Adaptive divergence, genetic connectivity, and post-parasitism morbidity in Darwin's small ground finch, *Geospiza fuliginosa*, on the island of Santa Cruz, Galápagos Archipelago.

Toby Heath Galligan

A thesis submitted in fulfilment of the requirements for the

Degree of Doctor of Philosophy

School of Biological Sciences

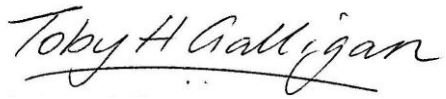
Faculty of Science and Engineering

Flinders University

I dedicate this work first to my beautiful Zonnetje, my family, and my friends – all of whom understand why I did it; and second to all the small ground finches – all of which will never understand why I did it.

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.



Toby Heath Galligan

10th January 2011

CONTENTS

THESIS SUMMARY

Speciation is arguably the most important problem in evolutionary biology. Following the biological species concept, speciation is the process by which populations of one species reduce inter-population mating – that is, gene flow – to the point where they become two reproductively isolated species. Gene flow can be reduced more or less incidentally by geographical isolation (i.e., allopatry), or by strong divergent selection on intrinsic barriers (e.g. immigrant inviability, divergent mate preference, or divergent mate recognition) in the same (symparty) or adjacent (parapatry) locations. In birds, the beak is used for foraging and mate recognition (e.g. song production); thereby, divergent niches or habitats can directly select for adaptive divergence in beak dimensions, while indirectly selecting for divergence in mate recognition. The significance of allopatric divergence has been long appreciated; however, the significance of sympatric and parapatric divergence remains debated (particularly in birds). Darwin's finches of the Galápagos Archipelago are a model system in which to study evolution in nature. On the island of Santa Cruz, Darwin's small ground finch *G. fuliginosa* has recently expanded its range from the arid lowlands into the humid highlands; the ecological contrast between these zones providing strong disruptive selection. Previous studies have shown evidence for adaptive divergence in this system (i.e., morphological clines along the ecological cline, environment-phenotype matching at the extreme zones, and more resightings across years of individuals with predicted trait values for each zone). My thesis has expanded on this work in five ways. First, I have used neutral molecular data to show high gene flow among all ecological zones on Santa Cruz; rejecting non-adaptive divergence in this system (Chapter 2). Second, I have shown the predicted breakdown of morphological clines under relaxed selection in a "benign" high rainfall year; which infers a central role for alternating strong and weak selection against immigrants as a mechanism of divergence in this system (Chapter 3). Third, I have revealed a loss of assortative pairing within highland-colonist *G. fuliginosa* in response to ecological opportunities and reduced interspecific competition that have followed range expansion (Chapter 4). Fourth, I

have demonstrated the importance of ecological contrasts in the formation of barriers to gene flow, by showing greater divergence in song and song discrimination between lowland and highland zones, than between localities within each zone, while controlling for geographical distance (Chapter 5). Fifth, I have shown that the introduced parasitic botfly *P. downsi*, which is causes high nestling mortality in Darwin's finches, also causes beak malformations that may significantly influence adaptation, mate recognition, and divergence in this system and this group of birds as a whole (Chapter 6). In synthesising my findings, I conclude while strong divergent selection exists between lowland and highland zones, intrinsic aspects of *G. fuliginosa* (e.g. high mobility) and Santa Cruz (e.g. no physical barriers between zones) can permit high levels of active dispersal, and probably gene flow, between zones (Chapter 3). In low rainfall periods, divergent selection and adaptive divergence is predicted to be strongest; whereas, in high rainfall years divergent selection is weakest and immigration of otherwise ill-adapted individuals is high, effectively reshuffling phenotypes among zones (Chapter 3). The long-term product of these counter processes requires further research. Yet, song discrimination in lowland *G. fuliginosa* in a high rainfall year suggests that partial barriers to gene flow may have arisen (Chapter 5).

STATEMENT OF AUTHORSHIP AND ACKNOWLEDGEMENT

This thesis represents original and independent research. I have performed all significant aspects of the research design, analysis, and interpretation.

I have presented this thesis as a series of manuscripts that are either published or "in preparation" for publication in scientific journals. Each chapter represents a separate manuscript. Published chapters are formatted according to the author guidelines for the journal of submission; bar tables and figures, which are imbedded in the text for ease of reading. The journal of submission for each manuscript is given on chapter title pages, where I have also acknowledged my collaborators as co-authors. Further acknowledgements are made at the end of each chapter. The contribution of collaborators is as follows:

Sonia Kleindorfer, my primary supervisor, instructed me in evolutionary ecology research theory, field techniques for the collection of data, and desk-top data analysis. Sonia contributed data collected prior to 2008, financial support for data collection in 2008, and comments on all manuscript drafts. Further, this research would not have been possible without Sonia's established connections with the Charles Darwin Research Station and Galápagos National Park in Ecuador.

Steve C. Donnellan, my secondary supervisor, instructed me in molecular genetics research theory, and field and laboratory techniques used to collect and analyse molecular data. Steve contributed comments to manuscript drafts were I have acknowledged him as co-author.

Frank J. Sulloway instructed me in evolutionary ecology research theory, experimental design, and statistical analysis for morphological and genetic data. Frank contributed comments to manuscript drafts were I have acknowledged him as co-author.

Terry Bertozzi and Alison J. Fitch instructed me in techniques used to collect and analyse molecular genetic data. Terry and Alison contributed comments to manuscript drafts were I have acknowledged them as co-authors.

Finally, the work represented by this thesis has adhered to the legal and ethical requirements of the Government of Australia, the Government of Ecuador, Charles Darwin Research Station (Ecuador), and Flinders University (Australia).

CHAPTER ONE

Introduction

Speciation

Speciation is arguably the most important problem in the study of evolutionary biology (Coyne and Orr 2004; Dieckmann *et al.* 2004; Grant and Grant 2008a; Price 2008). Speciation refers to the process by which a new species arises; this can occur if two or more populations of one species diverge in phenotype and/or genotype to an extent where they become reproductively isolated, cease exchanging genes freely, and thereby form two or more new species. Tens of millions of extant species and hundreds of millions of extinct species are proof of the significant influence speciation has on life. Speciation is the link between the occurrence of evolution (i.e., microevolution – genetic change within and between populations) and the vastness of diversity (i.e., macroevolution – genetic distinctness and disparity in higher taxa). As such, an enhanced understanding of the mechanisms for speciation is essential to an enhanced understanding of biodiversity and how best to conserve it.

Divergence with Gene Flow

It has long been appreciated that reproductive isolation can be achieved by completely restricting gene flow between populations by means of a physical structure in the landscape (Dobzhansky 1937; Mayr 1942, 1947). This is referred to as the *allopatric* mode of speciation, of which there are many examples that can be inferred in nature (reviewed in Coyne and Orr 2004). A good example of allopatric speciation would be two sister species each inhabiting different islands where expanses of water prevents interisland dispersal, and thereby, prevents gene flow (Mayr and Diamond 2001). In reality, many scenarios where allopatric speciation has been invoked, gene flow between divergent populations is likely to have been ongoing, but at potentially negligible levels (for example, dispersal between islands is not likely to be a singular event; *sensu* Petren *et al.* 2005). This raises two obvious questions: (1) *can speciation occur between populations where gene flow is not*

prevented by a physical structure in the landscape? (2) *And if so, how significant is speciation with gene flow?* The answers are: (1) yes – speciation *can* occur between populations where gene flow is not prevented by a physical structure in the landscape; and (2) unknown – the significance of speciation with gene flow remains unknown (reviewed in Coyne and Orr 2004; see also Nosil 2008).

We refer to modes of speciation with gene flow as *parapatric* – if populations inhabit separate geographical locations – or *sympatric* – if populations inhabit the same geographical location. For these modes, it is adaptation to differing habitats (parapatric), niches (sympatric), or sexual preferences (parapatric and sympatric) that drive divergence between populations, reducing gene flow over time, and results in reproductive isolation. More recently, the parapatric and sympatric modes are often referred to together as divergence with gene flow, which serves to highlight the key difference between these modes and the allopatric mode. Another key differences is that parapatric and sympatric speciation, unlike allopatric speciation that can be driven by non-adaptive processes (i.e., genetic drift, founder effects, and inbreeding), more often represent true ecological (Schluter 2000, 2001; Rundle and Nosil 2005) and adaptive (Dieckmann *et al.* 2004) speciation; where selection for adaptive divergence overrides the homogenising effect of gene flow.

Theoretically, divergence with gene flow is plausible (Endler 1977; Coyne and Orr 2004; Dieckmann *et al.* 2004; Gavrilets 2004; Von Doorn *et al.* 2009), but there is a scarcity of convincing examples in nature (reviews in Coyne and Orr 2004; Giraud *et al.* 2008; Price 2008; Rocha and Bowen 2008). This scarcity stems from the fact that in almost all scenarios where either parapatric or sympatric speciation can be invoked, so too can the more parsimonious allopatric speciation; for example, sister species with adjacent or overlapping distributions can be explained by secondary contact following speciation in allopatry (Coyne and Orr 2004). Therefore, the true significance of the parapatric and sympatric modes of speciation in nature remains unknown; particularly in groups like birds (reviewed in Price 2008).

Studying speciation in birds

Birds represent model organisms in which to study speciation (Mayr 1947, 1963; Lack 1947, 1976; Grant 1999; Grant and Grant 2008a; Price 2008). Birds are diverse (approximately 10,000 species), are easily identified in the field (by size, shape,

plumage, vocalisations, and behaviours), and generally sampled with little difficulty (because they are generally diurnal, non-reclusive, easily detectable, commonly encountered, and lack dangerous weapons). This is particularly so for the Passerines (Passeriformes) – the small to medium sized birds commonly referred to as the *song birds* or *perching birds*. Passerines also represent the most diverse group of birds; accounting for approximately half of all species. Perhaps above all, the key characteristic that makes birds ideal model organisms for divergence with gene flow research are their beaks (more accurately *bills*, however in the literature on Darwin's finches *beak* is traditionally used and therefore I use this term throughout this thesis).

A bird's beak has both an ecological and reproductive function; and thereby, links the two. Ecologically, the beak is used to acquire, manipulate, and consume food. Reproductively, the beak is used to attract, recognise, and select mates, via audible (song) and visual (size, shape, and colouration) cues. Because a bird's beak links foraging and mating behaviour, divergence in one can lead to divergence in the other (Grant 1999; Schluter 2001; Rundle and Nosil 2005; Grant and Grant 2008a; Benkman 2009). For example, if two habitats within a species' range differ in the primary type of food available, say the size of seeds, then selection would favour divergence in beak size to best adapt to foraging on large seeds in one habitat and small seeds in the other habitat. It follows that audible and visual cues that are dependent on beak size would simultaneously diverge between habitats also. For example, larger-beaked birds in one habitat may be physically constrained to sing lower frequency songs with slower trill rates and smaller-beaked birds in the other habitat may be physically constrained to sing higher frequency songs with faster trill rates (Podos 2001). Selection would also favour assortative mating between these two populations – that is, larger-beaked males and larger-beaked females more often mate than larger-beaked males and smaller-beaked females, and vice versa – so that offspring inherit the beak size adaptation favoured by their local habitat (Huber and Podos 2006). This process can continue in a positive feedback loop, increasing divergence and reducing gene flow to the point of speciation (i.e., ecological speciation: Schluter 2001; Rundle and Nosil 2005; Price 2008; or adaptive speciation: Dieckmann *et al.* 2004). Thus, a bird's beak represents a "magic trait"– that is, a trait that can facilitate reproductive isolation as a by-product of ecological divergence (Gavrilets 2004).

Of course there exist a number of alternative ecological, social, and biological mechanisms through which reproductive isolation can occur in birds and organisms in general (see Coyne and Orr 2004; Price 2008; Van Doorn *et al.* 2009); and an evolutionary ornithologist needs to be mindful of them all. However, the *beak as a magic trait* hypothesis is predicted to be particularly important in some groups of birds; including the species which I have studied here.

Darwin's Finches and the Galápagos Archipelago

Darwin's finches and the Galápagos Archipelago make arguably the finest system in which to study the dynamics of evolution in nature (Grant 1999; Schluter 2001; Grant and Grant 2008a). This statement is based on the following facts: (1) the Galápagos Archipelago is vastly isolated from other landmasses; (2) has a simple biotic community; and (3) is subject to an irregularly alternating wet and dry climate; and Darwin's finches (4) have adaptively radiated in the archipelago; (5) maintain high adaptive potential (i.e., behavioural and morphological flexibility); and (6) possess a "magic trait" for diversification to act on (i.e., a beak; Grant and Grant 2008a).

Expanding on these points, Darwin's finches represent 15 species of tanager (Thraupidae) belonging to the subfamily Tholospiza (Burns 2002). All are derived from a single common ancestor that arrived in the archipelago approximately two to three million years ago, and rapidly diversified in response to ecological opportunities and a lack of inter-specific competition (Lack 1947; Grant 1999; Grant and Grant 2008a). All but one species are endemic to the Galápagos Archipelago. Phenotypically, species differ greatest in the size and shape of their beaks, with almost all having a unique set of beak dimensions that are suited for a unique niche. Notable examples are: the fine pointed warbler-like beak of the insectivorous warbler finches *Certhidea* spp.; the large curved parrot-like beak of the folivorous vegetarian finch *Platyspiza crassirostris*; and the increasingly larger pyramidoid finch-like beaks of the granivorous small, medium, and large ground finches *Geospiza fuliginosa*, *Geospiza fortis*, and *Geospiza magnrostris* (respectively). However, considerable variation in beak dimensions can occur within species as well, which is best exemplified by populations of *Geospiza conirostris* and *Geospiza difficilis* inhabiting different islands (Grant and Grant 2008a).

The Galápagos Archipelago lies on the Equator approximately 1,000 km west of continental South America. Volcanic in origin, these islands first emerged from the Pacific Ocean approximately 10 million years ago (Christie *et al.* 1992; Sinton *et al.* 1996). The archipelago's isolation has restricted the diversity of organisms (particularly terrestrial ones) that have colonised it. Despite straddling the Equator, the Galápagos Archipelago is subject to a bi-seasonal climate influenced by ocean currents: specifically, a hot and wet season between January and May; and a cool and dry season for the rest of the year. In addition, climate in the Galápagos Archipelago is affected by the *El Niño*-Southern Oscillation, which irregularly brings brief high rainfall *El Niño* periods (spanning 1-2 years) to typically low rainfall *La Niña* periods (spanning 2-11 years) in the eastern Pacific Ocean (Snell and Rae 1999; see Chapter 3 [Fig. 2]).

Combined, the seasonal and annual climate in the Galápagos Archipelago is one of unpredictable extremes. As a result, the majority of organisms inhabiting these islands follow a boom-bust pattern of phenology. In the hot and wet season the islands' receive most of their annual rainfall and boom into life with mass plant growth and seeding, and subsequent mass reproduction in animals. In the cool and dry season the islands' receive no or very little rainfall and food production largely ceases, supply decreases, competition increases, and mortality among species increases. Alternating through *El Niño* and *La Niña* periods, this same bust-boom pattern observed annually is magnified across decades with dramatic effect on life in the Galápagos Archipelago (interestingly, what I have just described is only true for the terrestrial environment, and the marine environment responds in exactly the opposite direction: boom in the cool and dry season and in a *La Niña* year; bust in the hot and wet season and in a *El Niño* year).

Darwin's finches and the Galápagos Archipelago have enhanced our understanding of the interplay between evolution, ecology, and biology possibly more than any other system. This has been achieved through the work of many ingenious and determined researchers – David Lack, Peter Bowman, Peter Grant, Rosemary Grant, Ian Abbot, Lynette Abbott, Peter Boag, Lisle Gibbs, Laurene Ratcliffe, Dolph Schluter, Trevor Price, Ken Petren, Sonia Kleindorfer, Jeffery Podos, Andrew Hendry, Akie Sato, Lukas Keller, Sabine Tebbich, and Arhat Abzhanov: to name a few (their contributions are largely reviewed in Grant and Grant 2008a). Darwin's finches demonstrate how natural selection shapes

populations (Price *et al.* 1984; Gibbs and Grant 1987; Grant and Grant 1989; Grant and Grant 2002); how one species can adaptively radiate (speciate) into many others (Lack 1947; Grant 1999; Grant and Grant 2008a); and how speciation is a process, not an event (Grant and Grant 2008a). Work in this group has shown the central importance of character displacement and release (Boag and Grant 1984; Schluter *et al.* 1985; Grant and Grant 2006, 2010; Hendry *et al.* 2009); introgressive hybridisation (Grant 1993; Grant and Grant 1992, 1994, 1996, 2008b; Grant *et al.* 2005); and underlying genes (Abzhanov *et al.* 2004, 2006) for speciation. In addition, the link between ecological adaptation and reproduction isolation via beak morphology is apparent in Darwin's finches (Ratcliffe and Grant 1983, 1985; Christensen *et al.* 2006; Huber and Podos 2006; Podos 2001, 2010).

Darwin's finches and the Galápagos Archipelago have also been central to the divergence with gene flow debate. Traditionally, adaptive radiation of Darwin's finches has been regarded a text book example of allopatric speciation: where species largely diverged on separate islands and then established their present distributions (Lack 1947; Grant 1999; Grant and Grant 2008). However, in the last half of this decade, evidence has emerged that rejects a strict allopatric model – specifically, considerable gene flow between island populations (Petren *et al.* 2005) and species in sympatry (Grant *et al.* 2005); and suggest a potential important influence of within-island divergence – namely, adaptive divergence in a sympatric population of medium ground finch *G. fortis* (reviewed in de Leon 2010) and a parapatric population of small ground finch *G. fuliginosa* (reviewed in Kleindorfer and Mitchell 2009) both of which inhabit the central island of Santa Cruz.

Darwin's Small Ground Finch Geospiza fuliginosa *on the island of Santa Cruz*

Darwin's small ground *Geospiza fuliginosa* (Fig. 1), as its name suggests, is one of the smallest species of Darwin's finches (approximate mean weight = 14 g) and predominately forages close to or on the ground using the base of its beak to crush small seeds. *Geospiza fuliginosa* is the most abundant and widely distributed of Darwin's finches; the most recently split, evolutionarily (sister species to the medium ground finch *Geospiza fortis*; Petren *et al.* 1999); and the most generalist species, displaying a variety of foraging behaviours and consuming a diversity of prey (Bowman 1961; Kleindorfer *et al.* 2006).

Figure 1: Darwin's small ground finch *Geospiza fuliginosa* (male aged 3-4 years). Photograph by Frank J. Sulloway.

The island of Santa Cruz is the second largest (986 km^2) and highest island (850 m a.s.l.) in the Galápagos Archipelago. It is roughly circular in shape with its highest points in the centre (see Chapter 2 [Fig. 1]); it is also middle aged for the islands in the archipelago, with no obvious crater and a considerable deposit of soil in the

highlands. Rainfall (and precipitation from sea mists) increases with altitude on Santa Cruz; supporting four main ecological zones on the southern side of the island: running from the lowlands to the highlands they are the arid zone, transitional zone, agricultural zone, and humid zone. On the northern side of the island, the agricultural zone is absent, and the humid and transitional zones reduced due to southern prevailing winds and a rain shadow cast by the central peaks (at any given altitude the northern side receives less rainfall than the southern side). The extremes of the ecological gradient on Santa Cruz contrasts dramatically: dry-deciduous open forest and woodland in the arid zone; evergreen closed forest and shrubland in the humid zone. In addition, the biotic community and food productivity between these zones differs markedly; yet, *G. fuliginosa* forages and breeds in both. Therefore, *G. fuliginosa* is subjected to strong divergent natural selection within Santa Cruz.

Not having been recorded in the highlands prior to the 1960s, the current patterns of divergence in *G. fuliginosa* is the product of a recent range expansion from the lowlands. Range expansion was presumably facilitated by the invasion of small-seeding weeds – for which *G. fuliginosa* is preadapted to forage – and the local extinction of the sharp beaked finch *Geospiza difficilis* – which may have excluded *G. fuliginosa*; both changes the result of an increase in agriculture on Santa Cruz in the latter half of the 19th century. Therefore, *G. fuliginosa* on Santa Cruz are possible at a very early stage of adaptive divergence; an uncommon scenario in nature and one worth examining further.

Kleindorfer *et al.* (2006), Kleindorfer (2007), and Sulloway and Kleindorfer (in review) have shown evidence for adaptive divergence in this system. First, highland *G. fuliginosa* had longer beaks and shorter feet, and more often gleaned insects from understory foliage; whereas, lowland *G. fuliginosa* had shorter beaks and longer feet, and more often picked seed from the ground (i.e., environment-phenotype matching: Kleindorfer *et al.* 2006). Second, clines in beak length, foot size, and other traits were found along the ecological cline on the southern side of Santa Cruz (Sulloway and Kleindorfer in review). Third, morphological divergence was maintained over a six year period (i.e., 2000-2005: Kleindorfer *et al.* 2006; Sulloway and Kleindorfer in review). Fourth, individuals with morphological trait values predicted for the arid and humid zone were more often re-sighted in subsequent sampling years (i.e., trait utility: Sulloway and Kleindorfer in review). Fifth, highland *G. fuliginosa* had smaller clutch sizes, shorter renesting intervals, and

reduced behavioural conspicuousness in response to higher levels of depredation (Kleindorfer 2007).

However, the above evidence may not indicate *adaptation* in its classic sense – that is, a change in phenotype as a result of increased fitness on heritable traits. Phenotypic divergence between locations can also arise through phenotypic plasticity (i.e., an individual changes its phenotype to better match the environment) or matching habitat choice (i.e., an individual changes the environment to better match its phenotype; see Edelaar *et al.* 2008). While Phenotypic plasticity is unlikely because of the high heritability of morphological traits in Darwin's finches (e.g., bill length: Boag and Grant 1978; Boag 1983); matching habitat choice is a possible factor influencing phenotypic divergence in this system, particularly given the size of Santa Cruz and the dispersal ability of *G. fuliginosa*. Matching habitat choice can initiate and accelerate local adaptation, and may enable adaptive peak shifts (Edelaar *et al.* 2008; Holt and Barfield 2008); but it may also prevent classic adaptive divergence when selection against dispersal is negligible. Therefore, an enhanced understanding of divergence and dispersal in Santa Cruz's *G. fuliginosa* across space and time is required.

Darwin's finches and the introduced botfly Philornis downsi

A topic that impinges on all research in Darwin's finches is the impact of the introduced parasitic botfly *Philornis downsi* – identified as the greatest threat to the conservation of these birds (Causton *et al.* 2006). The larvae of *P. downsi* enter the nares of nestling and feed on blood and tissues. In some years, *P. downsi* is prevalent in 100 % of nests (Dudaniec *et al.* 2007) and causes 95 % nestling mortality (Fessl *et al.* 2006). Survivors of *P. downsi* parasitism are inflicted with nares and beak malformation; however, the implications for long-term survival and beak-centred divergence are presently unknown.

Objectives of my thesis

In my thesis, I will expand the examination of adaptive divergence, range expansion, and *P.downsi*-induced impact in *G. fuliginosa* on Santa Cruz. Specifically, I will:

1. Use neutral molecular data to examine population substructure and contemporary gene flow and validate adaptive divergence between ecological zones during periods of low rainfall;
2. Use morphological and neutral molecular data to examine dispersal behaviour across ecological zones, and the effect of dispersal on morphological clines in a "benign" high rainfall year;
3. Examine positive assortative pairing within lowland-source and highland-colonist populations for divergence in mating strategies following range expansion;
4. Examine song and response to song within and between lowland and highland zones for emerging barriers to gene flow;
5. Examine environmental predictors for *P. Downsi*-induced post-parasitism morbidity, and the effect of morbidity on beak dimensions, overall development, and foraging efficiency.

I will conclude my thesis with a synthesis of my findings and suggestions for future research.

References

Abzhanov, A., Protas, M., Grant, B. R., Grant P. R., and Tabin, C. J. 2004. Bmp4 and morphological variation of beaks in Darwin's finches. *Science.* 305: 1489-1465.

