IMPACT AND MOLECULAR ECOLOGY OF *Philornis downsi*:

AN INTRODUCED PARASITIC FLY OF BIRDS ON

THE GALÁPAGOS ISLANDS

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

DECLARATION

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

Rachael Yvonne Dudaniec

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THESIS ABSTRACT

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

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

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

STATEMENT OF AUTHORSHIP

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

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

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

Rachael Yvonne Dudaniec

CHAPTER 1: Introduction

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

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

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

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

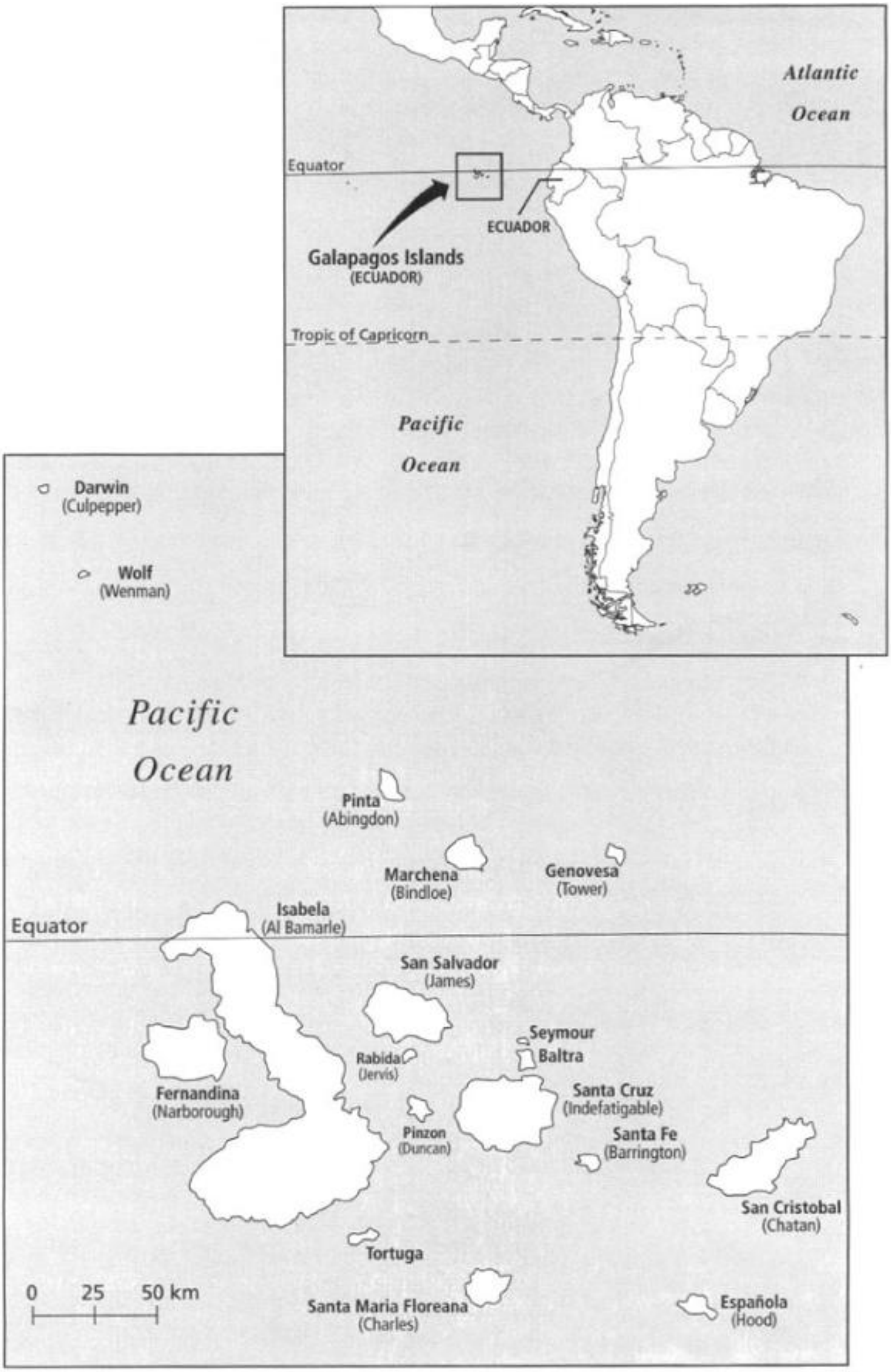

Figure 1.1 Map of the Galápagos Islands, Ecuador.

(a)

(b)

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

Avian host-parasite interactions

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

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

Genetics of island parasite invasions

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

(a)

(b)

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

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

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

Molecular insights into parasite ecology

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

(a)

(b)

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

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

The role of genetics in controlling parasitic flies

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

Thesis scope and objectives

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

Specifically, the aims of this study are to:

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

Organisation of the thesis

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