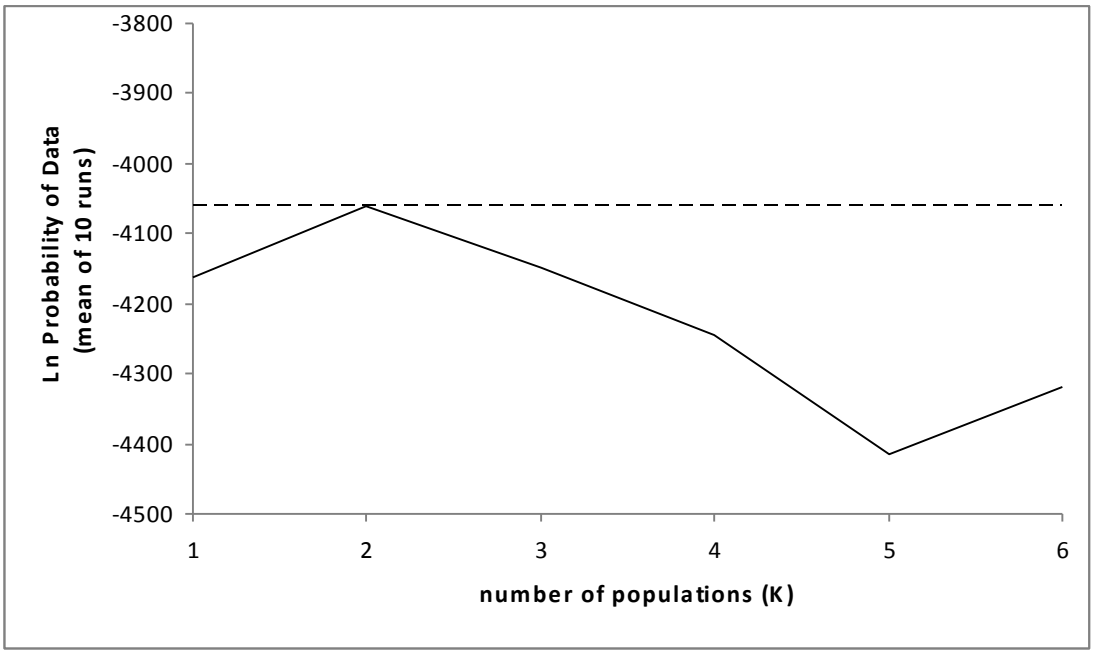
Appendix 7C. Mean logarithm of probability of the data for K = 1-6 estimated using the standard STRUCTURE model. Dashed line represents maximal logarithm of probability of the data.



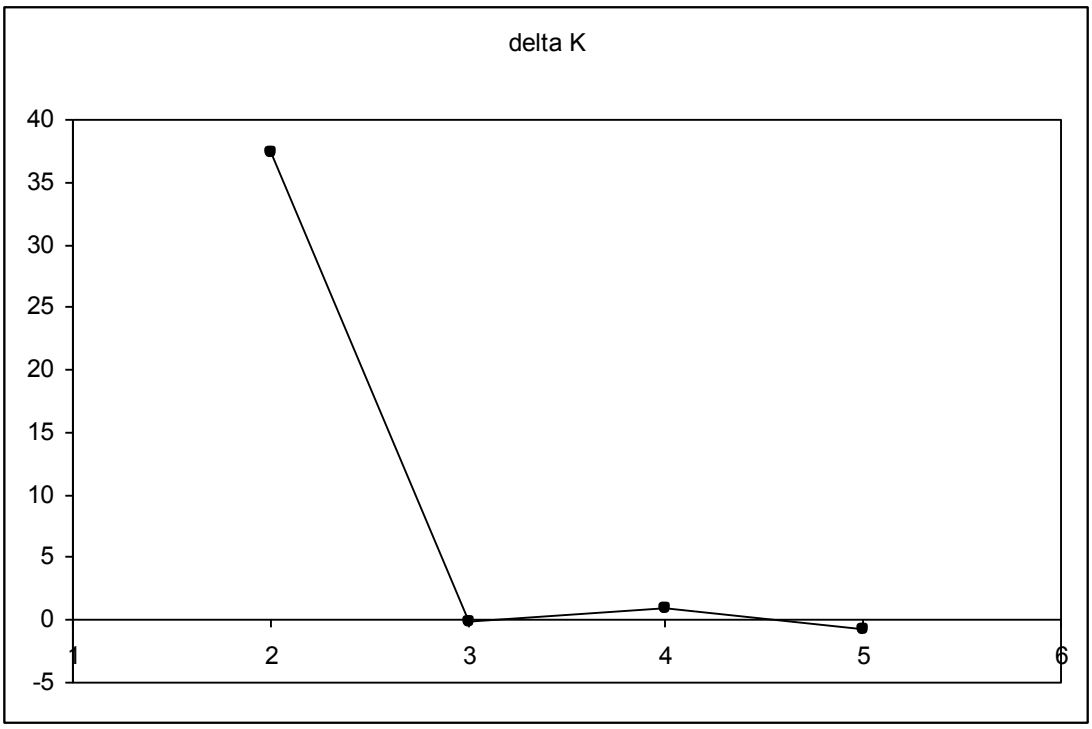
Appendix 7D: Delta K for K = 1-6, calculated by transforming logarithm of probability of the data estimated using the standard structure model.



Appendix 7E: Mean logarithm of probability of the data for K=1-6 estimated using the locprior model in STRUCTURE. Dashed line represents maximal logarithm of probability of the data.



Appendix 7F: Delta K for K = 1-6, calculated by transforming logarithm of probability of the data estimated using the LOCPRIOR model in STRUCTURE.



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