Abzhanov, A., Kuo, W. P., Hartmann, C., Grant, B. R., Grant P. R., and Tabin, C. J. 2006. The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. *Nature* **442**: 563-567.

Benkman, C. W., Smith, J. W., Keenan, P. C., Parchman, T. L., and Santisteban, L. 2009. A new species of the red crossbill (Fringillidae: Loxia) from Idaho. *Condor* **111**: 169-176.

Boag, P. T. 1983 The heritability of external morphology in Darwin's ground finches (Geospiza) on Isla Daphne Major, Galápagos. *Evolution* **37**: 877-894.

Boag, P. T. and Grant, P. R. 1978. The heritability of external morphology in Darwin's finches. *Nature* **274**: 793-794.

Boag, P. T. and Grant, P. R. 1984. The classical case of character release – Darwin's finches (*Geospiza*) on Isla Daphne Major, Galapagos. *Biological Journal of the Linnean Society* **22**: 243-287.

Bolnick, D. I. and Fitzpatrick, B. M. 2007. Sympatric speciation: models and empirical evidence. *Annual Review of Ecology, Evolution and Systematics.* **38**: 459-487.

Bowman, R. I. 1961. Morphological differentiation and adaptation in the Galapagos finches. *University of California Publications in Zoology* **58**: 1-302.

Burns, K. J., Hackett, S. J., and Klein, N. K. 2002. Phylogenetic relationships and morphological diversity in Darwin's finches and their relatives. *Evolution* **56**: 1240-1252.

Coyne, J. A. and Orr, H. A. 2004. *Speciation*. Sinauer Associates: Sunderland, M. A.

Causton CE, Peck SB, Sinclair BL, Roque-Albelo L, Hodgson CJ, Landry B. 2006. Alien insects: threats and implications for conservation of Galapagos Islands. *Annuals of Entomological Society of America* **99:** 121-143.

Christensen, R., Kleindorfer, S. and Robertson, J. 2006. Song is a reliable signal of beak morphology in Darwin's small tree finch *Camarhynchus parvulus*, and vocal performance predicts male pairing success. *J. Avian Biol.*, **6**: 617-624.

Christie, D. M., Duncan, R. A., McBirney, A. R., Richards, M. A., White, W. M. Harpp, K. S., and Fox, C. G. 1992. Drowned island downstream from the Galapagos hot spot imply extended speciation times. *Nature* **355:** 246-248.

de Leon, L. F., Bermingham, E., Podos, J. and Hendry, A. P. 2010. Divergence with gene flow as facilitated by ecological differences: within-island variation in Darwin's finches. *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**: 1041-1052.

Dieckmann, U., Doebeli, M., Metz, J. A. J. and Tautz, D. 2004. *Adaptive Speciation*. Cambridge University Press: Cambridge, U. K.

Dobzhansky, T. 1937. *Genetics and the Origin of Species*. Columbia University Press: New York, N. Y.

Dudaniec, R., Fessl, B. and Kleindorfer, S. 2007. Interannual and interspecific variation in intensity of the parasitic fly, Philornis downsi, in Darwin's finches. *Biological Conservation* **139**: 325-332.

Edelaar, P., Siepielski, A. M. and Clobert, J. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. *Evolution* **62**: 2462-2472.

Endler, J. A. 1977. *Geographic Variation, Speciation, and Clines* Princeton University Press: Princeton, N. J.

Fessl B, Kleindorfer S, Tebbich S. 2006. An experimental study on the effects of an introduced parasite in Darwin's finches. *Biological Conservation* **127:** 55-61.

Gavrilets, S. 2004. *Fitness Landscapes and the Origin of Species*. Princeton: Princeton, N. J.

Gibbs, H. L. and Grant, P. R. 1987. Oscillating selection on Darwin's finches. *Nature* **327**: 511-513.

Giraud, T., Refregier, G., Le Gac, M., de Vienne, D. M. and Hood, M. E. 2008. Speciation in fungi. *Fungal Genetics and Biology*. **45**: 791-802.

Grant, B. R. and Grant, P. R. 1989. *Evolutionary dynamics of a natural population: the large cactus finch of the Galápagos*. University of Chicago Press: Chicago, I. L.

Grant, B. R. and Grant, P. R. 1996. High survival of Darwin's finch hybrids: effect of beak morphology and diets. *Ecology* **77**: 500-509.

Grant, B. R. and Grant, P. R. 2010. Songs of Darwin's finches diverge when a new species enters the community. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 20156-20163.

Grant, P. R. 1993. Hybridisation of Darwin's finches on Isla Daphane Major, Galapagos. *Philosophical Transactions of the Royal Society B-Biological Sciences* **340**: 127-138.

Grant, P. R. and Grant, B. R. 1992. Hybridisation of bird species. *Science* **227**: 1056-1059.

Grant, P. R. and Grant, B. R. 1994. Phenotypic and genetic-effects of hybridisation in Darwin's finches. *Evolution* **48**: 297-316.

Grant, P. R. and Grant, B. R. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science* **296**: 707-711.

Grant, P. R. and Grant, B. R. 2006. Evolution of character displacement in Darwin's finches. *Science*

Grant, P. R. and Grant, B. R. 2008a. *How and Why Species Multiple: the Radiation of Darwin's Finches* Princeton University Press: Princeton, N. J.

Grant, P. R. and Grant, B. R. 2008b. Fission and fusion of Darwin's finches populations *Philosophical Transactions of the Royal Society B-Biological Sciences* **363**: 2821-2829.

Grant, P. R., Grant, B. R. and Petren, K. 2005. Hybridization in the recent past. *American Naturalist* **166**: 56-67.

Hendrey, A. P., Huber, S. K., De Leon, L. F., Herrel A. and Podos J. 2009. Disruptive selection in a bimodal population of Drawin's finches. *Proceeding of the Royal Society of London Series B* **276**: 753-759.

Holt, R. D. and Barfield, M. 2008. Habitat selection and niche conservatism. *Israel Journal of Ecology & Evolution* **54**: 295-309.

Huber, S.K., De León, L.F., Hendry, A.P., Bermingham, E. and Podos, J. 2007. Reproductive isolation of sympatric morphs in a population of Darwin's finches. *Proc. R. Soc. Lond. B.,* **274**: 1709-1714.

Huber, S. K. and Podos, J. 2006. Beak morphology and song features covary in a population of Darwin's finches (Geospiza fortis). *Biological Journal of the Linnean Society* 88: 489-498.

Kleindorfer, S. 2007. The ecology of clutch size variation in Darwin's Small Ground Finch Geospiza fuliginosa: comparison between lowland and highland habitats. *Ibis* **149**: 730-741.

Kleindorfer, S., Chapman, T. W., Winkler, H. and Sulloway, F. J. 2006. Adaptive divergence in contiguous populations of Darwin's Small Ground Finch (Geospiza fuliginosa). *Evolutionary Ecology Research* **8**: 357-372.

Kleindorfer, S. and Mitchell, J. 2009. Biological Networks: Rainforests, Coral Reefs, and the Galapagos Islands In: The Network Challenge: Strategy, Profit, and Risk in an Interlinked World. Kleindorfer, P. R., Wind, Y. and Gunther, R. E. (eds). Warton School Publishing: Philadelphia, PA

Lack, D. 1947. *Darwin's Finches*. Cambridge University Press: **Grant, P. R. 1999.** *Ecology and Evolution of Darwin's Finches*. Princeton University Press: Princeton, N. J.

Lack, D. 1976. *Island Biology Illustrated by the Land Birds of Jamaica*. University of California Press: Berkeley, C. A.

Mayr, E. 1942. *Systematics and the Origin of Species*. Columbia University Press: New York, N. Y.

Mayr, E. 1947. Ecological factors in speciation. *Evolution* **1**: 263-288.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press: Cambridge, M. A.

Mayr, E. and Diamond, J. 2001. *The Birds of Northern Melanesia: Speciation, Ecology, and Biogeography.* Oxford University Press: Oxford, U. K.

Nosil, P. 2008. Speciation with gene flow could be common. *Molecular Ecology* **17**: 2103-2106.

Petren, K., Grant, B. R., and Grant, P. R. 1999. A phylogeny of Darwin's finches based on microsatellite DNA length variation. *Proceeding of the Royal Society of London Series B* **266**: 321-329.

Petren, K., Grant, P. R., Grant, B. R. and Keller, L. F. 2005. Comparative landscape genetics and the adaptive radiation of Darwin's finches: the role of peripheral isolation. *Molecular Ecology* **14**: 2943-2957.

Podos, J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* 409: 185-188.

Podos, J. 2010. Acoustic discrimination of sympatric morphs in Darwin's finches: a behavioural mechanism for assortative mating? *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**: 1031-1039.

Price, T. D., Grant, P. R., Gibbs, H. L. and Boags P. T. 1984. Recurrent patterns of natural selection in a population of Drawin's finches. *Nature*. **309**: 787-789.

Price, T. 2008. *Speciation in Birds*. Roberts and Company: Greenwood Village, C. O.

Ratcliffe, L. M. and Grant, P. R. 1983. Species recognition in Darwin's finches (Geospiza Gould). I. Discrimination by morphological cues. *Animal Behaviour* **31**: 1139-1153.

Ratcliffe, L. M. and Grant, P. R. 1985. Species recognition in Darwin's finches (Geospiza Gould). III. Male responses to playback of different song types, dialects, and heterospecific songs. *Animal Behaviour* **33**: 290-307.

Rocha L. A. and Bowen, B. W. 2008. Speciation in coral-reef fishes. *Journal of Fish Biology.* **72**: 1101-1121.

Rundle, H. D. and Nosil, P. 2005. Ecological speciation. *Ecology Letters* **8**: 336-352.

Schluter, D., Price, T. D. and Grant, P. R. 1984. Ecological character displacement in Darwin's finches. *Science* **227**: 1056-1059.

Schulter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press: Oxford, U. K.

Schluter, D. 2001. Ecology and the origin of species. *Trends in Ecology & Evolution* **16**: 372-380.

Sinton, C. W., Christie, D. M., and Duncan, R. A. 1996. Geochronology of Galapagos seamounts. *Journal of geophysical Research* **101**: 13,689-13,700.

Snell, H. and Rae, S. 1999. The 1997-98 El Nino in Galapagos: can 34 years of data estimate 120 years of pattern? *Noticias de Galapagos* **60**: 11-20.

van Doorn, G. S., Edelaar, P. and Weissing, F. J. 2009. On the Origin of Species by Natural and Sexual Selection. *Science* **326**: 1704-1707.

CHAPTER TWO

High gene flow supports adaptive divergence in an island population of Darwin's small ground finch, *Geospiza fuliginosa*

Toby H. Galligan, Stephen C. Donnellan, Frank J. Sulloway,
Alison J. Fitch, Terry Bertozzi and Sonia Kleindorfer

Molecular Ecology (in preparation)

ABSTRACT

The divergence-with-gene-flow model of speciation has a strong theoretical basis with a growing number of plausible examples in nature, but remains hotly debated. Darwin's finches of the Galápagos Archipelago have played an important role in our understanding of speciation processes. Recent studies suggest that this group may also provide insights into divergence with gene flow. On the island of Santa Cruz, Darwin's small ground finch, *Geospiza fuliginosa*, has shown adaptive divergence across contrasting arid and humid ecological zones. Despite the short geographical distance between these zones, disruptive selection, strengthened in periods of low rainfall, is expected to maintain adaptive divergence. Conversely, in periods of high rainfall, when disruptive selection is predicted to be weakened, population divergence in adaptive traits is expected to break down. Because periods of low and high rainfall irregularly alternate, adaptive divergence can be assumed to degenerate and, importantly, regenerate *in situ.* Here, we use microsatellite allele frequency data to assess whether phenotypic divergence in this system (1) has occurred in the presence of gene flow; and (2) has led to overall genetic substructure within the population. Our results clearly showed a single panmictic population of *G. fuliginosa* with substantial contemporary dispersal (dispersal rate = 0.20), which is largely independent of ecological or geographical differences among the four ecological zones and 21 sites that were sampled. A notable exception is greater emigration from the agricultural zone, which likely is explained by avoidance of low quality habitat. We conclude that phenotypic divergence has occurred in the presence of gene flow, but has not lead to overall genetic divergence. Even low levels of gene flow reject genetic drift as a valid process generating phenotypic divergence. We discuss how our findings may support either classic adaptation, or matching habitat choice, or a combination of the two.

Keywords: divergence-with-gene-flow, adaptive divergence, parapatic divergence, within island dispersal, fluctuating disruptive selection, divergent selection, Darwin's finches.

INTRODUCTION

Recent speciation theory provides realistic models for how species can evolve via disruptive selection despite moderate (parapatric) to high (sympatric) levels of gene flow (Mayr 1966; Endler 1977; Rice and Hostert 1993; Schluter 2001; Kirkpatrick and Ravigne 2002; Dieckmann *et al.* 2004; Gavrilets 2004; Rundle and Nosil 2005; Gavrilets and Vose 2007; Gavrilets *et al.* 2007; van Doorn *et al.* 2009). Fundamental to many of these models is a differential exchange in genes that do and do not encode for traits under selection – that is, disruptive selection may restrict gene flow for maladaptive genes, but permit gene flow for neutral genes (Hey 2006). An ever-increasing number of empirical studies have used an array of methods to infer gene flow during speciation in a variety of organisms and natural circumstances (e.g., Smith *et al.* 1997; Wang *et al.* 1997; Emelianov *et al.* 2004; Schilthuizen *et al.* 2005; Smith *et al.* 2005; Barluenga *et al.* 2006; Panova *et al.* 2006; Savolainen *et al.* 2006; McCormack and Smith 2008; Niemiller *et al.* 2008; Crow *et al.* 2010; Li *et al.* 2010; Storchova *et al.* 2010). Nevertheless, the overall significance of speciation via divergence with gene flow remains hotly debated (Coyne and Orr 2004; Hey 2006; Coyne 2007; Grant and Grant 2008; Nosil 2008b; Price 2008; Fitzpatrick *et al.* 2009).

Many studies have examined alternative modes of speciation by contrasting gene flow in genes under selection and neutral genes between divergent populations (e.g., Smith *et al.* 1997; and reviewed in Nosil 2008a). Typically, gene flow in selected genes is measured indirectly using phenotypic trait differentiation, whereas gene flow in neutral genes is measured directly using neutral genetic marker differentiation. When phenotypic traits differ significantly between populations, but neutral genetic markers do not, then divergence with gene flow can be inferred. This approach has indeed demonstrated that divergence can be maintained in the presence of moderate gene flow, but that high gene flow constrains divergence (Smith *et al.* 1997), which has been confirmed experimentally (Nosil 2009). Therefore, it remains unclear how phenotypic divergence can be *initiated* in the presence of high gene flow.

Darwin's finches of the Galápagos Archipelago remain a constant source of evidence for how species evolve in nature (reviewed in Grant and Grant 2008). Presently, work on these finches is contributing to our understanding of how

divergence, and ultimately speciation, can occur in the presence of gene flow (de Leon *et al.* 2010; and reviewed therein). This line of investigation for Darwin's finches was initiated by Kleindorfer *et al.* (2006) who showed that adaptive divergence could occur within a single island population.

Concurrently, while examining genetic connectivity between species and island populations of species across the Galápagos Archipelago, Petren *et al.* (2005) showed that the adaptive radiation of Darwin's finches likely had occurred in the presence of considerable gene flow. While, a pattern of isolation by distance was detected in three species (suggesting the potential importance of peripheral island populations for divergence), phenotypic divergence was not shown to be constrained by gene flow overall (Petren *et al.* 2005). Hence, these findings suggested an adaptive radiation among Darwin's finches under conditions akin to parapatry between islands (*sensu* Smith *et al.* 1997; Coyne and Orr 2004) more so than allopatry (Lack 1947; Grant 1999; Petren *et al.* 2005; Grant and Grant 2008). In a second study, Grant *et al.* (2005) showed that closely related species inhabiting the same island frequently hybridised introgressively, and conclude that gene flow probably had occurred during character displacement in sympatry – the critical final step in the speciation process, traditionally viewed as occurring in the absence of gene flow (Lack 1947; Grant 1999; Grant and Grant 2008).

Extensive research conducted on an island population of medium ground finch, *Geospiza fortis*, with a bimodal distribution for beak size, has been focussed on enhancing our understanding of divergence with gene flow for speciation in Darwin's finches (reviewed in de León *et al.* 2010). In *G. fortis*, beak size bimodality is presumed to be an adaptation to food size bimodality within the same location; suggesting that divergence was generated in sympatry; and thus under presumably a high level of gene flow (Ford *et al.* 1973). However, such a pattern is also consistent with the secondary contact of populations that have diverged in allopatry (Grant and Grant 2008, 2010). Therefore, while this work has shown that phenotypic and genetic divergence can be maintained in the presence of gene flow (León *et al.* 2010; and reviewed therein), whether such divergence was *initiated* amid a high level of gene flow remains uncertain.

We use the system introduced by Kleindorfer *et al.* (2006) to examine evidence for divergence with gene flow for speciation in Darwin's finches. This system comprises a population of small ground finch, *Geospiza fuliginosa*, inhabiting

the relatively small (986 km^2) but elevated (869 m a.s.l.) island of Santa Cruz, on which four distinct ecological zones exist: the *arid* lowland, *transitional* midland, and *humid* highland zones generated by altitudinal differences in rainfall, and the *agricultural* midland zone generated by anthropogenic alteration (Fig. 1). In a typical year, the arid zone receives less than 250 mm of rain, the humid zone receives greater than 700 mm of rain, and the intervening transitional and agricultural zone receive intermediate levels. As a direct result of variation in rainfall, and anthropogenic alteration, each zone varies in its biotic community (particularly plant and invertebrate species) and productivity (see Tebbich *et al.* 2002). As a result, the ecological contrast between arid and humid zones is predicted to generate considerable disruptive selection. However, despite the ecological differences between zones, *G. fuliginosa* forages and breeds across the entire island (Kleindorfer *et al.* 2006; Kleindorfer 2007). Hence, the system we study has the key elements for divergence with gene flow: (1) a taxon continuously distributed over (2) a small geographical range where (3) disruptive selection is likely to be acting.

Previously we have found evidence for adaptive divergence in *G. fuliginosa* inhabiting the ecological extremes on Santa Cruz. Specifically, there is evidence for clines in ecologically-significant traits across zones – that is, clines in beak length and foot size (Kleindorfer *et al.* 2006; Sulloway and Kleindorfer in review); divergent phenotype-environment matching within arid and humid zones (Kleindorfer *et al.* 2006; Kleindorfer 2007); and utility of ecologically-significant traits within arid and humid zones (Sulloway and Kleindorfer in review). The origin of divergence in our system is parsimonious with a parapatric mode, as opposed to a sympatric mode, and thereby matches the pattern of divergence observed across island populations (*sensu* Petren 2005) on a finer scale. In addition, our most recent work suggests that morphological divergence is greatest during *La Nińa* low rainfall periods and can breakdown (morphological convergence) during *El Nińo* high rainfall events (Chapter 3). Therefore, as the climate in the Galápagos alternates between typical low rainfall periods of 2-11 years and irregular high rainfall events of 1-2 years (Snell and Rae 1999), divergence is subjected to regeneration and degeneration, accordingly (Chapter 3). This is an important point because it means that adaptive divergence in our system has most likely been initiated *in situ* among ecological zones on Santa Cruz; and not in allopatry. Therefore, by establishing gene flow between divergent populations, we can confirm that divergence with gene flow

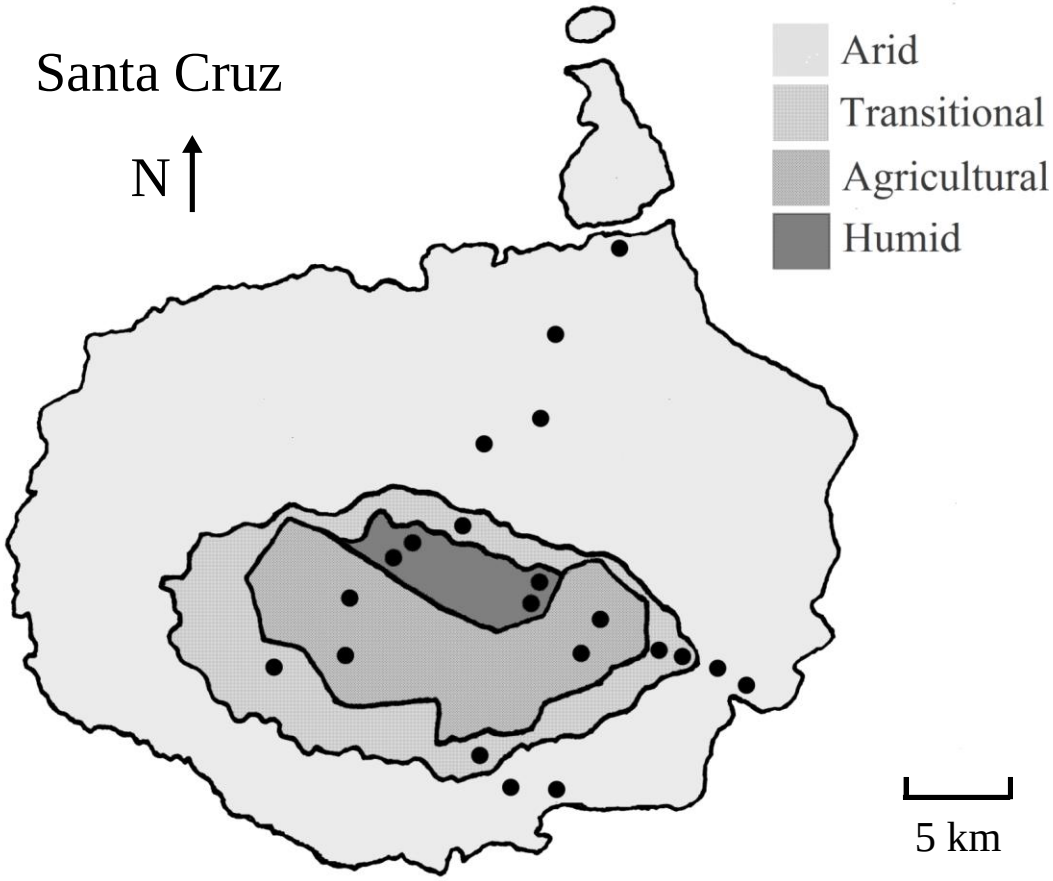


Figure 1: Map of Santa Cruz, Galápagos Archipelago, showing the distribution of four major ecological zones and the 21 sites (indicated by filled circles) sampled.

can be initiated in Darwin's finches within a single island.

We are mindful of the lack of evidence for within-island speciation in birds – namely, the scarcity of endemic sister species on isolated islands (Coyne and Price 2000). Therefore, we do not propose that the adaptive divergence with or without gene flow in *G. fuliginosa* on Santa Cruz would lead to speciation *in situ*. Rather, adaptive divergence within islands may facilitate speciation following differential dispersal by ecotypes among islands to preadapted habitats (Kleindorfer *et al.* 2006). Indeed, habitat choice has been shown in Darwin's finches during inter-island dispersal (Grant *et al.* 2001; Tonnis *et al.* 2005). In such cases adaptive divergence first initiated in close parapatry (within an island) could culminate in speciation in distant parapatry (between islands), via either increased disruptive selection or decreased gene flow between source and colonist populations.

Here, our aim is to test evidence for the significance of within island generated divergence for the pattern of adaptive radiation observed in Darwin's finches. Consequently, we examine microsatellite allele frequency data extensively sampled from *G. fuliginosa* on Santa Cruz to answer two fundamental questions: (1) has phenotypic divergence occurred in the presence of gene flow and (2) has phenotypic divergence led to genetic divergence between populations as reflected in neutral markers? The latter issue would bear on the second step in the process towards speciation – that is, the generation and maintenance of incipient reproductive isolation between adaptively divergent ecotypes.

METHODS

Study species and sites

Our study was conducted 2008, a high rainfall *El Niño* year, between January and May which coincided with the breeding season of *G. fuliginosa* in that year. We sampled from 21 sites across the island – encompassing multiple sites within all four ecological zones (Table 1; Fig. 1). We located our sites approximately 2 km apart along existing roads and tracks to maximise accessibility.

The arid zone on Santa Cruz is characterised by dry-deciduous open forest and woodland dominated by *Bursera graveolens*. The vegetation in the humid zone is evergreen and forms closed forest (*Scalesia pedunculata* dominated), closed shrubland (*Miconia robinsoniana* dominated), woodland (*Cinchona succirubra* dominated), and sedge-grassland. The transitional midlands is characterised by a mixture of dry-deciduous and evergreen open forest (*Pisonia floribunda* – *Piscidia carthagenensis* – *Psidium galapageium* codominate). The agricultural zone represents historic highland and transitional habitat that has been largely replaced by fields of introduced *Pennisetum purpureum* and stands of exotic trees, such as *Cinchona pubescens*, *Erythrina coarllodendron* and *Psidium guajava*.

Data collection

Finches were captured using mist nets. Juvenile finches, discriminated based on the presence of obvious gape flanges, soft feet, and short wing and/or tail feathers, were

not processed because those caught within the same site may be genetically related and thereby bias results.

Not all finches sampled were likely to contribute to the breeding population (i.e., immature finches and unpaired mature finches). However, *G. fuliginosa* is socially monogamous and typically sex ratios are equal (Grant 1999); further, mature finches have a greater survivorship than immature finches (Grant 1999). Thus, the entire population, as sampled in this study, is likely to largely represent the breeding population. This is expected to particularly so in an *El Niño* year, such as the one we have sampled, when greater rainfall and subsequent greater productivity presumably permits a greater breeding population than in other years.

Blood samples were collected from 518 individuals and stored on FTA® databasing paper. DNA was extracted for each individual from a 1 mm^2 disc of blood-soaked FTA® using a protocol modified from Smith and Burgoyne (2004). Specifically, each disc was washed in 500 µL lysis buffer for 30 min, 500 µL DNAzol® for 10 min, and two washes in 500 µL molecular grade water for 10 min discarding the solutions after each wash. DNA was released from the discs by incubating in 50 µL of 10 mM Tris containing 0.1 mM EDTA at 90ºC for 5 min.

We redesigned nucleotide sequences of PCR primer pairs for 12 autosomally inherited microsatellite loci – *Gf01, Gf03-09, Gf12-15* – first isolated and designed by Petren (1998) to enable multiplex genotyping of PCR products. A primer in each pair was labelled with one of four 5' labelled fluorescent tags: FAM (GeneWorks); NED, PET, or VIC (Applied Biosystems). 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^oC for 30 minutes. Capillary electrophoresis (ABI 3730 DNA analyser) was used to separate and analyse PCR multiplexes at the Australian Genome Research Facility Ltd, Adelaide. We used the program GeneMapper® version 3.7 (Applied Biosystems) to size PCR products for each locus.

We used microsatellite markers, as opposed to mitochondrial or nuclear markers, because of the former can be used to estimate contemporary gene flow (Paetkau *et al.* 1995; Piry *et al.* 2004) and have been shown to better resolve genetic

relationships among recently divergent species and populations of Darwin's finches (Petren *et al.* 1999; Petren *et al.* 2005; de León *et al* 2010).

Analysis of genetic diversity and differentiation

Initially we screened our genetic data set for typing and typographical errors using MICRO-CHECKER version 2.2.3 (van Oosterhout *et al.* 2004). We examined Hardy-Weinberg equilibrium (HWE; (Raymond and Rousset 1995) and genotypic disequilibrium (GD) across loci and sites using the Markov chain Monte Carlo (MCMC) method implemented in GENEPOP version 4.0 (Rousset 2008). The MCMC parameters were set at 10,000 iterations, 1000 batches, and a dememorisation of 10,000. To account for multiple comparisons, significance of deviations from HWE and GD were assessed using sequential Bonferroni corrections (Rice 1989).

Genetic diversity statistics were obtained from GENEPOP (Roussett 2008) and FSTAT version 2.9.3.2 (Goudet 1995). Observed and expected heterozygosities (H_O and H_E) were calculated using GENEPOP; whereas, allelic richness (*AR*) was calculated using FSTAT. We used both programs to calculate genetic differentiation among sites and ecological zones based on the (1) infinite allele mutation model F_{ST} (Weir and Cockerham 1984), and (2) the stepwise mutation model, R_{ST} (Slatkin 1995). We used an allele size test implemented in SPAGeDI version 1.3 (Hardy and Vekemans 2002) to determine which measure was more appropriate for our data. Briefly, F_{ST} is considered more appropriate than R_{ST}, unless $R_{ST} > F_{ST}$, which indicates that the rate of mutation is not negligible in relation to drift (Hardy *et al.* 2003). We obtained 95 % confidence intervals for global differentiation estimates using FSTAT.

Analysis of genetic structure

We examined genetic structure among sites using clustering analyses implemented in GENELAND version 3.1.4 (R Development Core Team 2004; Guillot *et al.* 2005). GENELAND uses Bayesian statistics and spatial data for each individual in the form of *x* and *y* coordinates to infer genetic clusters, assuming that the overall sample consists of *K* clusters that are in HWE and GD. We conducted multiple analyses using alternative genetic models to find parameter sets (i.e., chain length, burn-in, etc.) that allowed the MCMC to best explore the parameter space and establish

Table 1: Physical details and genetic statistics for the 21 sites studied: ecological zone; site number; locality name; coordinates (UTM; zone = 15); altitude (metres above sea level); geographical zone (GZ); sample size (*n*); allelic richness (*AR*, based on a minimal sample size of 8); mean expected and observed heterozygosity (H_E and H_O); and inbreeding coefficient (F_{IS}).

Ecological zone	Site	Locality	GZ	Coordinates (N, E)	Alt.	*n*	*AR*	H_E	H_O	F_{IS}
Humid (H)	1	*El Puntudo*	Central 1	0797223, 9929170	702	15	4.75	0.77	0.69	0.10
	2	*Media Luna*	Central 1	0797450, 9927147	605	14	6.51	0.77	0.71	0.09
	3	*Los Gemelos*	Central 1	0791254, 9931119	617	30	7.77	0.79	0.76	0.04
	4	*Los Gemelos*	Central 1	0790140, 9929998	569	35	6.51	0.78	0.72	0.07
Agricultural (AG)	5	*El Camote*	Central 2	0801700, 9927485	413	15	5.16	0.77	0.66	0.15
	6	*El Cascajo*	Central 2	0800134, 9925082	326	22	6.80	0.80	0.74	0.07
	7	*Santa Rosa*	Central 2	0788819, 9928198	447	21	5.80	0.77	0.67	0.13
	8	Tortoise Territory	Central 2	0788633, 9925920	331	32	7.17	0.71	0.66	0.08
Transitional (T)	9	*El Garrapatero*	South 2	0804672, 9926163	255	29	8.33	0.81	0.74	0.08
	10	*El Garrapatero*	South 2	0806750, 9925521	149	31	7.98	0.77	0.75	0.03
	11	*El Chato*	South 2	0785088, 9925622	198	32	7.71	0.79	0.71	0.10
	12	*Guaybillos*	South 2	0795374, 9921159	128	23	6.15	0.77	0.70	0.09
	13	*Mina Rojo*	Central 1	0793093, 9931805	590	29	6.38	0.80	0.78	0.02
Arid (AR)	14	*El Garrapatero*	South 1	0808374, 9925013	66	29	5.99	0.79	0.70	0.11
	15	*El Garrapatero*	South 1	0809269, 9923213	5	28	5.68	0.76	0.70	0.08
	16	*El Mirador**	South 1	0797380, 9918815	65	34	8.61	0.78	0.76	0.03
	17	CDRS**	South 1	0800270, 9917844	9	28	6.32	0.77	0.69	0.11
	18	Refuse site	North 1	0794217, 9935157	277	10	6.00	0.79	0.69	0.14
	19	*Mina Negro*	North 1	0796817, 9936484	254	36	7.25	0.77	0.74	0.04
	20	Goat-hunters' Track	North 2	0798818, 9940558	100	10	5.57	0.81	0.76	0.07
	21	*Itabaca Canal*	North 2	0802592, 9944812	10	15	3.87	0.75	0.68	0.09

* *El Mirador de los Túneles*; ** Charles Darwin Research Station, Academy Bay

convergence. Each analysis comprised 10 runs, with a *K* set to vary between 1 and 10 (the upper limit being more than twice the number of populations predicted based on ecological zones). Spatial data were entered as UTM coordinates with an uncertainty of 250 m (a distance greater than the maximum distance between mist nets within sample sites).

We used TRACER version 1.5 (Rambaut and Drummond 2007) to assess convergence in MCMC from our GENELAND runs. Specifically, we inspected the trace and effective sample size (ESS) of the log posterior densities and log likelihoods generated for each run. Convergence was deemed adequate if the trace plateaued before the end of the run, and the ESS was greater than 200. An uncorrelated D-model with $1x10^7$ iterations and a thinning of 0.1 % showed consistent MCMC convergence (after a burn-in of 100 iterations). Here, we comment only on the *K* inferred from these final analyses. Our GENELAND estimate of *K* was confirmed using additional genetic clustering programs (results not presented).

Genetic structure can be influenced by a pattern of isolation-by-distance where geographic distance between population extremes can limit gene flow. We assessed this possibility using a Mantel test implemented in IBDWS Version 3.16 (Jensen *et al.* 2005). Specifically, we compared matrices of F_{ST} and Euclidean geographical distances (km) between each site; assessing significance based on 30,000 randomisations of the genetic matrix.

Analysis of gene flow

We examined gene flow between sites directly using a frequency-based method (Paetkau *et al.* 1995) implemented in GENECLASS version 2 (Piry *et al.* 2004). GENECLASS detects first generation migrants within each sample and then assigned migrants to a likely sample population of origin. In this way, both the number of immigrants and emigrants per site is estimated. We used the resampling method of Paetkau *et al.* (2004) and the following parameters: likelihood criteria L = L_home; alpha value = 0.05; and 10,000 simulated individuals. The frequency-based method performs equally well as alternative Bayesian methods for the number of samples and loci that we used (Cornuet *et al.* 1999). We assessed the accuracy of our results using the "assign/exclude population as origin of individual" function in GENECLASS: we removed 5 individuals from each site to create a "to be assigned" population and used the remainder of individuals as a "reference population"

We examined differences in the proportion of migrants among ecological zones and across geographical distance using focussed regression by frequency analysis. We performed these analyses using the online Stats Toolpack of the Department of Obstetrics and Gynaecology, Chinese University of Hong Kong (Chang 1999). For gene flow among ecological zones, each zone was contrasted against all other zones combined (e.g., Arid = 3, Transition = -1, Agricultural = -1, and Humid = -1). We examined differences in gene flow within-zones and between-zones. For gene flow across geographical distance, sites were grouped based on geographical proximity (= geographical zones; see Table 1) and coded for two linear contrasts: one running south-north (South 1 = -5, South 2 = -3, Central 1 = -1, Central 2 = 1, North 1 = 3, and North 2 = 5); and the other, north-south (North 2 = -5, North 1 = -3, Central 2 = -1, Central 1 = 1, South 2 = 3, and South 1= 5). In the south-north direction, we measured gene flow by comparing the number of South 1 migrants and residents in each geographical zone; in the north-south direction, we compared the number of North 1 migrants and residents in each geographical zone. We report the combined effect size, r_z, of south-north and north-south analyses calculated using Fisher's *r* to *z* transformation, which was then transformed back to *r*. Finally, all analyses were performed separately for immigrants and emigrants, except for within ecological zones where immigration = emigration.

We did not examine gene flow using indirect genetic methods (e.g., comparison of allele frequencies between populations and reconstruction of gene trees) because such methods do not discriminate between contemporary and historic genetic connectivity. We were interested in measuring contemporary gene flow only. Further, recent simulations have raised concern regarding the accuracy of indirect estimates (Abdo *et al.* 2004; Slatkin 2005).

RESULTS

Of the 518 birds tested, no individual had missing data at more than 3 loci. After Bonferroni correction, we found a single deviation from HWE among loci (Table 2), but no deviations within sites (Table 3). We found 2 out of a possible 66 cases of genetic linkage in the global population and 2 out of a possible 1389 cases of genetic linkage within sites (first adjusted critical value = 0.0008). High heterozygosity and

low genetic diversity (F_{IS}) were common among the loci used (Table 2), as well as the sites examined (Table 1). We found no difference in F_{IS} among sites (Friedman's test: χ^2 = 20.83, df = 20, p = 0.407).

The allele size test found that R_{ST} was not significantly different to F_{ST} (p = 0.856, n = 518); therefore the latter was a more appropriate measure of genetic differentiation. F_{ST} values calculated between sites (Table 4) and ecological zones (Table 5) were close to zero. Global F_{ST} for sites (0.004 [-0.001 – 0.011]) and ecological zones (0.001 [-0.001 – 0.003]) were not significantly different from zero (confidence intervals overlap zero). Our GENELAND analysis detected a single genetic cluster within the island, with K = 1 consistently found across runs. We found no evidence of isolation-by-distance (Mantel's Z = 7.81, r = -0.08, n = 210, p = 0.764).

Twenty percent (106/518; mean number of migrants per site = 5.05) of all finches sampled were first generation migrants. At least one immigration and emigration event had occurred within and between each ecological zone, with one exception: no migrants were exchanged among the four sites within the humid zone (Table 6). We did not find a significant difference in immigration among zones

Table 2: Genetic diversity statistics for each microsatellite locus genotyped: number of individuals sampled (n); number of alleles (A); expected and observed heterozygosity (H_E and H_O); inbreeding coefficient (F_{IS}); and the probability of deviation from Hardy-Weinberg equilibrium (p; significant deviations italicised).

Locus	*N*	*A*	H_E	H_O	F_{IS}	*p*
Gf1	497	22	0.88	0.83	0.05	0.998
Gf3	491	18	0.85	0.77	0.10	1.000
Gf4	479	19	0.60	0.60	-0.01	0.895
Gf5	491	13	0.77	0.77	0.00	0.998
Gf6	476	14	0.63	0.56	0.10	1.000
Gf7	498	21	0.68	0.70	-0.03	0.807
Gf8	510	27	0.92	0.78	0.15	1.000
Gf9	503	16	0.68	0.64	0.06	0.995
Gf12	484	19	0.88	0.89	0.00	0.709
Gf13	509	17	0.89	0.97	-0.09	*<0.001*
Gf14	496	21	0.76	0.76	0.01	0.998
Gf15	493	30	0.84	0.37	0.56	1.000

(Table 7). However, we found a significant difference in emigration among zones: emigrants were ~3 times more likely to have originated from the agricultural zone than any other zone (Table 7). In addition, arid zone finches were ~4 times more likely to disperse within-zone than finches from all other zones (Table 7). Comparing dispersal within-zone and between-zones, for each ecological zone except the humid zone, we found no difference in either immigrants to zones (arid χ^2 = 0.20, p = 0.652, n = 42; transitional χ^2 = 2.83, p = 0.092, n = 29; agricultural χ^2 = 0.27, p = 0.607, n = 16) nor emigrants from zones (arid χ^2 = 0.02, p = 0.883, n = 37; transitional χ^2 = 2.27, p = 0.132, n = 27; agricultural χ^2 = 2.58, p = 0.108, n =31). In addition, we found no effect of geographical distance on immigration (Fishers r_z = 0.060) or emigration (r_z = 0.055). Importantly, GENECLASS could only assigned 4 out of 105 (3.81 %) of individuals to a single site

DISCUSSION

Our analysis of microsatellite allele frequency data showed neither population substructure nor a pattern of isolation by distance; therefore, we conclude that *G. fuliginosa* on Santa Cruz represents one panmictic population. In addition, we found no evidence that the ecological and geographical differences among zones reduced gene flow. In fact, overall gene flow from one ecological zone to another was high (rate of dispersal = 0.20; but see discussion below). We are therefore confident that the phenotypic divergence previously observed between arid and humid zone *G. fuliginosa* (Kleindorfer *et al.* 2006) on Santa Cruz has occurred in the presence of high gene flow.

Our findings are not surprising given: (1) the size of the island; (2) the high mobility of *G. fuliginosa*; and (3) the lack of physical barriers to dispersal within the island landscape. However, Kleindorfer *et al.* (2006) had predicted low levels of gene flow between the arid and humid zones based on resighting and recapture of marked finches (<0.02 observed dispersal rate). Although their estimate does not agree with our overall estimate of gene flow among sites, it does reflect estimates for

Table 3: The probabilities of deviation from Hardy-Weinberg equilibrium for each locus used and among all sites sampled. Significant deviations are italicised, however no deviation remained significant after sequential Bonferroni correction (adjusted critical value = 0.003). The mean number of individuals per site used to calculate probabilities for each locus is shown.

Site	*n*	*Gf1*	*Gf3*	*Gf4*	*Gf5*	*Gf6*	*Gf7*	*Gf8*	*Gf9*	*Gf12*	*Gf13*	*Gf14*	*Gf15*
1	14	0.999	0.529	0.992	0.199	0.916	0.511	0.275	0.999	0.470	0.343	0.910	0.999
2	14	0.840	0.776	0.148	0.466	0.658	0.254	0.669	0.948	0.970	0.153	0.798	1.000
3	13	0.168	1.000	0.942	0.942	1.000	0.639	0.999	0.502	0.927	0.071	0.738	1.000
4	20	0.918	0.266	0.253	0.168	0.365	0.865	0.986	0.888	0.728	0.653	0.510	1.000
5	29	0.713	0.170	0.726	0.842	1.000	0.622	1.000	0.827	0.081	*0.011*	0.426	1.000
6	30	0.339	0.861	0.089	0.986	0.185	0.317	0.927	0.813	0.979	0.056	0.194	1.000
7	27	0.896	0.806	0.872	0.664	0.999	0.979	1.000	0.990	0.111	*0.026*	0.636	1.000
8	27	0.992	0.997	0.370	0.906	0.148	0.418	0.993	0.203	0.637	0.271	0.198	1.000
9	29	0.743	0.838	0.693	0.552	0.363	0.198	0.934	0.238	0.641	0.366	0.476	1.000
10	34	0.644	0.987	0.167	0.504	0.503	*0.033*	0.990	0.705	0.398	0.867	0.895	1.000
11	21	0.792	0.798	0.910	0.708	0.651	0.432	0.967	0.976	0.802	0.284	0.678	1.000
12	31	0.814	0.999	0.610	*0.032*	0.952	0.767	0.934	0.945	0.258	0.098	0.168	1.000
13	31	0.871	0.991	0.144	0.984	0.758	0.790	0.991	0.825	0.720	0.051	0.967	1.000
14	20	0.827	0.772	0.460	0.236	0.971	0.108	1.000	0.955	0.488	0.268	0.926	1.000
15	32	0.904	0.512	0.577	0.965	0.728	0.517	0.977	0.184	0.520	0.353	0.271	0.998
16	26	0.983	0.906	0.551	0.250	1.000	0.565	0.996	0.386	0.738	0.056	0.477	1.000
17	27	0.903	0.257	0.336	0.271	0.875	0.957	0.667	0.197	0.821	0.058	0.975	1.000
18	9	0.549	0.926	0.224	0.722	0.993	0.507	0.964	0.747	0.744	0.555	0.874	1.000
19	34	0.355	0.992	0.999	0.797	0.992	0.543	0.903	0.388	0.196	*0.029*	0.136	1.000
20	10	0.938	0.940	0.785	0.423	0.769	0.799	0.750	0.874	0.305	0.435	*0.014*	1.000
21	15	0.728	0.911	0.147	0.578	0.962	0.789	0.993	0.802	0.409	0.720	0.207	1.000

Table 4: F_{ST} values for pairwise comparisons for all sites sampled arranged by ecological zones.

		Humid				Agricultural				Transitional					Arid						
	Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Humid	2	0.005																			
	3	0.012	0.005																		
	4	0.012	-0.007	0.000																	
Agricultural	5	0.009	0.002	0.011	0.003																
	6	0.000	0.003	0.000	0.006	0.013															
	7	0.003	-0.005	0.003	0.003	-0.004	0.007														
	8	0.014	0.001	0.000	0.005	0.004	0.010	0.002													
Transitional	9	0.004	-0.005	0.004	0.003	0.005	-0.003	0.007	0.006												
	10	0.016	0.005	0.004	0.008	0.008	0.008	0.004	0.001	0.007											
	11	0.000	-0.004	0.010	0.014	0.014	-0.003	0.016	0.020	0.003	0.019										
	12	0.006	-0.003	0.003	0.003	-0.005	0.008	-0.004	-0.002	0.008	0.009	0.012									
	13	-0.001	-0.006	0.003	-0.001	0.003	0.002	-0.003	0.004	0.001	0.006	0.007	0.000								
Arid	14	0.004	-0.004	0.003	0.008	0.003	0.010	-0.003	-0.002	0.008	0.008	0.015	-0.006	-0.003							
	15	0.010	-0.002	0.000	0.001	0.002	0.018	0.000	0.005	0.013	0.014	0.017	-0.001	0.006	0.005						
	16	0.003	0.002	-0.003	0.003	0.005	-0.004	0.001	0.004	0.000	0.002	0.004	0.003	0.000	0.003	0.008					
	17	0.022	0.011	0.003	0.009	0.005	0.005	-0.001	0.005	0.007	0.007	0.012	0.001	-0.004	-0.003	0.006	-0.001				
	18	0.002	-0.007	-0.003	0.006	0.003	0.002	-0.001	0.002	0.006	0.003	0.017	-0.002	0.001	-0.001	0.002	-0.001	-0.002			
	19	0.002	-0.003	0.002	0.001	-0.001	0.006	-0.004	0.001	0.003	0.000	0.008	-0.002	-0.004	-0.001	0.006	-0.001	-0.002	0.000		
	20	0.008	0.001	0.010	0.009	0.013	0.010	0.002	0.001	0.012	0.006	0.021	0.008	-0.001	0.000	0.011	0.006	-0.003	0.000	0.005	
	21	0.000	-0.007	0.001	-0.001	-0.007	0.009	-0.009	-0.002	0.005	0.001	0.013	-0.005	-0.008	-0.007	-0.007	0.000	-0.009	0.003	-0.003	-0.004

Table 5: F_{ST} values for pairwise comparisons for each ecological zone sampled.

	Humid	Agricultural	Transitional
Agricultural	>0.001		
Transitional	0.002	>0.001	
Arid	0.001	>0.001	0.002

gene flow between the arid and humid zones alone that were obtained in this study (0.04) and a second study (0.06-0.08: Chapter 3). Interestingly, our second study was conducted during a low rainfall *La Niña* period, in contrast to our present study in a high rainfall *El Niño* year, suggesting climate has no detectable effect on contemporary gene flow (Chapter 3).

In our present study, gene flow between each zone and all other zones did not differ, for the most part. This finding, in combination with no difference in gene flow within and between ecological zones overall, suggests finches readily disperse widely across the island and without fidelity to ecological zones. However, we found two notable exceptions to an otherwise homogeneous pattern of gene flow: (1) a greater proportion of emigrants originated from the agricultural zone than any other zone; and (2) a greater proportion of arid zone finches dispersed within the arid zone than to all other zones combined.

High emigration from the agricultural zone may be explained by a presumed lower habitat quality for this zone in comparison to other zones. The agricultural zone is unique in that it the vegetation has been almost entirely altered by humans. Following our description in the Methods, the area encompassed by the agricultural zone was once covered in the diverse plant communities of the humid and upper

Table 6 (overleaf): Bidirectional estimates of gene flow between 21 sites (n = 518). Rows indicate the number of immigrants per site from sites listed in the column headings; and columns indicate the number of emigrants per site to sites listed in the row headings. Also shown are the ecological zones each site belongs to, site and zone sample sizes, and the total number of immigrants (*TI* on the right) and emigrants (*TE* at the bottom) per site and for the entire population (*TI* = *TE*; at the bottom and on the right).

		Humid (*n*=94)				Agricultural (*n*=90)				Transitional (*n*=144)					Arid (*n*=190)								
	Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	*TI*
Humid	1 (*n*=15)	-	0	0	0	0	0	0	2	0	0	0	1	0	1	0	0	0	0	0	0	0	4
	2 (*n*=14)	0	-	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	4
	3 (*n*=30)	0	0	-	0	0	0	1	0	0	1	0	0	0	1	3	0	0	0	0	0	0	6
	4 (*n*=35)	0	0	0	-	0	1	0	1	0	0	0	0	2	0	0	1	0	0	0	0	0	5
Agricultural	5 (*n*=15)	0	0	0	0	-	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	4
	6 (*n*=22)	0	0	0	1	0	-	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	4
	7 (*n*=21)	0	0	1	1	1	0	-	0	1	0	0	0	0	0	0	0	0	0	0	0	0	4
	8 (*n*=32)	0	0	0	0	0	0	1	-	1	0	0	2	0	0	0	0	0	0	0	0	0	4
Transitional	9 (*n*=29)	0	0	1	1	1	0	1	1	-	0	1	0	0	0	0	0	0	0	0	0	0	6
	10 (*n*=31)	0	0	0	1	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	1	0	2
	11 (*n*=32)	0	0	0	1	0	1	0	1	1	0	-	0	0	0	0	0	0	0	1	0	0	5
	12 (*n*=23)	0	0	0	1	1	1	0	1	1	0	0	-	0	1	0	0	3	0	0	0	1	10
	13 (*n*=29)	0	0	0	0	0	0	0	1	0	0	1	0	-	0	1	2	0	0	0	0	1	6
Arid	14 (*n*=29)	0	1	0	0	0	0	0	0	0	0	0	1	0	-	1	0	0	1	0	0	0	4
	15 (*n*=28)	0	0	0	0	1	0	1	1	0	1	0	1	1	0	-	0	0	0	0	0	3	9
	16 (*n*=34)	0	0	1	1	0	1	0	2	0	1	0	0	0	0	0	-	0	0	0	0	0	6
	17 (*n*=28)	0	0	0	0	0	2	0	0	0	0	0	0	2	1	0	1	-	1	1	0	0	8
	18 (*n*=10)	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	-	0	0	1	3
	19 (*n*=36)	0	0	0	0	0	0	1	1	0	0	0	2	0	1	0	1	1	0	-	0	0	7
	20 (*n*=10)	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	-	0	3
	21 (*n*=15)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	-	2
	TE	0	1	3	7	6	6	5	14	4	5	3	7	8	5	6	6	5	4	2	2	7	106

transitional forests; but today, it is largely covered in a single species of tall and densely growing introduced grass, with isolated stands of mostly introduced trees. While the agricultural zone harbours many exotic species, native plants and animals are observably rare in the agricultural zone. Certainly, *G. fuliginosa* is one of a few species of Darwin's finch that inhabits the agricultural zone. In comparison to all other zones, the agricultural zone has a lower abundance of *G. fuliginosa* during their breeding season, male territories are larger, and nest sites are further apart (Galligan and Kleindorfer unpublished data). A lack of prey diversity and abundance combined with a scarcity of feeding sites (open ground with grasses and herbs) and nesting sites (native trees with fruticose lichens) in the agricultural zone, is expected to result in a low quality habitat for *G. fuliginosa* (*sensu* Wiedenfeld 2008). Thus, high emigration from the agricultural zone to other zones may be evidence of passive, or even active, avoidance of a poor quality habitat. This idea is supported further by a non-significant trend for greater emigration between-zones than dispersal within-zone for the agricultural zone (*phi* = 0.30, *n* = 518, *p* = 0.108).

We had thought, despite genetic exchange among ecologically dissimilar zones (for the reasons given above), that gene flow in *G. fuliginosa* would be greater within zones than between zones, because in theory strong disruptive selection would favour assortative mating of ecomorphs and disfavour immigration between habitats. A greater level of gene flow within the arid zone in relation to all other zones at first

---

Table 7 (overleaf): Focussed regression by frequency analysis for the number of dispersal events within and among ecological zones, and across geographical zones (*n* = 518). For analysis of ecological zones, each zone was contrasted against all other zones combined (e.g., Arid = -3, Transition = 1, Agricultural = 1, and Humid = 1). For analysis of geographical distance, each zone was coded for 2 linear contrasts: south-north (Arid = -5, Transition = -3, Agricultural. = -1, Humid = 1, North1 = 3, and North2 = 5); and north-south (North2 = = -5, North1 = -3, Humid = -1, Agric. = 1, Trans. = 3, and Arid = 5). In the south-north direction, we measured gene flow by comparing the number of South 1 migrants and residents in each geographical zone; in the north-south direction, we compared the number of North 1 migrants and residents in each geographical zone. All analyses was performed separately for immigrants and emigrants, except within zones where immigration = emigration. Italicised *p*-values indicate significance after Bonferroni sequential corrections within groups of analyses.

	Contrast	X^2	*rho*	O.R.	*p*
Immigration	Arid vs. all other zones combined	0.75	0.04	0.80	0.39
among ecological zones	Transitional vs. all other zones combined	0.08	0.01	1.08	0.77
	Agricultural vs. all other zones combined	0.09	0.01	0.91	0.77
	Humid vs. all other zones combined	1.08	0.05	1.35	0.30
	Geographical distance (south-north)	3.01	0.08	-	0.08
	Geographical distance (north-south)	0.80	0.04	-	0.37
Emigration	Arid vs. all other zones combined	4.38	0.09	0.58	0.04
among ecological zones	Transitional vs. all other zones combined	0.06	0.01	0.94	0.81
	Agricultural vs. all other zones combined	19.19	0.19	3.09	*>0.001*
	Humid vs. all other zones combined	1.99	0.06	0.62	0.16
	Geographical distance (south-north)	3.70	0.08	-	0.05
	Geographical distance (north-south)	0.53	0.03	-	0.46
Dispersal	Arid	9.95	0.14	4.22	*0.002*
within ecological zones	Transitional	0.63	0.03	0.63	0.43
(immigration = emigration)	Agricultural	0.79	0.04	0.51	0.38
	Humid	4.61	0.09	0.00	0.03

appears to be evidence for gene flow with ecological zone fidelity. However, beyond this study we have no evidence of assortative mating of ecomorphs (Chapter 4), nor reduced immigration between habitats (but see Chapter 3); and here we found no difference between within-zone and between-zone immigration, or emigration, for arid zone finches; therefore, greater gene flow within the arid zone is an artefact of a greater discrepancy between within-zone and between-zone gene flow in other zones. This greater discrepancy is most apparent when comparing the proportion of immigrants, within-zone and between-zones, for the arid (0.07 and 0.09, respectively) and agricultural (0.02 and 0.06, respectively) zones. Indeed, within the humid zone no gene flow was detected (therefore: 0.00 and 0.04, respectively).

Ignoring the humid zone results, we found no significant difference in the rate of either immigration or emigration between within-zones and-between zones; that is, gene flow showed neither ecological zone fidelity nor infidelity. Non-significant trends detected in the transitional zone for greater emigration to other zones than within-zone (*phi* = 0.29), and greater immigration from other zones than within-zone (*phi* = 0.31), suggest that gene flow between different zones, which is apparent in the humid zone, occurs more frequently than gene flow within the same zone. However, caution is required in this interpretation, as the power for our within-zone and between-zones analyses was low.

Our interpretation of gene flow needs to be taken with a degree of caution. GENECLASS has been shown to accurately detect first generation migrants in real data with low genetic differentiation (i.e., 100 % accuracy for F_{ST} = 0.06 and 78 % accuracy for F_{ST} = 0.04: Berry *et al.* 2004). However, accuracy swiftly drops in simulated data to approximately 20 % for F_{ST} values close to zero (Cornuet *et al.* 1999); the observed level of genetic differentiation in *G. fuliginosa* between among sites and ecological zones. Our own test of accuracy revealed that GENECLASS2 could only designate ~4 % of individuals to a single site. Therefore, we can have little if any confidence in our estimates of gene flow.

The effect of low genetic differentiation aside, resampling methods implemented in GENECLASS are designed to reduce Type I errors (i.e., misassignment of residents as immigrants) at an obvious cost to Type II errors (i.e., misassignment of immigrants as residents); therefore, we expect that true gene flow in *G. fuliginosa* among sites and zones is higher than we report.

Extremely low genetic differentiation makes obtaining accurate estimates of contemporary gene flow using any genetic method difficult. As an alternative direct measure of gene flow, mark-recapture/resighting techniques can be employed. We have data on recaptured and resighted finches (discussed above) that suggest very little gene flow between two sets of sites approximately 18 km apart (equivalent to Sites 3 and 4, and 14 and 15 in our study). However, the probability of recording every dispersal event using such methods is low. Given the size of Santa Cruz, the high mobility of *G. fuliginosa*, and the lack of physical barriers to dispersal within the landscape; in addition to low F_{ST} among sites and no population substructure; and typically high gene flow in birds (Avise 1994); we are confident that gene flow in *G. fuliginosa* within Santa Cruz is high. However, only future studies dedicated to extensive mark-recapture and resighting can provide a more accurate estimate.

High gene flow excludes genetic drift from contributing to phenotypic divergence between populations, and thereby validates selection as a significant influence. Our results corroborate previous findings that showed differences in foraging behaviour (Kleindorfer *et al.* 2006) and resighting probability (Sulloway and Kleindorfer in review) across ecological zones, which were thought to be the products of adaptation. However, adaptation in our system may not be entirely *adaptation* in its classic sense – that is, a change in phenotype as a result of increased fitness on heritable traits. Phenotypic divergence between locations can also arise through phenotypic plasticity (i.e., an individual changes its phenotype to better match the environment) or matching habitat choice (i.e., an individual changes the environment to better match its phenotype; see Edelaar *et al.* 2008). Phenotypic plasticity is an unlikely explanation for morphological divergence observed in our system, because of the high heritability of many morphological traits in Darwin's finches (e.g., bill length: Boag and Grant 1978; Boag 1983). However, matching habitat choice is a possible factor influencing phenotypic divergence in this system, given the contrast among ecological zones on Santa Cruz and the ability of *G. fuliginosa* to disperse widely and freely across the entire island (Edelaar *et al.* 2008).

Thus, a considerable level of *adaptive* divergence in *G. fuliginosa* on Santa Cruz may be generated by the movement of individuals among ecological zones to optimise a match between their phenotype and the environment (i.e., dispersal down an individual selection gradient [Armsworth and Roughgarden 2005a, b]); rather than selective mortality of maladapted individuals. As we have discussed above, a lack of

dispersal among arid zone sites may provide evidence for matching habitat choice in this system. Certainly, evidence found to support classic adaptation between arid and humid zone *G. fuliginosa* on Santa Cruz – that is, phenotype matching (Kleindorfer *et al.* 2006) and trait utility (Sulloway and Kleindorfer *et al.* in review) – can equally support matching habitat choice. Even evidence for adaptive divergence in clutch size (Kleindorfer 2007) and song characteristics (Chapter 5) may be explained (although less likely) by matching habitat choice. However, there is strong evidence that selection was responsible for increased beak size and changing beak shape in *G. fuliginosa* between 2000 and 2005 (Sulloway and Kleindorfer in review). Given that classic adaptation and matching habitat choice are not mutually exclusive; therefore, both may be acting in our system at present.

Matching habitat choice can initiate and accelerate local adaptation, and may enable adaptive peak shifts (Edelaar *et al.* 2008; Holt and Barfield 2008). For *G. fuliginosa* on Santa Cruz, range expansion into the humid zone occurred recently (< 50 years ago) facilitated by expansion of small-seeding plants (Chapter 4) and the local extinction of the sharp-beaked finch *Geospiza difficilis* (Kleindorfer *et al.* 2006; Kleindorfer 2007; Kleindorfer and Mitchell 2009). It is highly likely that matching habitat choice played an important role in the initial colonisation events (the success of colonists would have relied on pre-adaptations to the humid zone (Mayr 1965; Chapter 4) and thereby biased the mean phenotype of the founding population. Fifty years later, matching habitat choice may maintain a significant influence on the distribution of phenotypic variance among ecological zones during low rainfall periods. Alternatively, matching habitat choice may have initiated and accelerated the formation of a (classic) adaptive cline across ecological zones; most pronounced during low rainfall periods. Therefore, *G. fuliginosa* on Santa Cruz represents a unique opportunity to explore the evolution and interplay between classic adaptation and matching habitat choice. Our future work needs to assess the relative contribution of each process by examining the strength and pattern (random or non-random) of (1) mortality and reproduction and (2) dispersal among ecological zones; as well as, the roles of competition, imprinting, and heritability on habitat choice (Edelaar *et al.* 2008).

Matching habitat choice has been previously documented in Darwin's finches on a finer scale. Specifically, on the island of Daphne Major, male *G. fortis* with larger beak depths would establish territories with an abundance of their preferred

food source – the large-seed-producing *Tribulus sp.*; whereas males with smaller beak depths would establish territories where *Tribulus sp.* was scarce (Price 1987). At a broader scale, habitat choice (not necessarily for a match with phenotype) was shown for interisland dispersal in *G. fortis* (Grant *et al.* 2001) and the warbler finch species *Certhidea spp.* (Tonnis *et al.* 2005).

Whereas phenotypic divergence can be maintained among ecological zones by the adaptive responses discussed above, the underlying disruptive selection in our system has not favoured non-random mating among ecological zones. A lack of deviations from HWE and no significant difference in gene diversity among sites are genetic indicators of random mating. That being said, we sampled the entire population and not just the breeding population. Further, we have evidence for positive assortative pairing (Chapter 4) and discrimination of local and foreign song (Chapter 5) in the arid zone finches. However, these findings are not reproduced in the humid zone (Chapter 4 and 5), indicating (at most) one-way reproductive isolation in our system. An alternative explanation for a lack of genetic divergence is that insufficient time has passed for it to reach a detectable level. Unlike our estimates of dispersal among sites (number of first generation migrants), genetic population structure was determined using both contemporary and historic genetic connectivity; and therefore, may reflect recent shared ancestry. Given the presumed recent history of divergence in Santa Cruz *G. fuliginosa*, and the average mutation rates for eukaryotes (i.e., $10x^{-3}$–$10x^{-5}$/base/generation), a "too soon" explanation for the lack of genetic divergence is possible. The aim of our study was to use genetic data, specifically microsatellite allele frequency data, to answer two fundamental questions pertaining to the observed phenotypic divergence in *G. fuliginosa* inhabiting contrasting ecological zones on Santa Cruz: (1) has phenotypic divergence occurred in the presence of gene flow? and (2) has phenotypic divergence led to genetic divergence? Our findings suggest that: (1) phenotypic divergence has occurred in the presence of gene flow, supporting a classic adaptive divergence and/or a matching habitat choice hypothesis, the relative contributions of each remain to be tested; and (2) phenotypic divergence has not led to genetic divergence, but may maintain the potential to do so as long as disruptive selection is strong enough and local adaptation builds up over time. Our study also provides evidence for the initiation of the first stage of the non-allopatric models of speciation – that is adaptive divergence amid high gene flow.

ACKNOWLEDGEMENTS

We thank the Galápagos National Park and the Charles Darwin Research Station for the opportunity to conduct this research; Max Planck Institute for Ornithology, American Bird Conservancy, Conservation International, Galápagos Conservation Fund, and Flinders University for their generous financial support; TAME airlines for financial assistance with airfares; Zonnetje Auburn, Mari Cruz Jaramillo, Birgit Fessl, Michael Dvorak, Jody O'Connor, Sarah Huber, Jennifer Koop, and Ryan Buss for their assistance in the field; Steven Myers, Mike Gardner, Kathy Saint, Leanne Wheaton, and Ken Petren for their assistance in the laboratory. This research was conducted under Flinders University animal ethics approval (E189).

REFERENCES

Abdo, Z., Crandall, K. A. and Joyce, P. 2004. Evaluating the performance of likelihood methods for detecting population structure and migration. *Molecular Ecology* **13**: 837-851.

Avise, J. C. 1994. *Molecular Markers, Natural Selection, and Evolution.*Chapman and Hill: New York, N. Y.

Armsworth, P. R. and Roughgarden, J. E. 2005a. Disturbance induces the contrasting evolution of reinforcement and dispersiveness in directed and random movers. *Evolution* **59**: 2083-2096.

Armsworth, P. R. and Roughgarden, J. E. 2005b. The impact of directed versus random movement on population dynamics and biodiversity patterns. *American Naturalist* **165**: 449-465.

Barluenga, M., Stolting, K. N., Salzburger, W., Muschick, M. and Meyer, A. 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* **439**: 719-723.

Berry, O., Tocher, M. D. and Sarre, S. D. 2004. Can assignment tests measure dispersal? *Molecular Ecology* **13**: 551-561.

Boag, P. T. 1983 The heritability of external morphology in Darwin's ground finches (Geospiza) on Isla Daphne Major, Galápagos. *Evolution* **37**: 877-894.

Boag, P. T. and Grant, P. R. 1978. The heritability of external morphology in Darwin's finches. *Nature* **274**: 793-794.

Chang, A. 1999. *Stats Toolbox* Hong Kong. http://department.obg.cuhk.edu.hk/

Cornuet, J. M., Piry, S., Luikart, G., Estoup, A. and Solignac, M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* **153**: 1989-2000.

Coyne, J. A. 2007. Sympatric speciation. *Current Biology* **17**: R787-R788.

Coyne, J. A. and Orr, H. A. 2004. *Speciation*. Sinauer Associates: Sunderland, M. A.

Coyne, J. A. and Price, T. D. 2000. Little evidence for sympatric speciation in island birds. *Evolution* **54**: 2166-2171.

Crow, K. D., Munehara, H. and Bernardi, G. 2010. Sympatric speciation in a genus of marine reef fishes. *Molecular Ecology* **19**: 2089-2105.

de Leon, L. F., Bermingham, E., Podos, J. and Hendry, A. P. 2010. Divergence with gene flow as facilitated by ecological differences: within-island variation in Darwin's finches. *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**: 1041-1052.

Dieckmann, U., Doebeli, M., Metz, J. A. J. and Tautz, D. 2004. *Adaptive Speciation*. Cambridge University Press: Cambridge, U. K.

Edelaar, P., Siepielski, A. M. and Clobert, J. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. *Evolution* **62**: 2462-2472.

Emelianov, I., Marec, F. and Mallet, J. 2004. Genomic evidence for divergence with gene flow in host races of the larch budmoth. *Proceedings of the Royal Society B-Biological Sciences* **271**: 97-105.

Endler, J. A. 1977. *Geographic Variation, Speciation, and Clines* Princeton University Press: Princeton, N. J.

Fitzpatrick, B. M., Fordyce, J. A. and Gavrilets, S. 2009. Pattern, process and geographic modes of speciation. *Journal of Evolutionary Biology* **22**: 2342-2347.

Ford, H. A., Parkin, D. T. and Ewing, A. W. 1973. Divergence and evolution in Darwin's finches. *Biological Journal of the Linnean Society* **5**: 289-295.

Gavrilets, S. 2004. *Fitness Landscapes and the Origin of Species*. Princeton: Princeton, N. J.

Gavrilets, S. and Vose, A. 2007. Case studies and mathematical models of ecological speciation. 2. Palms on an oceanic island. *Molecular Ecology* **16**: 2910-2921.

Gavrilets, S., Vose, A., Barluenga, M., Salzburger, W. and Meyer, A. 2007. Case studies and mathematical models of ecological speciation. 1. Cichlids in a crater lake. *Molecular Ecology* **16**: 2893-2909.

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal of Heredity* **86**: 485-486.

Grant, P. R. 1999. *Ecology and Evolution of Darwin's Finches*. Princeton University Press: Princeton, N. J.

Grant, P. R. and Grant, B. R. 2008. *How and Why Species Multiple: the Radiation of Darwin's Finches* Princeton University Press: Princeton, N. J.

Grant, P. R. and Grant, B. R. 2010. Sympatric speciation, immigration, and hybridisation in island birds. In: The Theory of Island Biogeography Revisted. Losos, J. B., Ricklefs, R. and MacArthur, R. H. (eds). Princeton University Press: Princeton, N. J.

Grant, P. R., Grant, B. R. and Petren, K. 2001. A population founded by a single pair of individuals: establishment, expansion, and evolution. *Genetica* **112**: 359-382.

Grant, P. R., Grant, B. R. and Petren, K. 2005. Hybridization in the recent past. *American Naturalist* **166**: 56-67.

Guillot, G., Mortier, F. and Estoup, A. 2005. GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes* **5**: 712-715.

Hardy, O. J., Charbonnel, N., Freville, H. and Heuertz, M. 2003. Microsatellite allele sizes: A simple test to assess their significance on genetic differentiation. *Genetics* **163**: 1467-1482.

Hardy, O. J. and Vekemans, X. 2002. SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**: 618-620.

Hey, J. 2006. Recent advances in assessing gene flow between diverging populations and species. *Current Opinion in Genetics & Development* **16**: 592-596.

Holt, R. D. and Barfield, M. 2008. Habitat selection and niche conservatism. *Israel Journal of Ecology & Evolution* **54**: 295-309.

Jensen, J. L., Bohonak, A. J. and Kelley, S. T. 2005. Isolation by distance, web service. *Bmc Genetics* **6**:

Kirkpatrick, M. and Ravigne, V. 2002. Speciation by natural and sexual selection: Models and experiments. *American Naturalist* **159**: S22-S35.

Kleindorfer, S. 2007. The ecology of clutch size variation in Darwin's Small Ground Finch *Geospiza fuliginosa*: comparison between lowland and highland habitats. *Ibis* **149**: 730-741.

Kleindorfer, S., Chapman, T. W., Winkler, H. and Sulloway, F. J. 2006. Adaptive divergence in contiguous populations of Darwin's Small Ground Finch (*Geospiza fuliginosa*). *Evolutionary Ecology Research* **8**: 357-372.

Kleindorfer, S. and Mitchell, J. 2009. Biological Networks: Rainforests, Coral Reefs, and the Galapagos Islands In: The Network Challenge: Strategy, Profit, and Risk in an Interlinked World. Kleindorfer, P. R., Wind, Y. and Gunther, R. E. (eds). Warton School Publishing: Philadelphia, PA

Lack, D. 1947. *Darwin's Finches*. Cambridge University Press: Cambridge, U. K.

Li, J. W., Yeung, C. K. L., Tsai, P. W., Lin, R. C., Yeh, C. F., Yao, C. T., Han, L. X., Hung, L. M., Ding, P., Wang, Q. S. and Li, S. H. 2010. Rejecting strictly allopatric speciation on a continental island: prolonged postdivergence gene flow between Taiwan (*Leucodioptron taewanus*, Passeriformes Timaliidae) and Chinese (*L. canorum canorum*) hwameis. *Molecular Ecology* **19**: 494-507.

Mayr, E. 1965. The nature of colonisation in birds. In: The Genetics of Colonising Species. Baker, H. G. and Stebbins, G. L. (eds). Academic press: New York, N. Y.

Mayr, E. 1966. Sympatric Speciation. *American Naturalist* **100**: 637-650.

McCormack, J. E. and Smith, T. B. 2008. Niche expansion leads to small-scale adaptive divergence along an elevation gradient in a medium-sized passerine bird. *Proceedings of the Royal Society B-Biological Sciences* **275**: 2155-2164.

Niemiller, M. L., Fitzpatrick, B. M. and Miller, B. T. 2008. Recent divergence with gene flow in Tennessee cave salamanders (Plethodontidae: Gyrinophilus) inferred from gene genealogies. *Molecular Ecology* **17**: 2258-2275.

Nosil, P. 2008a. Ernst Mayr and the integration of geographic and ecological factors in speciation. *Biological Journal of the Linnean Society* **95**: 26-46.

Nosil, P. 2008b. Speciation with gene flow could be common. *Molecular Ecology* **17**: 2103-2106.

Nosil, P. 2009. Adaptive Population Divergence in Cryptic Color-Pattern Following a Reduction in Gene Flow. *Evolution* **63**: 1902-1912.

Paetkau, D., Calvert, W., Stirling, I. and Strobeck, C. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* **4**: 347-354.

Paetkau, D., Slade, R., Burden, M. and Estoup, A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* **13**: 55-65.

Panova, M., Hollander, J. and Johannesson, K. 2006. Site-specific genetic divergence in parallel hybrid zones suggests nonallopatric evolution of reproductive barriers. *Molecular Ecology* **15**: 4021-4031.

Petren, K. 1998. Microsatellite primers from Geospiza fortis and cross-species amplification in Darwin's finches. *Molecular Ecology* **7**: 1782-1784.

Petren, K., Grant, P. R. and Grant, B. R. 1999. A phylogeny of Darwin's finches based on microsatellite DNA length variation. *Proc. R. Soc. Lond. B* **266**: 321-329.

Petren, K., Grant, P. R., Grant, B. R. and Keller, L. F. 2005. Comparative landscape genetics and the adaptive radiation of Darwin's finches: the role of peripheral isolation. *Molecular Ecology* **14**: 2943-2957.

Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L. and Estoup, A. 2004. GENECLASS: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity* **95**: 536-539.

Price, T. 1987. Diet variation in a population of Darwin's finches. *Ecology* **68**: 1015-1028.

Price, T. 2008. *Speciation in Birds*. Roberts and Company: Greenwood Village, C. O.

R Development Core Team. 2004. *R-project*. Auckland, N. Z.

Rambaut, A. and Drummond, A. J. 2007. *Tracer v1.5*.

Raymond, M. and Rousset, F. 1995. GENEPOP (version 1.2) - Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**: 248-249.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Ecvolution* **43**: 223-244.

Rice, W. R. and Hostert, E. E. 1993. Laboratory experiments on speciation: what have we learned in forty years? *Evolution* **47**: 1637-1653.

Rousset, F. 2008. GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8**: 103-106.

Rundle, H. D. and Nosil, P. 2005. Ecological speciation. *Ecology Letters* **8**: 336-352.

Savolainen, V., Anstett, M. C., Lexer, C., Hutton, I., Clarkson, J. J., Norup, M. V., Powell, M. P., Springate, D., Salamin, N. and Baker, W. J. 2006. Sympatric speciation in palms on an oceanic island. *Nature* **441**: 210-213.

Schilthuizen, M., Cabanban, A. S. and Haase, M. 2005. Possible speciation with gene flow in tropical cave snails. *Journal of Zoological Systematics and Evolutionary Research* **43**: 133-138.

Schluter, D. 2001. Ecology and the origin of species. *Trends in Ecology & Evolution* **16**: 372-380.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**: 457-462.

Slatkin, M. 2005. Seeing ghosts: the effect of unsampled populations on migration rates estimated for sampled populations. *Molecular Ecology* **14**: 67-73.

Smith, L. M. and Burgoyne, L. A. 2004. Collecting, archiving and processing DNA from wildlife samples using FTA® databasing paper. *BMC Ecology* **4**: 4-15.

Smith, T. B., Calsbeek, R., Wayne, R. K., Holder, K. H., Pires, D. and Bardeleben, C. 2005. Testing alternative mechanisms of evolutionary divergence in an African rain forest passerine bird. *Journal of Evolutionary Biology* **18**: 257-268.

Smith, T. B., Wayne, R. K., Girman, D. J. and Bruford, M. W. 1997. A role for ecotones in generating rainforest biodiversity. *Science* **276**: 1855-1857.

Snell, H. and Rae, S. 1999. The 1997-98 El Nino in Galapagos: can 34 years of data estimate 120 years of pattern? *Noticias de Galapagos* **60**: 11-20.

Storchova, R., Reif, J. and Nachman, M. W. 2010. Female heterogamety and speciation: reduced introgression of the Z chromosome between two species of nightingales. *Evolution* **64**: 456-471.

Tebbich, S., Taborsky, M., Fessl, B. and Dvorak, M. 2002. The ecology of tool-use in the woodpecker finch (Cactospiza pallida). *Ecology Letters* **5**: 656-664.

Tonnis, B., Grant, P. R., Grant, B. R. and Petren, K. 2005. Habitat selection and ecological speciation in Galapagos warbler finches (*Certhidea olivacea* and *Certhidea fusca*). *Proceedings of the Royal Society B-Biological Sciences* **272**: 819-826.

van Doorn, G. S., Edelaar, P. and Weissing, F. J. 2009. On the Origin of Species by Natural and Sexual Selection. *Science* **326**: 1704-1707.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**: 535-538.

Wang, R. L., Wakeley, J. and Hey, J. 1997. Gene flow and natural selection in the origin of *Drosophila pseudoobscura* and close relatives. *Genetics* **147**: 1091-1106.

Weir, B. S. and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358-1370.

Wiedenfeld, D. A. and Jiménez-Uzcátegui, G. A. 2008. Critical problems for bird conservation in the Galápagos Islands. *Cotinga* **29**: 22-27.

CHAPTER THREE

High rainfall event relaxes selection against immigrants and removes morphological clines in Darwin's small ground finch, *Geospiza fuliginosa*

Toby H. Galligan, Frank J. Sulloway, Stephen C. Donnellan,
Terry Bertozzi, Alison J. Fitch, and Sonia Kleindorfer

PLoS Biology (in preparation)

ABSTRACT

Disruptive natural selection favours adaptive divergence between populations and active individual dispersal to pursue environment-phenotype matching. Both responses increase and decrease with selection intensity; however, dispersal between populations is also influenced by geographical proximity and landscape barriers. Therefore, under disruptive selection and free dispersal, it is the strength of selection against immigrants in each population that determines whether phenotypic divergence or convergence is exhibited. Selection against immigrants is positively related to disruptive selection: as disruptive selection relaxes, so does selection against immigrants. We test this idea in Darwin's small ground finch, *Geospiza fuliginosa*, on the island of Santa Cruz, Galápagos Archipelago. On Santa Cruz the ecosystem is strongly influenced by rainfall, which increases with altitude and shows significant annual and seasonal variation. The past decade has been characterised by a prolonged low rainfall condition with infrequent high rainfall events. In low rainfall years, <250 mm rain falls in the arid lowlands, but >700 mm of rain falls in the humid highlands. This ecological contrast is associated with morphological clines in *G. fuliginosa* across altitude-dependent ecological zones. Here, we show no difference in mean morphological traits among all four ecological zones on Santa Cruz in a high rainfall year, when ~700 mm of rain fell in the lowlands and ~1500 mm fell in the highlands. To explain how clines may have been broken down, we use morphological and molecular genetic data to examine patterns of dispersal between lowlands and highlands in both low and high rainfall years. Our findings, although not conclusive, suggest no increase in dispersal between lowlands and highlands in the high rainfall year. Therefore, we propose that relaxed selection against otherwise ill-adapted immigrants under the "benign" conditions of the high rainfall year has removed morphological clines in *G. fuliginosa* inhabiting different ecological zones.

Keywords: relaxed disruptive selection, selection against immigrants, immigrant inviability, morphological convergence, within island dispersal, Darwin's finches, El Niño-Southern Oscillation

INTRODUCTION

Dispersal behaviour is an adaptation that enables individuals to change local selection regimes, and thereby alter fitness outcomes (Olivieri *et al.* 1995; Clobert *et al.* 1997, 2001; Bowler and Benton 2005; Edelaar *et al.* 2008). Under disruptive selection generated by heterogeneity in the landscape, populations may show adaptive divergence (e.g. Hendry 2001; Langerhans *et al.* 2003; Ferrari *et al.* 2006; Schlotfeldt and Kleindorfer 2006; Berner *et al.* 2009; Mila *et al.* 2009; Myers *et al.* 2010); and individuals may show active inter-population dispersal, to achieve environment-phenotype matching (e.g. Jones and Probert 1980; Cruz *et al.* 2004; Garant *et al.* 2005; Edelaar *et al.* 2008; Bolnick *et al.* 2009). Selection intensity influences the magnitude of these responses in the same direction – so that, as disruptive selection increases (or decreases) so does adaptive divergence and active dispersal. However, the magnitude of active dispersal (unlike adaptation) is also positively influenced by geographic proximity of populations and the ease of movement between them (i.e., a lack of barriers to dispersal). Thus, for populations under disruptive selection and conditions permitting unrestricted dispersal, the key evolutionary process determining whether differences in phenotypes are exhibited, is the strength of selection against immigrants in each population.

Selection against immigrants, or immigrant inviability, was only conceptually introduced recently (Hendry 2004; Nosil *et al.* 2005); however, the idea has earlier origins (e.g. Mallet 1989; Funk 1989; Nagy and Rice 1997; Via 1999; Hendry 2000) and numerous contemporary examples (e.g. Lowry *et al.* 2008; Matute *et al.* 2009; Tobler 2009; Wellenreuther *et al.* 2010; MacColl and Chapman 2010; Westberg *et al.* 2010). In a review of the literature, where the contribution of various reproductive barriers was quantified within study systems, Nosil *et al.* (2005) showed that the contribution of immigrant inviability was ubiquitous and frequently principal (9/20 studies). This reproductive barrier can be defined simply as the lower survival or fecundity of ill-adapted immigrants (Nosil *et al.* 2005). Ill-adaptation is directly related to the degree of adaptive divergence between populations (note that ill-adaptation can occur in one population only); hence, selection against immigrants is expected to be strong when disruptive selection is strong (or strengthened); and weak when disruptive selection is weak (or relaxed). This idea can be tested in parallel systems where the intensity of disruptive selection differs between them; or in a

single system where the intensity of disruptive selection regularly oscillates in time.

The Galápagos archipelago represents a system that has oscillating selection intensity across both space and time (see Grant 1999; Grant and Grant 2008). For terrestrial organisms, spatial and temporal variation in rainfall is the key selection pressure as it directly affects the diversity and abundance of prey (Grant 1999; Grant and Grant 2008). Spatially, the amount of rainfall received increases with altitude, generating four major altitudinal-dependent ecological zones on elevated islands: *arid* lowlands, *transitional* midlands, *agricultural* midlands, and *humid* highlands (Fig. 1). Temporally, the climate alternates between prolonged *La Nińa* periods of low rainfall (2-11 years) and brief *El Nińo* periods of high rainfall (1-2 years: Snell and Rae 1999; Fig. 2). Combined, spatial and temporal variation in rainfall results in a strong ecological

Figure 1: Map of Santa Cruz, Galápagos Archipelago, showing the distribution of four major ecological zones and the 21 sites (indicated by filled circles) sampled in our study.

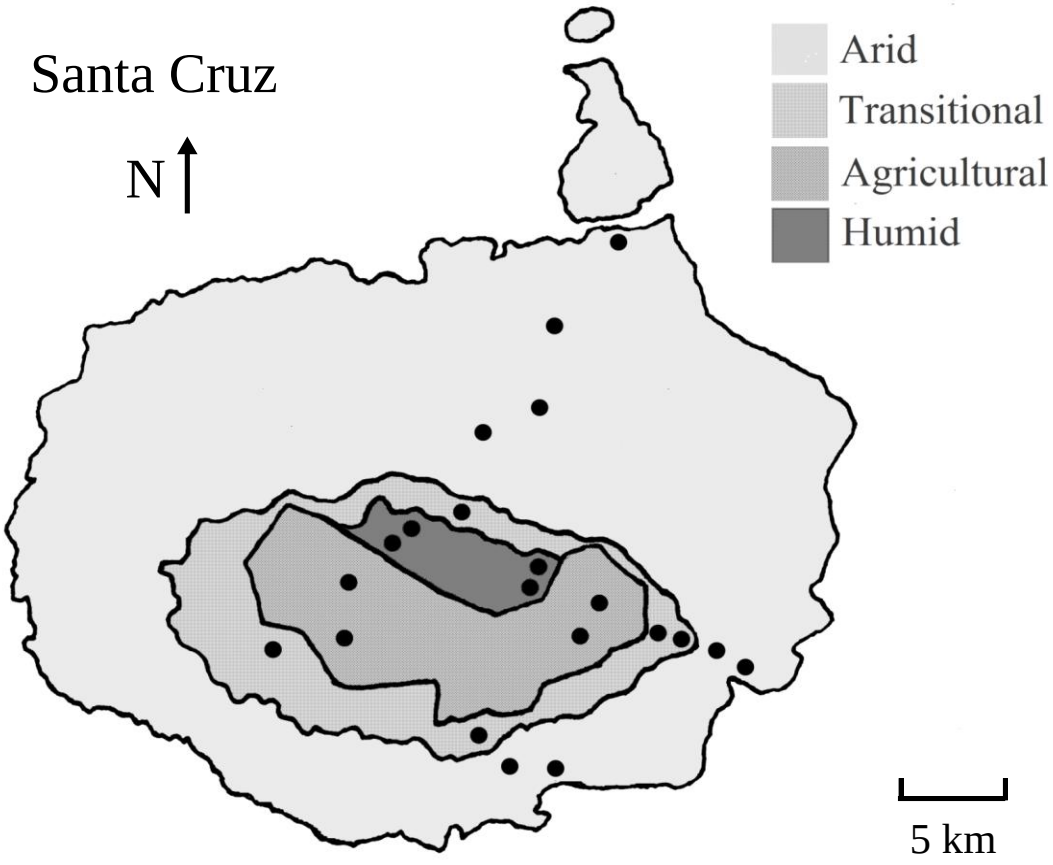
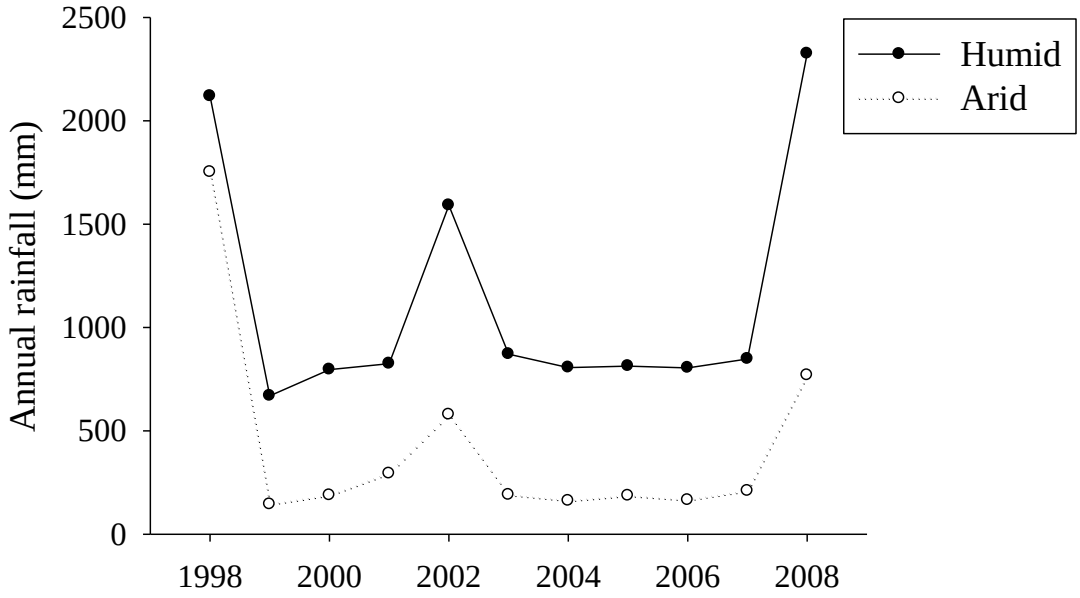


Figure 2: Annual rainfall recorded between 1998 and 2008 for the arid lowlands (Academy Bay: N, E = 0800270, 9917844; Altitude = 2 m a.s.l.) and humid highlands (Ballavista: 0795374, 9921159; 194 m a.s.l.) on Santa Cruz, Galápagos Archipelago.



contrast between the arid and humid zones, which is most pronounced during periods of low rainfall, when the wet season in the arid zone is almost non-existent and annual rainfall is below the level that defines a desert – i.e., less than 250 mm – but the wet season still occurs in the humid zone and annual rainfall is greater than 700 mm (Fig. 2). Such a contrast presumably generates strong disruptive selection on organisms that inhabit both zones; and thereby, strong selection against immigrants. Conversely, during periods of high rainfall, when the arid zone has wet seasons and receives greater than 500 mm of rain annually (Fig. 2), the ecological contrast between the arid and humid zones is weakened, independent of the difference in annual rainfall between zones. During these brief "benign" periods, there is a boom prey supply in the arid zone, and thereby, disruptive selection and selection against immigrants is predicted to be relaxed.

During the past decade of low rainfall (Fig. 2), Darwin's small ground finch, *Geospiza fuliginosa*, on the elevated island of Santa Cruz, Galápagos Islands, has

shown adaptive divergence among ecological zones – specifically, there was evidence for linear clines in beak length, tarsus thickness, foot length, and claw length (Kleindorfer *et al.* 2006; Sulloway and Kleindorfer in review); phenotype-environment matching (Kleindorfer *et al.* 2006; Kleindorfer 2007); and trait utility (Sulloway and Kleindorfer in review). Gene flow among ecological zones is expected to be high; therefore, adaptive divergence is predicted to be the result of classic adaptation and/or habitat matching choice (Chapter 2).

The year 2008 was one of high rainfall on Santa Cruz (Fig. 2), which provided an opportunity to examine the immediate effect of relaxed selection against immigrants on dispersal and morphological clines in *G. fuliginosa*. In 2008, we specifically tested whether the linear clines observed during low rainfall period were absent (had broken down) in a high rainfall year, resulting in no difference in mean trait values among *G. fuliginosa* inhabiting different ecological zones. To explain the mechanism for potential breakdown of clines, we examined differences in dispersal between arid and humid zones in low rainfall (2004 and 2005) and high rainfall (2008) periods. We specifically tested two alternative hypotheses against the null hypothesis (no difference in dispersal between sampling periods): (1) a unidirectional increase in dispersal in the high rainfall year; and (2) a bidirectional increase in dispersal in the high rainfall year. We ask: are morphological clines removed by a one-way invasion of the humid zone by arid zone finches, or vice versa (our first alternative hypothesis); by mass two-way movement of finches among ecological zones (our second alternative hypothesis); or by relaxed selection on otherwise ill-adapted immigrants among ecological zones (our null hypothesis)?

METHODS

Sample and site details

We collected morphological and molecular genetic data from Darwin's small ground finch, *Geospiza fuliginosa*, during two low rainfall years, 2004 (January-February) and 2005 (February), and one high rainfall year, 2008 (January to May). In each year the sampling periods coincided with the period of highest annual rainfall (wet season), prey supply, and breeding activity in *G. fuliginosa*. The lag between the

Table 1: Study sites: ecological zone; locality; coordinates (UTM; zone = 15); altitude (metres above sea level); and sample size (morphological, microsatellite) for 2004/2005 ($N_{2004/2005}$) and 2008 (N_{2008}).

Ecol. Zone	Locality	N, E	Alt.	$N_{2004/2005}$	N_{2008}
Humid	*El Puntudo*	0797223, 9929170	702	-	15, -
	Media Luna	0797450, 9927147	605	-	16, -
	Los Gemelos	0791254, 9931119	617	35, 30	36, 29
	Los Gemelos	0790140, 9929998	569	43, 34	50, 37
Agricultural	*El Camote*	0801700, 9927485	413	44, -	18, -
	El Cascajo	0800134, 9925082	326	-	27, -
	Santa Rosa	0788819, 9928198	447	-	23, -
	Tortoise Territory	0788633, 9925920	331	-	39, -
Transitional	*El Garrapatero*	0804672, 9926163	255	-	29, -
	El Garrapatero	0806750, 9925521	149	-	40, -
	El Chato	0785088, 9925622	198	-	53, -
	Guaybillos	0795374, 9921159	128	-	34, -
	Mina Rojo	0793093, 9931805	590	-	30, -
Arid	*El Garrapatero*	0808374, 9925013	66	34, 22	32, 29
	El Garrapatero	0809269, 9923213	5	30, 15	33, 28
	*El Mirador**	0797380, 9918815	65	-	34, -
	CDRS**	0800270, 9917844	9	-	42, -
	Mina Rojo	0793093, 9931805	590	-	30, -
	Refuse site	0794217, 9935157	277	-	11, -
	Mina Negro	0796817, 9936484	254	-	40, -
	Goat-hunters' Track	0798818, 9940558	100	-	12, -
	Itabaca Canal	0802592, 9944812	10	-	16, -

* *El Mirador de los Túneles*; **Charles Darwin Research Station, Academy Bay

onsets of wet season rains, the boom in prey supply, and breeding in Darwin's finches is short (Grant 1999).

In 2004 and 2005, we sampled from 5 sites on the southern side of the island of Santa Cruz: 2 sites each in the arid and humid zones and 1 site each in the

agricultural zone and humid zones (Fig. 1; Table 1). In 2008, we sampled from 21 sites which spanned the entire island: 8 sites in the arid zone, 5 sites in the transitional zone, and 4 each in the agricultural (Fig. 1; Table 1). Due to southern prevailing winds and a rain shadow cast by the central peaks, rainfall at a given altitude on the northern side of Santa Cruz is lower than on the southern side. As a result, on the northern side the arid and transitional extend to a greater altitude and the agricultural zone is absent (Table 1). Nevertheless, ecological zones maintain distinct ecological communities: the arid zone is characterised by dry-deciduous open forest and woodland dominated by *Bursera graveolens*; the humid zone is characterised by evergreen closed forest (*Scalesia pedunculata* dominated), closed shrubland (*Miconia robinsoniana* dominated), and fern-sedge grassland; the transitional zone is characterised by a mixture of dry-deciduous and evergreen species that form an open forest (*Pisonia floribunda – Piscidia carthagenensis – Psidium galapageium* codominated); the agricultural zone is characterised by humid and transitional habitat that had been largely replaced by fields of introduced *Pennisetum purpureum* and stands of exotic trees, such as *Psidium guajava*, *Cinchona pubescens*, and *Erythrina coarllodendron*.

Geospiza fuliginosa forages and breeds in all four ecological zones (Bowman 1961; Kleindorfer *et al.* 2006; Kleindorfer 2007).

Data Collection

Geospiza fuliginosa were sampled using mist nets. Only mature finches were processed because juvenile finches could bias results by not having fully developed traits and being closely related within sites. We discriminated juveniles based the presence of obvious gape flanges, soft feet, and short wing and/or tail feathers.

We recorded the following 11 morphological measurements from adult birds: (1) beak length (feathers) (culmen tip to the feather-line); (2) beak length (naris) (culmen tip to the anterior edge of the naris); (3) beak depth (at the feather line); (4) beak width (at the feather line); (5) tarsus length (length of the tarsometatarsus); (6) tarsus depth; (7) tarsus width; (8) wing length (carpal joint to tip of the ninth primary); (9) Kipp's distance (the distance between the primaries and secondaries); (10) foot length (middle toe tip to hind toe tip); and (11) foot and claw length (middle claw tip to hind claw tip). We used dial callipers with an accuracy of 0.1 mm for all measurements; except wing length and Kipp's distance, which were measured

with a stop rule with an accuracy of 1.0 mm. The two foot length measurements were taken from an impression of the individual's foot in plasticine. All measurements were taken on the right side of the bird.

Measurements were taken by S.K. in 2004/2005 and T.H.G. in 2008. Inter-measurer reliability for Darwin's finches could not be analysed because S.K. and T.H.G. did not measure the same individuals. Inter-measurer reliability was analysed on a morphologically similar Australian species, red-browed finch *Neochmia temporalis* (Estrildae), using skins from the South Australian Museum collection. S.K. and T.H.G independently measured 4 beak dimensions (as above) on the same 10 skins. For beak length (naris), beak width, and beak depth the person-to-person difference was within the normal range of error from repeated measurements by the same person (< 0.20 mm ~ ½ a standard deviation). However, for beak length feather, T.H.G.'s average measurements were 1.46 mm shorter than S.K.'s, which is up to 3.84 standard deviations of the normal range of error from repeated measurements by the same person. Thus, while we analyse differences between 2004/2005 and 2008 datasets to comment on temporal changes in morphology, we interpret our results with caution.

We collected blood to obtain DNA. Blood was stored on FTA® databasing paper in the field and DNA was extracted later in the laboratory using a modified protocol published by Smith and Burgoyne (2004). Specifically, we washed a 1 mm^2 disc of blood-soaked FTA® databasing paper for each individual in: (1) 500 µL of lysis buffer for 30 min; (2) 500 µL DNAzol® for 10 min; (3) 500 µL molecular grade water (MGW) for 10 min; and (4) 500 µL MGW for 10 min. The supernatant was removed after every wash. Discs were resuspended in 50 µL 1x Tris Low EDTA (10 mM Tris, 0.1 mM EDTA) and incubated at 90 ºC for 5 min to release DNA.

Petren (1998) designed 16 microsatellite primer pairs for Darwin's Finches. We redesigned polymerase chain reaction (PCR) primers for eleven autosomally inherited pairs – *Gf01*, *Gf03-07*, *Gf09*, and *Gf12-15* – to enable PCR products to be genotyped in two multiplexes. Loci were distinguished by one of four 5' labelled fluorescent tags: FAM (GeneWorks); NED, PET, or VIC (Applied Biosystems). PCR amplification (15 µL) was performed using: 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°C, followed by 40 cycles of 94°C for 45 seconds,

annealing at 54^{o}C for 45 seconds and extension at 72^{o}C for 1 minute, with a final extension temperature of 72^{o}C for 30 minutes. PCR multiplexes were separated and analysed using capillary electrophoresis (ABI 3730 DNA analyser) at the Australian Genome Research Facility Ltd, Adelaide. Alleles for each locus were sized and scored using GeneMapper® Software Version 3.7 (Applied Biosystems) with manual editing.

Morphological data analysis

We used standardised measurements (*z*-scores) to reduce our 11 morphological measurements into 4 composite traits to avoid multicollinearity for regression analysis. Specifically, we calculated the average *z*-score for (1) beak length (feathers), beak length (naris), beak depth, and beak width, which we dubbed *beak size*; (2) tarsus length, wing length, and Kipp's distance, which we dubbed *body size*; (3) tarsus width and tarsus depth, which we dubbed *tarsus thickness*; and (4) foot length and foot and claw length, which we dubbed *foot size*. We entered our total sample of finches across years and rotated the result with a Promax method. We considered *z*-score composite traits superior to components derived through principal component analysis for three reasons: (1) trait groupings are designated in respect to an organisms biology and thereby increase interpretability; (2) trait groupings are designated independent of data and therefore can be used across data sets; and (3) fewer individuals are excluded because of missing data as each composite trait is calculated independently of the other three composites. We checked the appropriateness of our choice of composite traits with principal component analysis in PASW version 18 (SPSS Inc. 2009). In addition to our composite traits, we analysed beak length (naris) separately given the observed adaptive divergence in this trait during low rainfall years (Kleindorfer *et al.* 2006; Sulloway and Kleindorfer in review).

To examine morphological trends across habitats in 2008, we used multiple regression analyses with *a priori* contrasts implemented in PASW. Considering the four ecological zones – arid, transitional, agricultural, and humid – we generated and tested a linear (-3, -1, +1, +3), a quadratic (-1, +1, +1, -1), and a cubic (-1, +3, -3, +1) contrast code sequence. Finding a significant linear trend would indicate a cline from arid to humid zones; a significant quadratic trend would indicate a contrast in trait values between the extreme (arid and humid) and central (transitional and

agricultural) zones; and a cubic trend would indicate a distinctive non-clinal pattern with respect to ecological zones. Beak length (naris) and composite traits were each individually regressed against a model with all three contrasts entered. In addition, we compared mean values for each trait among ecological zones using *t*-tests (PASW).

For our analysis of change in morphology between 2004/2005 and 2008, we compared mean beak length (naris) and composite trait values for the arid, agricultural, and humid zones across years using *t*-tests (PASW). In this analysis we used only 2008 data collected at sites that were also sampled in 2004/2005, with the exception of data collected at the agricultural zone site *El Cascajo*, which was required to bolster the sample size for the agricultural zone site *El Camote* in 2008 (see Table 1). The distance between our *El Cascajo* and *El Camote* sites was approximately 2 km, which was comparable to the distance between our two humid or two arid zone sites.

We also used *t*-tests to examine arid-humid zone divergence within time periods to establish whether our data accurately represented mean trait values measured in periods of low (2000-2005) and high (2008) rainfall.

Finally, the significance level for all multiple comparisons was adjusted using sequential Bonferroni corrections (Rice 1989).

Microsatellite data analysis

Blood samples from 2004/2005 were only collected from the arid and humid zones; hence across time periods, we only analysed genetic data from these two ecological zones. We screened our genetic data for genotypic and typographical errors using MICRO-CHECKER version 2.2.3 (van Oosterhout *et al.* 2004). Next, we used a Markov chain Monte Carlo (MCMC) method implemented in GENEPOP version 4.0 (Rousset 2008) to check for departures from Hardy-Weinberg equilibrium and genotypic disequilibrium across loci and sample populations. The MCMC parameters were set at 10,000 iterations, 1000 batches, and a dememorisation of 10,000. In tests with multiple comparisons, the significance level was adjusted using sequential Bonferroni corrections (Rice 1989).

We obtained statistics for genetic diversity among loci and sample populations using GENEPOP (Roussett 2008) and FSTAT version 2.9.3.2 (Goudet 1995). Observed and expected heterozygosities, H_O and H_E, were calculated using

GENEPOP; whereas, allelic richness, *AR*, was calculated using FSTAT. We used both programs to calculate genetic differentiation, F_{ST}, between ecological zone populations sampled in the same time period. We used F_{ST}, rather than R_{ST}, because this measure was shown to be more appropriate in this system (Chapter 2). We obtained 95 % confidence intervals for F_{ST} estimates using FSTAT.

Dispersal was measured directly using a genetic assignment method. We opted for the use of a direct method over an indirect method because the former estimates noneffective dispersal (i.e., total dispersal), as opposed to effective dispersal (i.e., only dispersal associated with gene flow), which is not independent of historical gene flow or common ancestry (Lowe *et al.* 2004). Thus, for populations that show divergence with gene flow, such as *G. fuliginosa* on Santa Cruz (Chapter 2), indirect methods would avoid confounding historical factors and provide an accurate estimate of contemporary dispersal.

We used a frequency-based method (Paetkau *et al.* 1995) implemented in GENECLASS version 2 (Piry *et al.* 2004) to estimate dispersal. GENECLASS has been shown to accurately measure dispersal when genetic differentiation is low (78 % accuracy for F_{ST} = 0.04; Berry et al 2004). The frequency-based method performs equally well as Bayesian methods for the number of samples and loci used in our study (Cornuet *et al.* 1999). GENECLASS detects first generation migrants within designated populations and then assigns migrants to a likely population of origin. We used the resampling method of (Paetkau *et al.* 2004) with likelihood criteria L = L_home, an alpha value of 0.05, and 10,000 simulated individuals. We examined differences in the number of migrants between ecological zones and climatic conditions using a Chi-square test. We assessed the accuracy of our results using the "assign/exclude population as origin of individual" function in GENECLASS: we removed 10 individuals from each habitat within sampling period to create a population "to be assigned" and used the remainder of individuals within sampling period as "reference populations".

RESULTS

We obtained morphological data from 188 individuals in 2004/2005 and 630 individuals in 2008 (Table 1; see Table 2 for variable means). We obtained

microsatellite data from 101 individuals in 2004/2005 and 122 individuals in 2008 (Table 1; see Table 3 for descriptive statistics). Less than 10 % of individuals had missing data for any one variable or locus. No individuals were resampled within or across years.

The total sample sizes for *z*-scored trait composites were as follows: beak size n = 810; body size n = 753; tarsus thickness n = 796; and foot size n = 767. Principle component analysis found 4 components with eigenvalues greater than 1.0 that mirrored our 4 *z*-scored trait composites almost completely (Table 4). A single discrepancy between the two data reduction methods regarded the higher loading for tarsus length with foot size measures than other body size measures in our principal component analysis. We were content that our placement of tarsus length – a standard body size indicator – with other body size measures for *z*-scored trait composites made more sense biologically. Collectively, principal components explained 68.0 % of the variance in the data without being highly correlated (r < 0.5); however, the total sample size for any component was n = 710, with the reduction in sample size being due to missing data.

Morphology trends in 2008

In the regression analysis on the entire 2008 dataset, we found 7 out of 15 significant trends for morphological traits (Table 5). Specifically, beak size showed a positive quadratic trend, and negative cubic trends; body size showed a positive cubic; tarsus thickness showed a negative quadratic trend, and positive cubic and linear trends; foot size showed a negative cubic trend (Table 5; see also Fig. 3). The overall models explained 2.5 % of the variance in beak size, 1.7 % in body size, 5.0 % in tarsus thickness, and 1.3 % in foot size (note: the significant positive linear trend in beak length observed by Kleindorfer *et al.* (2006) explained 5.0 % of the variance). We found no significant trends for beak length (naris) (overall model explained < 1 % of the variance; Table 5).

Our pairwise comparison of mean trait values for each of 4 ecological zones in 2008 showed significant differences in 7 out of 30 tests (Table 6; Fig. 3). Specifically, agricultural zone finches had significantly larger beaks than finches from all other zones; whereas, humid zone finches had significantly thicker tarsi than finches from all other zones; and agricultural zone finches had significantly longer

Table 2: Mean ± standard deviation (mm) and sample size for 11 morphological measurements (M) among four ecological zones in two sampling periods (i.e., 2004/2005 and 2008). Measurements are: beak length feathers (BLF); beak length naris (BLN); beak depth (BD); beak width (BW); tarsus length (TL); tarsus depth (TD); tarsus width (TW); wing length (WL); Kipp's distance (KD); foot size (FL); and foot size claws (FCL).

	Arid zone		Transitional zone		Agricultural zone		Humid zone	
M	2004/2005	2008	2004/2005	2008	2004/2005	2008	2004/2005	2008
BLF	14.3 ± 0.8, 64	12.8 ± 0.7, 220	-	12.8 ± 0.7, 185	14.5 ± 0.7, 44	13.0 ± 0.5, 107	14.5 ± 0.9,79	12.7 ± 0.7, 117
BLN	8.4 ± 0.4, 64	8.3 ± 0.4, 220	-	8.3 ± 0.4, 185	8.4 ± 0.4, 44	8.3 ± 0.5, 107	8.6 ± 0.4, 79	8.3 ± 0.5, 116
BD	7.7 ± 0.4, 64	7.6 ± 0.3, 219	-	7.6 ± 0.4, 183	7.6 ± 0.3, 44	7.6 ± 0.3, 107	7.7 ± 0.3, 77	7.5 ± 0.4, 117
BW	6.9 ± 0.3, 64	6.7 ± 0.4, 218	-	6.6 ± 0.4, 183	6.8 ± 0.3, 44	6.9 ± 0.3, 107	6.9 ± 0.3, 77	6.6 ± 0.4, 117
TL	19.4 ± 1.5, 64	19.5 ± 0.8, 210	-	19.5 ± 0.9, 183	19.3 ± 1.0, 44	19.5 ± 0.8, 107	19.5 ± 0.8, 77	19.7 ± 0.7, 114
TD	1.8 ± 0.2, 64	1.9 ± 0.1, 210	-	1.9 ± 0.2, 183	1.7 ± 0.1, 44	1.8 ± 0.2, 107	1.8 ± 0.1, 77	2.0 ± 0.1, 115
TW	1.2 ± 0.1, 64	1.2 ± 0.1, 210	-	1.2 ± 0.1, 180	1.3 ± 0.1, 44	1.2 ± 0.1, 106	1.2 ± 0.1, 77	1.2 ± 0.1, 115
WL	62.0 ± 2.0, 64	60.0 ± 2.0, 206	-	62.0 ± 2.0, 172	62.0 ± 2.0, 44	61.0 ± 2.0, 83	62.0 ± 2.0, 77	61.0 ± 2.0, 110
KD	8.0 ± 2.0, 64	10.0 ± 1.0, 206	-	10.0 ± 1.0, 171	8.0 ± 2.0, 44	10.0 ± 1.0, 83	9.0 ± 3.0, 77	10.0 ± 1.0, 108
FL	33.5 ± 1.5, 63	32.6 ± 1.6, 208	-	32.6 ± 1.7, 167	33.2 ± 1.7, 44	33.0 ± 1.2, 102	33.0 ± 1.4, 75	32.6 ± 1.6, 108
FCL	25.4 ± 1.3, 63	24.7 ± 1.2, 209	-	24.5 ± 1.2, 168	25.1 ± 1.2, 44	24.9 ± 1.1, 102	24.9 ± 1.0, 75	24.7 ± 1.4, 108

Table 3: Genetic diversity statistics for each microsatellite locus genotyped: number of individuals sampled (*N*); number of alleles (*A*); allelic richness (*AR*, based on a minimal sample size of 201); expected and observed heterozygosities (H_E and H_O); inbreeding coefficient (F_{IS}); and the probability of a deviation from Hardy-Weinberg equilibrium (*p*).

Locus	*N*	*A*	*AR*	H_E	H_O	F_{IS}	*P*
Gf1	201	22	14.13	0.879	0.851	0.032	0.900
Gf3	205	16	10.81	0.850	0.746	0.122	1.000
Gf4	201	18	6.05	0.538	0.547	-0.018	1.000
Gf5	217	11	8.28	0.750	0.742	0.011	1.000
Gf6	219	10	5.35	0.601	0.566	0.058	1.000
Gf7	213	23	14.68	0.715	0.770	-0.077	0.205
Gf9	213	17	9.45	0.657	0.662	-0.007	0.803
Gf12	211	16	12.94	0.889	0.886	0.003	0.387
Gf13	217	16	11.74	0.853	0.866	-0.015	0.992
Gf14	209	19	10.90	0.754	0.699	0.073	0.992
Gf15	206	21	10.85	0.792	0.252	0.682	1.000

feet than transitional zone finches (Table 6; Fig. 3). Notably, beak length (naris) did not differ among ecological zones in 2008.

Change in morphology between 2004/2005 and 2008

The sample size for these analyses was comparable across ecological zones (2004/2005 vs. 2008 = arid 64:65; agricultural 44:45; humid 78:86).

Both our 2004/2005 and 2008 data subsets showed the same morphological trends as were observed in the whole sample collected between 2000 and 2004 (see Kleindorfer *et al.* 2006) and in 2008 (our present study), respectively – that is, in 2004/2005, humid zone finches had significantly longer beaks (t = 2.75, df = 142, p= 0.007) and shorter feet (t = -2.63, df = 138, p = 0.010) than arid zone finches; and in 2008, finches showed no significant differences in morphology between arid and humid zones ($p > 0.1$). Our comparison of mean trait values for each of 3 ecological zones across sampling periods showed significant differences in 8 out of 15 tests (Table 7; Fig. 4).

Table 4: Loadings for 11 morphological measurements (M) across 4 components (PC1–PC2) calculated for our entire data set (n = 818) using principal component analysis with a promax rotation method. Measurements are: beak length to feathers (BLF); beak length to naris (BLN); beak depth (BD); beak width (BW); tarsus length (TL); tarsus depth (TD); tarsus width (TW); wing length (WL); Kipp's distance (KD); foot size (FL); and foot and claw length (FCL).Only loadings over 0.40 are shown. Also indicated are the 4 *z*-scored composite traits, to which each measurement was designated for analysis.

M	PC1	PC2	PC3	PC4	Composite trait
BLF	0.79				*Beak size*
BLN	0.70				
BD	0.70	0.40			
BW	0.68	0.43			
WL	0.43		0.41		*Body size*
KD			0.80		
TL		0.65	0.41		
FL	0.41	0.91			*Foot size*
FCL	0.43	0.88			
TD				0.81	*Tarsus thickness*
TW				0.76	

Across all ecological zones, finches had significantly smaller beaks in 2008 than in 2004/2005 (Table 7; Fig. 4). In the arid and humid zones, finches had significantly thicker tarsi in 2008 than in 2004/2005 (Table 7; Fig. 4). In the arid zone alone, finches had significantly larger bodies and shorter feet in 2008 than in 2004/2005; whereas, in the humid zone alone, finches had significantly shorter beaks in 2008 than in 2004/2005 (Table 7; Fig. 4).

Change in dispersal between 2004/2005 and 2008

We found no deviations from either Hardy-Weinberg equilibrium or genotypic disequilibrium among loci (Table 3) and within samples (Table 8). During both low (2004/2005) and high rainfall (2008) periods, we found low genetic differentiation between ecological zones (Table 8). We found no significant difference in genetic

Table 5: The strength and statistical significance of linear, quadratic, and cubic clinal trends for bill length naris (BLN) and four composite morphological traits in small ground finch, *Geospiza fuliginosa*, inhabiting four ecological zones (arid, transitional, agricultural, and humid) on Santa Cruz, Galápagos Islands, during a high rainfall year (2008).Contrast code sequences for each clinal trend are: linear (-3, -1, +1, +3); quadratic (-1, +1, +1, -1); and cubic (-1, +3, -3, +1). Partial correlations reflect each orthogonal trend, controlled for the other two trends.

Trait	*n*	*Partial r* (linear)	*Partial r* (quadratic)	*Partial r* (cubic)
Beak length (naris)	627	0.02	0.03	0.04
Beak size	623	-0.00	0.12**	-0.13***
Body size	567	0.06	-0.03	0.11**
Tarsus thickness	610	0.12**	-0.19***	0.12**
Foot size	580	0.03	0.02	-0.11**

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

differentiation (F_{ST}) values for 2004/2005 and 2008; which were also not significantly different from zero (see confidence intervals: Table 8). The number of first generation migrants from the arid to the humid zones, and vice versa, was not significantly different in 2004/2005 and in 2008 (Table 9). However, GENECLASS2 assigned no more than 20 % (2/10) of individuals from each sampling period to a single habitat.

DISCUSSION

The linear clines in morphology that were observed in *G. fuliginosa* across ecological zones during a period of low rainfall (2000-2005: Kleindorfer *et al.* 2006; Sulloway and Kleindorfer in review; see also this present study [2004/2005]) were either absent (i.e., beak length naris and foot size), or overshadowed by non-linear trends

Figure 3: Mean (± standard error) values for five morphological traits sampled across three ecological zones (where: agric. = agricultural; and trans. = transitional) in the high rainfall period (2008).

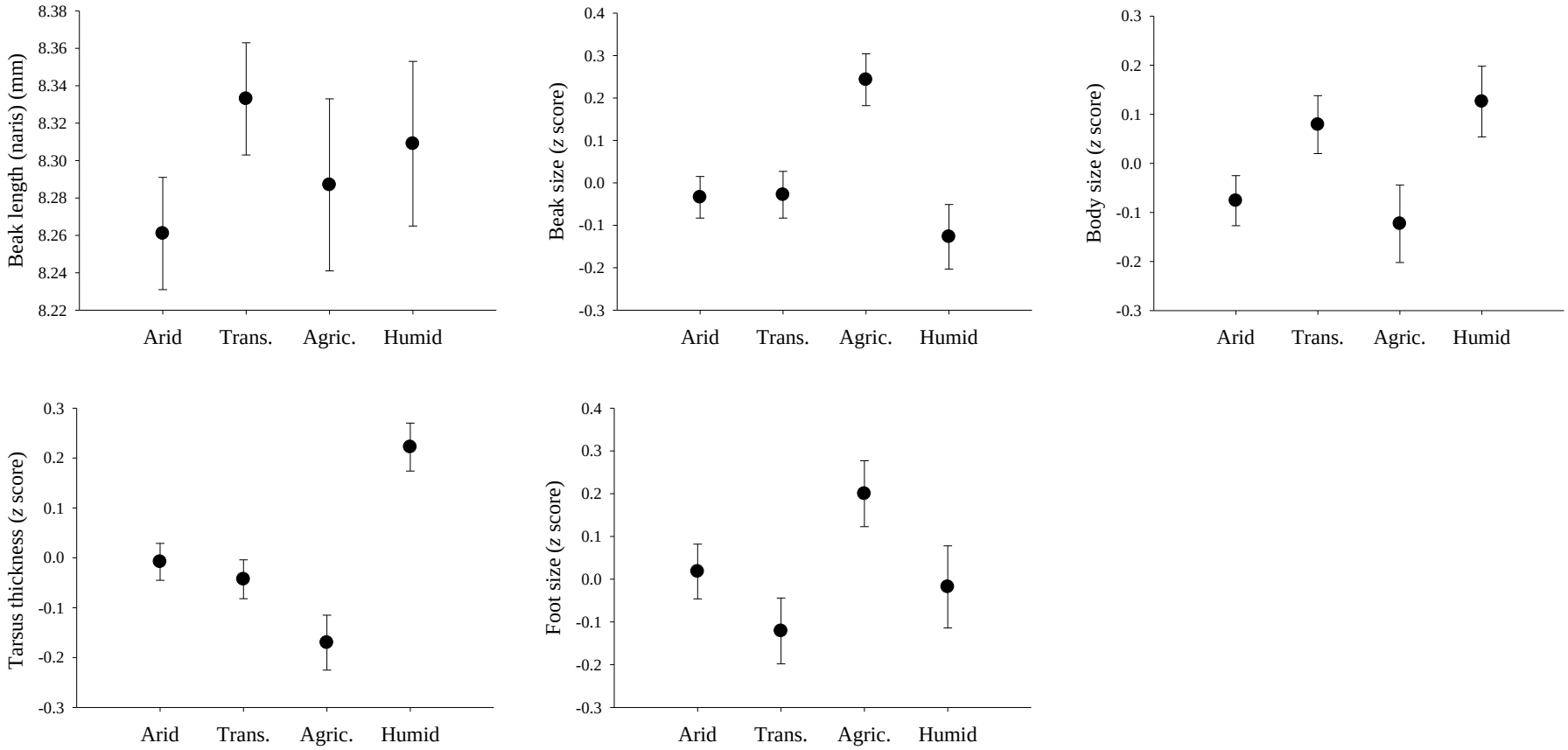


Table 6: Zonal differences in beak length (naris) and four composite traits in small ground finch, *Geospiza fuliginosa*, sampled in a high rainfall year (2008) on Santa Cruz, Galápagos Islands. Analyses contrasted between two of four ecological zones: arid (Ar); transitional (T); agricultural (Ag); and humid (H). Positive t-values indicate an increase in trait size from the first to the second zone in each contrast. P-values for significant differences after Bonferroni sequential correction are in bold italics. Also provided are the point-biserial correlation (r_{pb}) – a measure of effect size, the mean difference, and the standard error of the mean difference (MD ± SE). Beak length (naris) is measured in mm, and the composite traits are measured in standardised scores.

Trait	Contrast	*t*	df	*p*	r_{pb}	MD ± SE
Beak length	Ar vs. T	-1.67	403	0.095	-0.08	-0.1 ± 0.0
(naris)	Ar vs. Ag	-0.49	325	0.627	-0.03	-0.0 ± 0.1
	Ar vs. H	-0.91	334	0.361	-0.05	-0.1 ± 0.1
	T vs. Ag	0.86	290	0.392	0.05	0.1 ± 0.1
	T vs. H	0.46	299	0.645	0.03	0.0 ± 0.1
	Ag vs. H	-0.34	221	0.735	-0.02	-0.0 ± 0.1
Beak size	Ar vs. T	-0.08	399	0.934	-0.00	-0.0 ± 0.1
	Ar vs. Ag	-3.55	323	***0.001***	-0.19	-0.3 ± 0.1
	Ar vs. H	1.08	332	0.282	0.06	0.1 ± 0.1
	T vs. Ag	-3.14	288	***0.002***	-0.18	-0.3 ± 0.1
	T vs. H	1.08	297	0.280	0.06	0.1 ± 0.1
	Ag vs. H	3.79	221	***<0.001***	0.25	0.4 ± 0.1
Body size	Ar vs. T	-1.99	375	0.047	-0.10	-0.2 ± 0.1
	Ar vs. Ag	0.50	287	0.621	0.03	0.1 ± 0.1
	Ar vs. H	-2.32	312	0.021	-0.13	-0.2 ± 0.1
	T vs. Ag	1.99	252	0.048	0.12	0.2 ± 0.1
	T vs. H	-0.51	277	0.613	-0.03	-0.1 ± 0.1
	Ag vs. H	-2.33	189	0.021	-0.17	-0.3 ± 0.1
Tarsus	Ar vs. T	0.63	388	0.525	0.03	0.7 ± 0.1
Thickness	Ar vs. Ag	2.40	314	0.017	0.13	0.2 ± 0.1
	Ar vs. H	-3.74	323	***<0.001***	-0.20	-0.2 ± 0.1
	T vs. Ag	1.85	284	0.065	0.11	0.1 ± 0.1
	T vs. H	-4.27	293	***<0.001***	-0.24	-0.3 ± 0.1
	Ag vs. H	-5.34	219	***<0.001***	-0.34	-0.4 ± 0.1
Foot size	Ar vs. T	1.39	372	0.164	0.07	0.1 ± 0.1
	Ar vs. Ag	-1.72	307	0.087	-0.10	-0.2 ± 0.1
	Ar vs. H	0.31	313	0.753	0.02	0.4 ± 0.1
	T vs. Ag	-2.78	267	***0.006***	-0.17	-0.3 ± 0.1
	T vs. H	-0.84	273	0.402	-0.05	-0.1 ± 0.1
	Ag vs. H	1.76	208	0.081	0.12	0.2 ± 0.1

Table 7: Temporal differences in beak length (naris) and four composite traits in small ground finch, *Geospiza fuliginosa*, sampled in low (2004/2005) and high (2008) rainfall years on Santa Cruz, Galápagos Islands. Analyses performed within three ecological zones: arid; agricultural; and humid; as well as for all zones combined. Positive t-values indicate an increase in trait size over time. Significant *p*-values after Bonferroni sequential correction are in bold italics. Also provided are the point-biserial correlation (r_{pb}) – a measure of effect size, the mean difference, and the standard error of the mean difference (MD ± SE; calculated as: 2008 – 2004/2005). Beak length to naris is measured in mm and the composite traits are measured in standardised scores.

Zone	Trait	*T*	df	*p*	r_{pb}	MD ± SE
Arid	Beak length (naris)	-0.82	127	0.415	-0.07	-0.1 ± 0.1
	Beak size	-7.24	127	***<0.001***	-0.54	-0.8 ± 0.1
	Body size	2.51	122	***0.014***	0.22	0.3 ± 0.1
	Tarsus Thickness	4.98	126	***<0.001***	0.41	0.7 ± 0.1
	Foot size	-3.78	123	***<0.001***	-0.32	-0.6 ± 0.2
Agric.	Beak length (naris)	-1.94	87	0.055	-0.20	-0.2 ± 0.1
	Beak size	-2.91	87	***0.005***	-0.30	-0.4 ± 0.1
	Body size	1.82	82	0.072	0.20	0.3 ± 0.1
	Tarsus Thickness	0.74	86	0.463	0.08	0.1 ± 0.2
	Foot size	-0.21	86	0.835	-0.02	-0.0 ± 0.2
Humid	Beak length (naris)	-2.98	163	***0.003***	-0.23	-0.2 ± 0.1
	Beak size	-7.12	162	***<0.001***	-0.49	-0.8 ± 0.1
	Body size	1.96	156	0.052	0.16	0.2 ± 0.1
	Tarsus Thickness	5.36	160	***<0.001***	0.39	0.5 ± 0.1
	Foot size	-0.93	153	0.353	-0.07	-0.1 ± 0.2
All zones	Beak length (naris)	-3.34	381	***0.001***	-0.17	0.2 ± 0.1
	Beak size	-10.18	380	***<0.001***	-0.46	0.7 ± 0.1
	Body size	3.66	364	***<0.001***	0.19	-0.3 ± 0.1
	Tarsus Thickness	6.43	376	***<0.001***	0.31	-0.5 ± 0.1
	Foot size	-2.84	366	***0.005***	-0.15	0.3 ± 0.1

Figure 4: Mean (± standard error) values for five morphological traits sampled across three ecological zones (where agric. = agricultural) in a low (2004/2005) and high (2008) rainfall period.

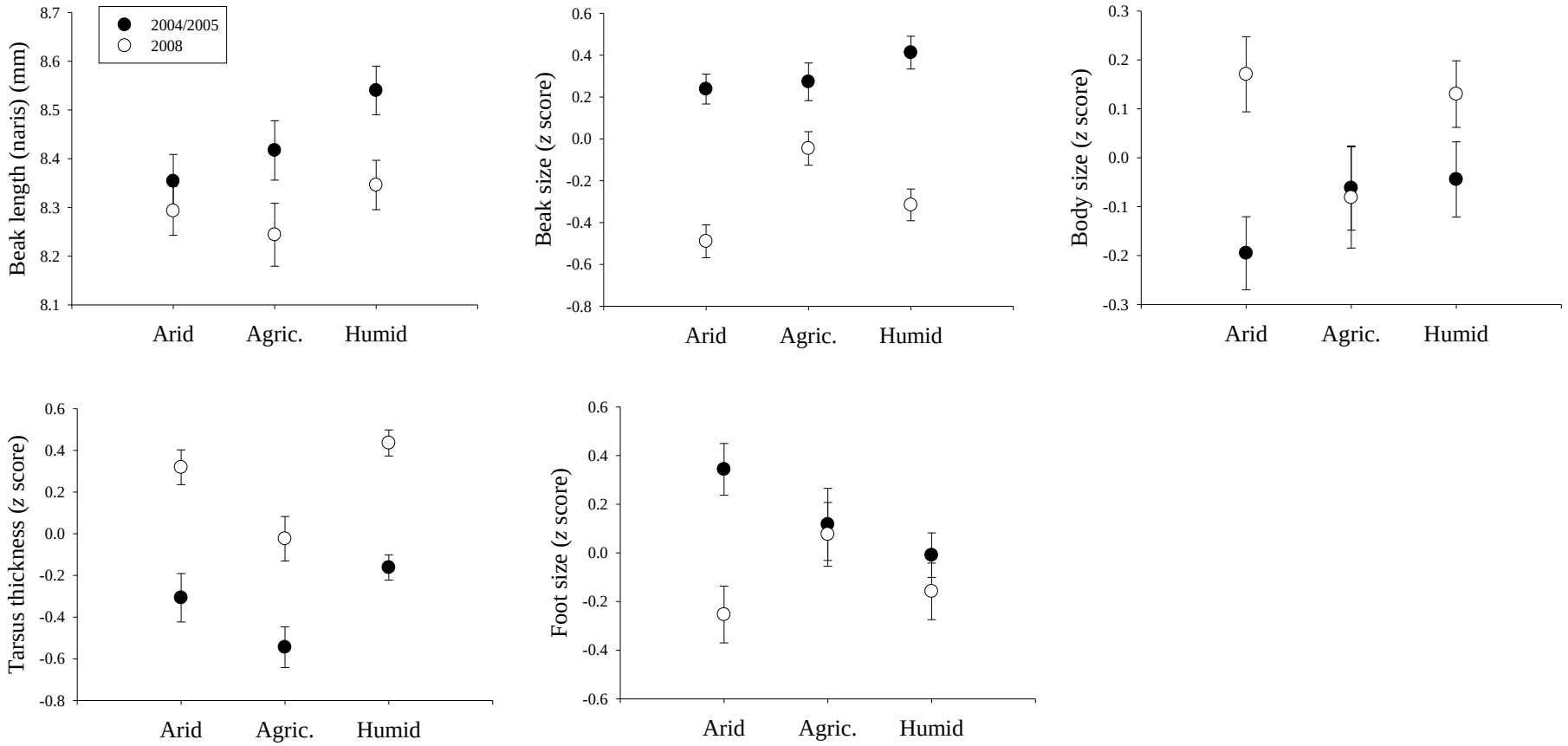


Table 8: Genetic diversity and genetic differentiation statistics for arid (Ar) and humid (H) zone small ground finch *Geospiza fuliginosa* sampled in low (2004/2005) and high rainfall (2008) years on Santa Cruz, Galápagos Islands. Statistics are number of individuals sampled (*N*); mean allelic richness (*AR*; based on a minimal sample size of 30); inbreeding coefficient (F_{IS}); probability of a deviation from Hardy-Weinberg equilibrium (*p*); and fixation index with 95 % confidence intervals (F_{ST}).

Years	Zone	*n*	*AR*	H_E	H_O	F_{IS}	*p*	F_{ST}
2004/2005	Ar	37	13.49	0.73	0.57	0.09	0.732	0.003
	H	64	13.92	0.74	0.72	0.09	0.654	(-0.003 – 0.009)
2008	Ar	57	12.29	0.77	0.74	0.09	0.484	0.002
	H	65	12.33	0.74	0.73	0.06	0.536	(-0.001 – 0.006)

Table 9: The number of, and statistical difference between, first generation small ground finch, *Geospiza fuliginosa*, migrants (F_0) detected in the arid (Ar) and humid (H) zones for low (2004/2005) and high (2008) rainfall years on Santa Cruz, Galápagos Islands. Analysis was performed using a frequency-based method implemented in GENECLASS. The percentage of individuals correct assigned to their population of origin also given.

	# F_0 migrants		*Chi-square test*	
	Ar → H	H → Ar	X^2, df	*P*
2004/2005	4	4	1.38, 3	0.710
2008	4	3		

(i.e., tarsus thickness) in the high rainfall year of 2008. Body size (r = 0.11) and foot size (r = 0.11) were best explained by a cubic trend, suggesting a random non-clinal pattern in these traits; tarsus thickness (r = 0.19) was best explained by a quadratic trend, suggesting a contrast between extreme and central zones; and beak size could be explained by cubic (r = 0.12) as well as quadratic (r = 0.13) trends. No trend could explain beak length (naris) because no difference was found between zones.

Few morphological traits differed significantly among ecological zones. Agricultural zone finches had larger beaks than finches from all other zones and

longer feet than transitional zone finches; whereas, humid zone finches had thicker tarsi than finches from all other zones. Analysis of morphological differences between sampling periods suggested that the mean beak size had not become larger in agricultural zone finches; rather that it had become smaller across all ecological zones in 2008, but to a lesser extent in the agricultural zone. Similarly, the mean tarsus thickness had not become larger in humid zone finches only, but larger across all zones in 2008, but to a greater extent in the humid zone. However, caution is required in these interpretations because inter-measurer reliability was not analysed. Beak length feather and tarsus depth was consistently larger across ecological zones in 2008 than in 2004/2005 (see Table 2); therefore it is possible that these traits were measured in a significantly different manner by S.K. and T.H.G (see Methods).

The most notable findings in 2008 were shorter beaks in humid zone finches and shorter feet in arid zone finches. Humid zone finches had a mean beak length (naris) similar to that of arid zone finches sampled during the low rainfall period; and arid zone finches had a mean foot size similar to that of humid zone finches sampled during the low rainfall period (Kleindorfer *et al.* 2006; Sulloway and Kleindorfer in review; see also Table 2). Therefore, the linear clines in ecologically significant traits among *G. fuliginosa* inhabiting the arid, agricultural, and humid zones observed during low rainfall years (2000-2005) no longer existed in the high rainfall year (2008). We can think of three possible explanations for no difference in mean trait values; we discuss the evidence for each in turn below.

Adaptive Convergence

Immediately, morphological convergence as a result of adaptation seems improbable: because that would mean the same selection regime that shaped divergence in *G. fuliginosa* between 2000 and 2005, also shaped convergence in 2008. Moreover, if the effects of that divergent selection regime continued as the low rainfall period continued and resources were further depleted, we would expect to see morphological divergence stronger than that reported by previous studies (Kleindorfer *et al.* 2006; Sulloway and Kleindorfer in review); whereas we found the opposite.

The divergent selection regime could have changed in the final years of the low rainfall period – for example, as seed biomass decreased across years, individuals with small beaks may be favoured, because they avoid interspecific

competition with the larger beaked medium ground *Geospiza fortis*. However, there is no indirect evidence for similar selection pressures among ecological zones in low rainfall years; given that rain received in the humid zone was never less than 500 mm annually (see Fig. 2). In other words, "low rainfall" conditions never actually occur in the humid zone (nor agricultural zone, which replaces historical humid forest). More specifically: while prey abundance in the arid zone is known to decrease as periods of low rainfall continue (Grant 1999), there is no evidence for the magnitude of this effect in the humid zone – where annual rainfall is not only greater, but more constant throughout the year. Thus, we return to the initial problem: the same divergent selection regime causing both adaptive divergence and no difference in mean trait values. In addition, our analyses only included mature individuals; hence, our finding of no difference in mean trait values cannot be explained by relaxed selection on juveniles born in the high rainfall year of 2008.

Parasite-induced convergence

Smaller beak dimensions in *G. fuliginosa* can be attributed to beak malformations caused during the nestling phase by the feeding behaviour of the recently introduced parasite *Philornis downsi* (Chapter 6). More specifically, finches that clearly showed evidence of parasitism (i.e., deformed nares), had shorter beak length (naris), shorter beak length (feathers), and shallower beak depth (Chapter 6 [Table 2]). These malformed finches made up one third of the total sample in 2008. However, we found no difference in the number of malformed finches among ecological zones; nor a difference between mean beak dimensions in malformed finches inhabiting different ecological zones (Chapter 6); and therefore, do not expect that post-parasitism morbidity has – caused the breakdown of morphological clines in *G. fuliginosa*.

However, an increase in *P. downsi* parasitism across years is a possible explanation for the observed difference in beak length (naris) and beak size between 2004/2005 and 2008. While there is no data to test this hypothesis directly, Dudaniec *et al.* (2007) found a significant increase in *P. downsi* intensity in the nests of six Darwin's finch species between 2000 and 2005. However, it is difficult to say how an increase in parasite intensity at nests relates to the frequency of beak malformation in the adult population, particularly when high parasite intensities result in high nestling mortality (Fessl *et al.* 2006); and therefore, whether it has

contributed substantially to the significant reduction in beak dimensions between 2004/2005 and 2008.

Dispersal-mediated clinal breakdown

We have evidence for and against dispersal-mediated clinal breakdown. Comparing dispersal between arid and humid zones, we found no difference between low and high rainfall years; therefore, intra-island dispersal in *G. fuliginosa* appears to be independent of the climatic condition in the archipelago. In addition, dispersal rates were low (0.06-0.08). Because selection is expected to disfavour immigrants in low rainfall years, the removal of clines is not expected to take place before 2008. Is it possible for rates of this magnitude to breakdown morphological clines at the onset of the wet season in 2008? To test this idea we split the data set by sampling period and exchanged 7 % of lowland and highland individuals in each sampling period to simulate a dispersal rate of 0.07. We focussed our analyses on beak length (naris). In 2004-2005, we swapped 5 of the longest beaked highland finches with 6 of the shortest beaked lowland finches; and in 2008, we swapped 8 of the shortest beaked highland finches with 15 of the longest beaked lowland finches. The result was the removal of the linear cline in 2004-2005 (r = 0.09, n = 187, p = 0.24) and the formation of a linear cline in 2008 (r = 0.24, n = 335, $p < 0.001$). Therefore, the dispersal rates that we reported can breakdown morphological clines within a single year; providing those finches represent the morphological extremes of the population and disperse to ecological zones predicted to match their morphology. However, the 15 individuals identified as first generation in this study do not meet these criteria; in fact, if we place the 8 individuals identified first generation immigrants in 2008 into their ecological zone of origin, the linear trend for beak length (naris) remains nonsignificant (r = 0.08, n = 149, p = 0.32). It is important to note that we have measured noneffective dispersal – that is, dispersal with and without gene flow – therefore, effective dispersal (dispersal with gene flow) is likely to have less of an effect.

Our finding that dispersal was too low to generate convergence may nevertheless be misleading for two reasons. First, we only sampled from two locations separated by 18 km, and not from closer intermediate sites, and therefore we may have overlooked potentially important dispersal. Further, the accuracy of assignment methods for the detection of dispersal has been shown to increase with

the number of sites sampled (Paetkau *et al.* 2004). However, in a second study conducted in 2008, we analysed dispersal among 21 sites across Santa Cruz (including four arid zone sites from the northern side of the island) and found that the dispersal rate remained low between the arid and humid zones (0.04: Chapter 2). The overall rate among all zones was somewhat greater (0.20); but even this rate would be too low to remove morphological clines observed in 2005 in a single year. In addition, the effects of the higher rate of dispersal observed between all zones (0.20) would be mitigated by the fact that most of this additional dispersal involves birds from neighbouring zones that, when clinal differences were still intact, would have been similar morphologically.

The second reason why our estimates of the efficacy of dispersal to remove clines (here and in our second study) may be misleading is far more important: namely, that is, our estimates of dispersal are inaccurate. The accuracy of estimates obtained from genetic assignment methods is positively related to the genetic differentiation (e.g., F_{ST}) among the groups being examined (Cornuet 1999). Genetic differentiation between the arid and humid zone *G. fuliginosa* is extremely low (F_{ST} = 0.001: Chapter 2). Cornuet (1999) showed that GENECLASS estimates for simulated data were only 20 % accurate for groups with genetic differentiation close to zero. Our own test of accuracy revealed that GENECLASS could only designate 20 % of individuals in each sampling period to one habitat. Therefore, we can have little if any confidence in our estimates of dispersal.

When genetic differentiation is low, obtaining accurate estimates of dispersal using genetic techniques is difficult. We chose to use GENECLASS because this program is, to the best of our knowledge, the most suited for measuring contemporary dispersal when genetic differentiation is low (Cornuet et al 1999; Berry *et al.* 2004; Anderson and Meilke 2010). More accurate estimates of dispersal may be obtained using direct methods in the field (mark-recapture-resighting); but demand extensive effort (Lowe et al 2004). Recapture and resighting data for *G. fuliginosa* suggest low dispersal (0.04) between arid and humid zones (Kleindorfer *et al.* 2006), which agrees with our estimates obtained above. However, logic would suggest that *G. fuliginosa* on Santa Cruz should show higher dispersal among sites and ecological zones. We base this supposition on the following considerations: (1) the relatively small size of Santa Cruz; (2) the high mobility of *G. fuliginosa*; (3) the lack of physical barriers to dispersal within the island landscape; (4) low F_{ST} among

sites and zones (Chapter 2); (5) no within-island population substructure (Chapter 2); and (6) high gene flow in birds in general (Avise 1994). Yet, only a dedicated mark-recapture-resighting study in the future can resolve this issue.

This being said, presuming overall high dispersal does not explain why no difference in mean trait values was seen in 2008, when the same argument for why dispersal was high in the high rainfall year would also hold in a low rainfall year. We speculate that, while the level of dispersal might be constant across differing climatic conditions, in the "benign" high rainfall year selection against immigrants is relaxed. Relaxed selection against immigrants would permit individuals to establish territories and search for mates in environments they would otherwise be ill-adapted to. Further, we expect that *G. fuliginosa* are able to determine, at the onset, whether a wet season will be one of low or high rainfall using various climate cues; and actively choose to either retain old breeding territories in low rainfall years, or explore novel and potentially better breeding territories in high rainfall years. Certainly, such alternating behaviour may be favoured.

We have two pieces of evidence to support this hypothesis. First, during the dry season *G. fuliginosa* form flocks. On the small island of Pinta, flock can include up to 200 individuals (Schluter 1982) – presumably on Santa Cruz, flocks can be even larger. Anecdotal evidence suggests that flocks on Santa Cruz centre on the agricultural zone, where there exists a constant source of food and water. Flocking behaviour is a response to unpredictable resources; the location and exploitation of which is increased in the presence of many individuals. However, birds that form flocks that contain a mix of similar and dissimilar phenotypes are expected to be favoured because detection and competition for specific food types is thereby optimised. Therefore, flocks of *G. fuliginosa* are expected to be made-up of ecomorphs from all zones (at least from the southern side of the island). At the start of the wet season, flocks breakup, males spread-out among ecological zones to secure old or new breeding territories based on present rainfall; and females follow to select mates. Therefore, at the onset of every wet season, many *G. fuliginosa* disperse to breeding grounds from a presumably mixed pool of phenotypes. In our analysis of dispersal among 21 sites on Santa Cruz, we found that emigration rate from the agricultural zone is greater than from all other zones combined, and occurs to all other zones (Chapter 2). These findings appear to represent the breakup of dry season flocks at the start of the wet season.

Second, at the beginning of the wet season in 2008 *G. fuliginosa* were observed to be common in the high altitude *Miconia* shrubland at *Media Luna*, but completely absent at this site in the middle and end of the wet season. Our sample of 16 individuals from *Media Luna* included large proportions of older males (4-5 years old: 0.31) and females (0.56); and while males appeared to be defending territories during sampling, these territories were abandoned (perhaps due to unsuitable vegetation structure for nest building or the inclement weather at that altitude). Therefore, it seemed that even older males that are assumed to retain breeding territories for life (*sensu* Grant and Grant 1989), gamble on territories in novel environments in the first weeks of the breeding season in a high rainfall year.

Conclusions

Adaptation and dispersal are two major mechanisms by which populations can diverge and converge in phenotype. For observed breakdown of morphological clines in *G. fuliginosa* inhabiting different ecological zones on Santa Cruz between 2000-2005 and 2008, we suggest that dispersal is the most likely candidate, or at least a significant contributing factor. However, due to a potentially high level of gene flow among *G. fuliginosa* on Santa Cruz, we cannot be completely certain. Based on geographical setting, finch biology, and anecdotal behavioural evidence, we tentatively suggest that considerable dispersal actually occurs constantly across climatic conditions; but in "benign" high rainfall years, selection against immigrants is relaxed, permitting a substantial reshuffling of phenotypes. In turn, a breakdown of morphological clines is observed. An obvious inference based on this idea – which merits further testing – is that during low rainfall periods immigrant inviability is likely an important reproductive barrier for intra-island adaptive divergence in *G. fuliginosa.*

ACKNOWLEDGEMENTS

We thank the following: the Galápagos National Park and the Charles Darwin Research Station for the opportunity to conduct this research; Max Planck Institute for Ornithology, American Bird Conservancy, Conservation International, Galápagos Conservation Fund, and Flinders University for their generous financial support;

TAME airlines for assistance with reduced airfare to and from the archipelago; Zonnetje Auburn, Mari Cruz Jaramillo, Birgit Fessl, Michael Dvorak, Jody O'Connor, Sarah Huber, Jennifer Koop, and Ryan Buss for their assistance in the field; Steven Myers, Mike Gardner, Kathy Saint, Leanne Wheaten, and Ken Petren for their assistance in the laboratory. This research was conducted under the ethics approval granted by Flinders University (E189).

REFERENCES

Anderson, C. S. and Meikle, D. R. 2010. Genetic estimates of immigration and emigration rates in relation to population density and forest patch area in *Peromyscus leucopus*. *Conservation Genetics*, 11: 1593-1605.

Avise, J. C. 1994. *Molecular Markers, Natural Selection, and Evolution.*Chapman and Hill: New York, N. Y.

Berner, D., Grandchamp, A. C. and Hendry, A. P. 2009. Variable progress towards ecological speciation in parapatry: stickleback across eight lake-stream transitions. *Evolution* **63**: 1740-1753.

Berry, O., Tocher, M. D. and Sarre, S. D. 2004. Can assignment tests measure dispersal? *Molecular Ecology* **13**: 551-561.

Bolnick, D. I., Snowberg, L. K., Patenia, C., Stutz, W. E., Ingram, T. and Lau, O. L. 2009. Phenotype-dependent native habitat preference facilitates divergence between parapatric lake and stream stickleback. *Evolution* **63**: 2004-2016.

Bowler, D. E. and Benton, T. G. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews* **80**: 205-225.

Bowman, R. I. 1961. Morphological differentiation and adaptation in the Galapagos finches. *University of California Publications in Zoology* **58**: 1-302.

Broquet, T. and Petit, E. J. 2009. Molecular Estimation of Dispersal for Ecology and Population Genetics. *Annual Review of Ecology Evolution and Systematics* **40**: 193-216.

Clobert, J., Danchin, E., Dhondt, A. A. and Nichols, J. D. 2001. *Dispersal*. Oxford University Press: Oxford.

Clobert, J., Ims, R. and Rousset, F. 1997. Causes, mechanisms and consequences of dispersal. In: Metapopulation Biology: Ecology, Genetics, and Evolution. Hanski, I. and Gaggiotti, O. E. (eds). Academic Press San Diego, C. A.

Cornuet, J. M., Piry, S., Luikart, G., Estoup, A. and Solignac, M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* **153**: 1989-2000.

Cruz, R., Vilas, C., Mosquera, J. and Garcia, C. 2004. Relative contribution of dispersal and natural selection to the maintenance of a hybrid zone in Littorina. *Evolution* **58**: 2734-2746.

Dudaniec, R., Fessl, B. and Kleindorfer, S. 2007. Interannual and interspecific variation in intensity of the parasitic fly, *Philornis downsi*, in Darwin's finches. *Biological Conservation* **139**: 325-332.

Edelaar, P., Siepielski, A. M. and Clobert, J. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. *Evolution* **62**: 2462-2472.

Ferrari, J., Godfray, H. C. J., Faulconbridge, A. S., Prior, K. and Via, S. 2006. Population differentiation and genetic variation in host choice among pea aphids from eight host plant genera. *Evolution* **60**: 1574-1584.

Fessl, B., Kleindorfer, S. and Tebbich, S. 2006. An experimental study on the effects of an introduced parasite in Darwin's finches. *Biological Conservation* **127**: 55-61.

Funk, D. J. 1998. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. *Evolution* **52**:1744-175.

Garant, D., Kruuk, L. E. B., Wilkin, T. A., McCleery, R. H. and Sheldon, B. C. 2005. Evolution driven by differential dispersal within a wild bird population. *Nature* **433**: 60-65.

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal of Heredity* **86**: 485-486.

Grant, P. R. 1999. *Ecology and Evolution of Darwin's Finches*. Princeton University Press: Princeton, N. J.

Grant, B. R. and Grant, P. R. 1989. *Evolutionary dynamics of a natural population: the large cactus finch of the Galápagos*. University of Chicago Press: Chicago, I. L.

Grant, P. R. and Grant, B. R. 2008. *How and Why Species Multiple: the Radiation of Darwin's Finches* Princeton University Press: Princeton, N. J.

Hendry, A. P., Wenburg, J. K., Bentzen, P., Volk, E. C. and Quinn, T. P. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science* **290**: 516-518.

Hendry, A. P. 2001. Adaptive divergence and the evolution of reproductive isolation in the wild: an empirical demonstration using introduced sockeye salmon. *Genetica* **112**: 515-534.

Hendry, A. P. 2004. Selection against migrants contributes to the rapid evolution of ecologically dependent reproductive isolation. *Evolutionary Ecology Research* **6**: 1219-1236.

Jones, J. S. and Probert, R. F. 1980. Habitat selection maintains a deleterious allele in a heterogeneous environment. *Nature* **287**: 632-633.

Kleindorfer, S. 2007. The ecology of clutch size variation in Darwin's Small Ground Finch *Geospiza fuliginosa*: comparison between lowland and highland habitats. *Ibis* **149**: 730-741.

Kleindorfer, S., Chapman, T. W., Winkler, H. and Sulloway, F. J. 2006. Adaptive divergence in contiguous populations of Darwin's Small Ground Finch (*Geospiza fuliginosa*). *Evolutionary Ecology Research* **8**: 357-372.

Langerhans, R. B., Layman, C. A., Langerhans, A. K. and Dewitt, T. J. 2003. Habitat-associated morphological divergence in two Neotropical fish species. *Biological Journal of the Linnean Society* **80**: 689-698.

Lowe, A., Harris, S. and Ashton, P. 2004. *Ecological Genetics: Design, Analysis, and Application*. Blackwell Publishing: Carlton, Australia.

Lowry, D. B., Rockwood, R. C. and Willis, J. H. 2008. Ecological reproductive isolation of coast and inland races of Mimulus guttatus. *Evolution* **62**: 2196-2214.

MacColl, A. D. C. and Chapman, S. M. 2010. Parasites can cause selection against migrants following dispersal between environments. *Functional Ecology* **24**: 847-856.

Mallet, J. 1989. The genetics of warning colour in Peruvian hybrid zones of *Heliconius erato* and *H. melpomene*. *Proceedings of the Royal Society of London. B* **236**: 163-185.

Matute, D. R., Novak, C. J. and Coyne, J. A. 2009. Temperature-based extrinsic reporductive isolation in two species of *Drosophila*. *Evolution* **63**: 595-612.

Mila, B., Wayne, R. K., Fitze, P. and Smith, T. B. 2009. Divergence with gene flow and fine-scale phylogeographical structure in the wedge-billed woodcreeper, *Glyphorynchus spirurus*, a Neotropical rainforest bird. *Molecular Ecology* **18**: 2979-2995.

Myers, S., Brown, G. and Kleindorfer, S. 2010. Divergence in New Holland Honeyeaters (*Phylidonyris novaehollandiae*): evidence from morphology and feeding behavior. *Journal of Ornithology* **151**: 287-296.

Nagy, E. S., and Rice., K. J. 1997. Local adaptation in two subspecies of an annual plant: implications for migration and gene flow. *Evolution* **51**: 1079-1089.

Olivieri, I., Michalakis, Y. and Gouyon, P. H. 1995. Metapopulation genetics and the evolution of dispersal. *American Naturalist* **146**: 202-228.

Paetkau, D., Calvert, W., Stirling, I. and Strobeck, C. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* **4**: 347-354.

Paetkau, D., Slade, R., Burden, M. and Estoup, A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* **13**: 55-65.

Petren, K. 1998. Microsatellite primers from Geospiza fortis and cross-species amplification in Darwin's finches. *Molecular Ecology* **7**: 1782-1784.

Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L. and Estoup, A. 2004. GENECLASS: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity* **95**: 536-539.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Ecvolution* **43**: 223-244.

Rousset, F. 2008. GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8**: 103-106.

Schluter, D. 1982. Distributions of Galápagos ground finches along an altitudinal gradient: the importance of food supply. *Ecology* **63**: 1504-1517.

Schlotfeldt, B. E. and Kleindorfer, S. 2006. Adaptive divergence in the Superb Fairy-wren (*Malurus cyaneus*): a mainland versus island comparison of morphology and foraging behaviour. *Emu* **106**: 309-319.

Smith, L. M. and Burgoyne, L. A. 2004. Collecting, archiving and processing DNA from wildlife samples using FTA® databasing paper. *BMC Ecology* **4**: 4-15.

Snell, H. and Rae, S. 1999. The 1997-98 El Nino in Galapagos: can 34 years of data estimate 120 years of pattern? *Noticias de Galapagos* **60**: 11-20.

SPSS Inc., an IBM Company. 2009. *PAWS 18.0*. Chicago, IL.

Tobler, M., Riesch, R., Tobler, C. M., Schulz-Mirbach, T. and Plath, M. 2009. Natural and sexual selection against immigrants maintains differentiation among micro-allopatric populations. *Journal of Evolutionary Biology* **22**: 2298-2304.

Tonnis, B., Grant, P. R., Grant, B. R. and Petren, K. 2005. Habitat selection and ecological speciation in Galapagos warbler finches (*Certhidea olivacea* and *Certhidea fusca*). *Proceedings of the Royal Society B-Biological Sciences* **272**: 819-826.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**: 535-538.

Via, S. 1999. Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. *Evolution* **53**: 1446-1457.

Wellenreuther, M., Vercken, E. and Svensson, E. I. 2010. A role for ecology in male mate discrimination of immigrant females in Calopteryx damselflies? *Biological Journal of the Linnean Society* **100**: 506-518.

Westberg, E., Poppendieck, H. H. and Kadereit, J. W. 2010. Ecological differentiation and reproductive isolation of two closely related sympatric species of Oenanthe (Apiaceae). *Biological Journal of the Linnean Society* **101**: 526-535.

CHAPTER FOUR

Loss of assortative pairing following colonisation of a new environment by Darwin's small ground finch, *Geospiza fuliginosa*

Toby H. Galligan and Sonia Kleindorfer

Evolutionary Ecology Research (2010) 12: 1-10

ABSTRACT

Organism: Darwin's small ground finch, *Geospiza fuliginosa*.

Field sites: The arid lowlands (0-100 m a.s.l.) and the humid highlands (500-600 m a.s.l.) of the island of Santa Cruz, Galápagos Archipelago.

Background: Positive assortative mating tends to maintain adaptations and individual niche specialisation. However, adaptations and niche specialisations are not always favoured across generations. In such cases, a loss of assortative mating could increase offspring adaptive potential and thereby offspring fitness. Range expansion into a novel habitat, with novel selection pressures, presents a scenario where assortative mating may be lost via relaxed selection on mate choice.

Hypothesis: A loss of assortative mating should be favoured in the highland-colonist population of *G. fuliginosa*.

Methods: We measured the beaks of 23 nesting pairs from the highland and lowland populations in January & March, 2001 & 2002. We used correlation analysis to examine assortative pairing for beak length. We determined the distribution of beak lengths for females and males within each ecological zone to rule out limited mates as a mechanism for the loss of assortative pairing.

Results: As predicted, we found positive assortative pairing for beak length in the lowland-source population but not the highland-colonist population. In addition, we found no evidence for limited mates.

Keywords: homogamy, parapatry, colonisation, selection intensity, reproductive isolation

INTRODUCTION

Positive assortative mating (or simply *assortative mating*), where individuals with similar phenotypes reproduce more often than individuals with dissimilar phenotypes, is common in natural populations (Bateson 1983; Christensen and Kleindorfer 2007). The likely explanation for the frequent occurrence of assortative mating is that it tends to maintain heritable adaptations from parents to offspring (Bateson 1983). Such adaptations may optimise resource exploitation and/or reduce interspecific competition (following individual niche specialisation theory: Bolnick *et al.* 2002). In this way, assortative mating can increase an individual's fitness via an increase in its progeny's fitness. Adaptations and niche specialisations that have enabled individuals to reach reproductive maturity are likely to be favoured in their progeny while reproduction between individuals of dissimilar phenotypes may dilute these traits.

However, the same adaptations and niche specialisations are not always favoured in an individual and its progeny. First generation individuals may overexploit resources or overcrowd niches, reducing the fitness of the second generation individuals. In such cases, heterotypic offspring of parents with dissimilar phenotypes could exploit different or broader niches, and thereby accrue higher fitness than homotypic offspring of parents with similar phenotypes (*sensu* hybrid advantage: Grant and Grant 1994, 1996). This scenario would favour a breakdown or loss of assortative mating with the consequence of adaptive shifts within lineages towards underexploited resources and available niches.

A loss of assortative mating can evolve in a number of ways. First, the cues used for mate choice can breakdown without a change in mate preference. Second, disassortative mating can be directly selected for – as is the case in the "overexploited resources" scenario we have described above. Third, a loss of assortative mating can be selected for (indirect selection for disassortative mating) – as would be the case if mates were limited and phenotype matching were therefore constrained. Fourth, a loss of assortative mating could simply *not be selected against* – that is, *selection on mate choice is relaxed* and mate preference is allowed to drift. Under relaxed selection on mate choice, neither homotypic nor heterotypic offspring would have an advantage and would therefore have similar fitness.

Range expansion into a novel habitat is a scenario that favours the loss of assortative mating. Here, the source and colonist populations would be subject to different environments and therefore different selection regimes. Consequently, the suite of adaptations the source population possesses would not all be favoured in the colonist population. As described above, a loss of assortative mating among the colonists could increase adaptive potential within lineages. However, not all adaptations would be disfavoured because an organism is not expected to colonise a habitat for which it is not pre-adapted (Mayr 1965; Tonnis *et al.* 2005). Therefore, in a novel habitat both the generation of new adaptations via disassortaitve mating and the maintenance of pre-adaptations via assortative mating would be favoured leading to relaxed selection on mate choice. In fact, novel habitats may generally exert relaxed selection because they offer ecological opportunities and reduced intraspecific competition.

Darwin's small ground finch (*Geospiza fuliginosa*) on the island of Santa Cruz, Galápagos Archipelago, provides an opportunity to examine differences in mating strategies between source and colonist populations. This is because (1) assortative mating is known in Darwin's finches (Grant 1999; Huber *et al.* 2007); and (2) *G. fuliginosa* on the island of Santa Cruz underwent a recent range expansion (post-1960) from the arid coastal lowlands to the humid central highlands (Bowman 1961, Kleindorfer *et al.* 2006, Kleindorfer 2007, Kleindorfer and Mitchell 2009). We think the range expansion in *G. fuliginosa* was facilitated by anthropogenic alteration to the highlands, which increased the abundance of preferred prey for *G. fuliginosa* – namely, plants that produce small seed – and the local extinction of the ecologically similar sharp-beaked ground finch (*G. difficilis*). Presently, highland *G. fuliginosa* are undergoing niche expansion and showing character shifts in ecologically significant traits (beak length, foot span: Kleindorfer *et al.* 2006); thus, we predict that assortative mating has been relaxed in highland *G. fuliginosa*.

We focus our examination on assortment for beak length, because of the adaptive significance of beak length in Darwin's finches (e.g. Bowman 1961; Grant 1999; Kleindorfer *et al.* 2006); its high heritability (e.g. Boag and Grant 1978; Boag 1983); and its role in mate selection (e.g. Christensen *et al.* 2006; Huber and Podos 2006; Christensen and Kleindorfer 2007; Huber *et al.* 2007; but see Grant and Grant 2008). We do not have data on maternity and paternity, and therefore examine assortative pairing rather than assortative mating.

Following our hypothesis that assortative mating would be lost in a novel habitat, we predict that lowland *G. fuliginosa* will show assortative pairing for beak length, but highland *G. fuliginosa* will not. We test our hypothesis using correlation analysis. In addition, we compare the distribution of female and male beak length within each ecological zone to reject the hypothesis that a loss of assortative pairing in the colonist population is the result of a limited number of potential mates with similar phenotypes. We address two alternative hypotheses for a loss of assortative pairing (breakdown of mate choice cues and selection for disassortative pairing) in the Discussion.

MATERIALS AND METHODS

Study site and species

This study was conducted between January and March in 2001 and 2002 on the island of Santa Cruz. The arid lowlands (0-100 m a.s.l.) are dry-deciduous open forest dominated by *Palo Santo* (*Bursera graveolens*); the humid highlands (500-600 m a.s.l.) are evergreen closed forest dominated by *Scalesia* trees (*Scalesia pedunculata*). *Geospiza fuliginosa* was not present in the highlands before the 1960s (Bowman 1961), but is presently as commonly encountered in the novel highland habitat as the source lowland habitat, and has been observed to breed in highland zone since 2000 (Kleindorfer 2007; Kleindorfer et al 2009a, b).

Darwin's finches are socially monogamous (Grant and Grant 1989). Males establish territories, construct display nests, and sing to attract females (Grant and Grant 1989). Females visit several male territories prior to pairing (Grant and Grant 1989; Kleindorfer 2007). Levels of extra-pair paternity (EPP) are unknown for *G. fuliginosa* on the island of Santa Cruz. For medium ground finch (*Geospiza fortis)* on Daphne Major Island, 20 % of offspring were the result of EPP (Keller *et al.* 2001); EPP was less than 8 % in the cactus finch (*Geospiza scandens*: Petren *et al.* 1999). Therefore, females may pay assortatively with the social-pair male, but disassortatively with the extra-pair male, although this remains to be tested.

Analysis of assortative pairing

We used mist-netting to catch breeding pairs at active nests within four randomly allocated plots (100 x 200 m) in the lowlands and highlands. Individuals were marked with a unique combination of colour bands, which were used to confirm pairings after release. Each colour-banded individual belonged exclusively to one nesting pair. We measured beak length from the anterior edge of the right naris to the beak-tip using dial callipers to an accuracy of 0.01 mm (see Kleindorfer *et al.* 2006).

Beak length was normally distributed, also between the sexes (Kolmogorov-Smirnov test). We measured the level of assortment in each ecological zone by calculating Pearson correlation coefficient (r_p) for female and male beak length. We tested for a significant difference between r_p by converting these values to *z*-scores and calculating z_{obs} using the formula outlined in Pallant (2007). A significant difference is indicated by a z_{obs} that is either less than or equal to -1.96 or greater than or equal to 1.96. We conducted a power analysis on this difference following the method (q [effect size] = $z_1 - z_2$) and table provided by Cohen (1988).

In addition, we calculated Spearman rank correlation (r_s) for female beak length and the absolute value of male residuals of beak length (generated from regression analysis) to determine whether assortment was *true*, as opposed to *apparent* (Crespi 1989; Arnquist *et al.* 1996). *True assortment*, indicated by a nonsignificant r_s value and a symmetrical distribution around the regression line, means that females and males with short beaks are paired at the same frequency as females and males with long beaks (Arnquist *et al.* 1996). *Apparent assortment* indicated by a significant r_s value and a triangular distribution around the regression line (an increase or decrease in variance in male beak length with an increase in female beak length), means that there is a female preference for either short or long beaks in males (Arnquist *et al.* 1996). Thus, true and apparent assortments have different evolutionary consequences. We used PASW version 18.0 (SPSS Inc., 2009) for all statistical analyses.

Analysis of beak length distribution

We used non-targeted mist-netting to assess the distribution of beak length for females and males in each ecological zone. We sampled from the same plots described above and used colour banding to avoid resampling individuals. We tested

the significance of the difference in beak length between sexes in each ecological zone.

RESULTS

Analysis of assortative pairing

We collected data from a total of 23 nesting pairs (2001, $n = 11$; and 2002, $n = 12$). Split by ecological zone, we examined data from 12 lowland pairs and 11 highland pairs. We found no significant differences in beak length within either ecological zone across years; and therefore, pooled data for further analysis. As we predicted, we found positive assortative pairing for beak length in the lowlands ($r_p = 0.80$, $p = 0.006$; Fig. 1a), but not in the highlands ($r_p = 0.07$, $p = 0.847$; Fig. 1b). We found high confidence (power = 0.94) that these r values were significantly different ($z_{obs} = 1.99$). Assortment in the lowlands was confirmed to be true ($r_s = -0.07$, $p = 0.855$).

Analysis of beak length distribution

We collected beak length from: 30 females (2001, $n = 17$; and 2002, $n = 13$) and 64 males (2001, $n = 37$; and 2002, $n = 27$) in the lowlands; and 32 females (2001, $n = 22$; and 2002, $n = 10$) and 55 males (2001, $n = 38$; and 2002, $n = 17$) in the highlands. We found no significant difference in beak length between years for data split by sex and ecological zone. A two-way ANOVA found a significant effect of ecological zone ($F = 17.54$, df = 1, $p < 0.001$), but not sex ($F = 0.17$, df = 1, $p = 682$) or the interaction term ($F = 0.01$, df = 1, $p = 0.910$) on beak length. In addition, the variance around the mean beak length was not significantly different between females and males in either ecological zone (Levene's test: lowlands $F = 3.14$, $p = 0.080$; highlands $F = 1.83$, $p = 0.179$). Percentage distributions of female and male beak length (to an accuracy of 0.1 mm) for each ecological zone are shown in Fig. 2.

DISCUSSION

We predicted that a colonist population in a novel habitat would have loss of assortative mating under conditions of relaxed selection for assortment. Our findings

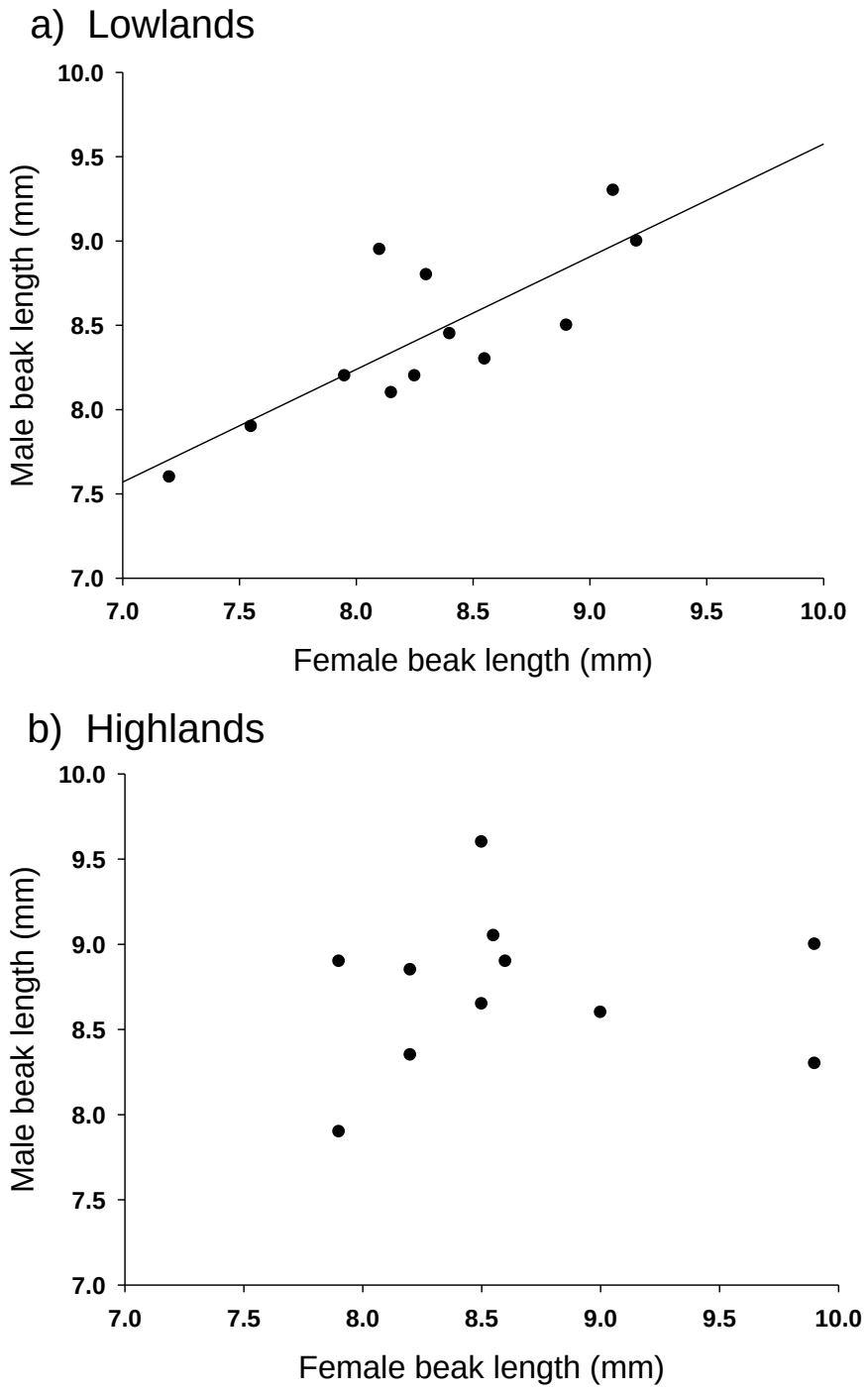


Figure 1: The relationship between female and male beak length (mm) for breeding pairs of Darwin's small ground finch (*G. fuliginosa*) in (a) the arid lowlands, and (b) the humid highlands on the island of Santa Cruz, Galápagos Archipelago (2001-2002).

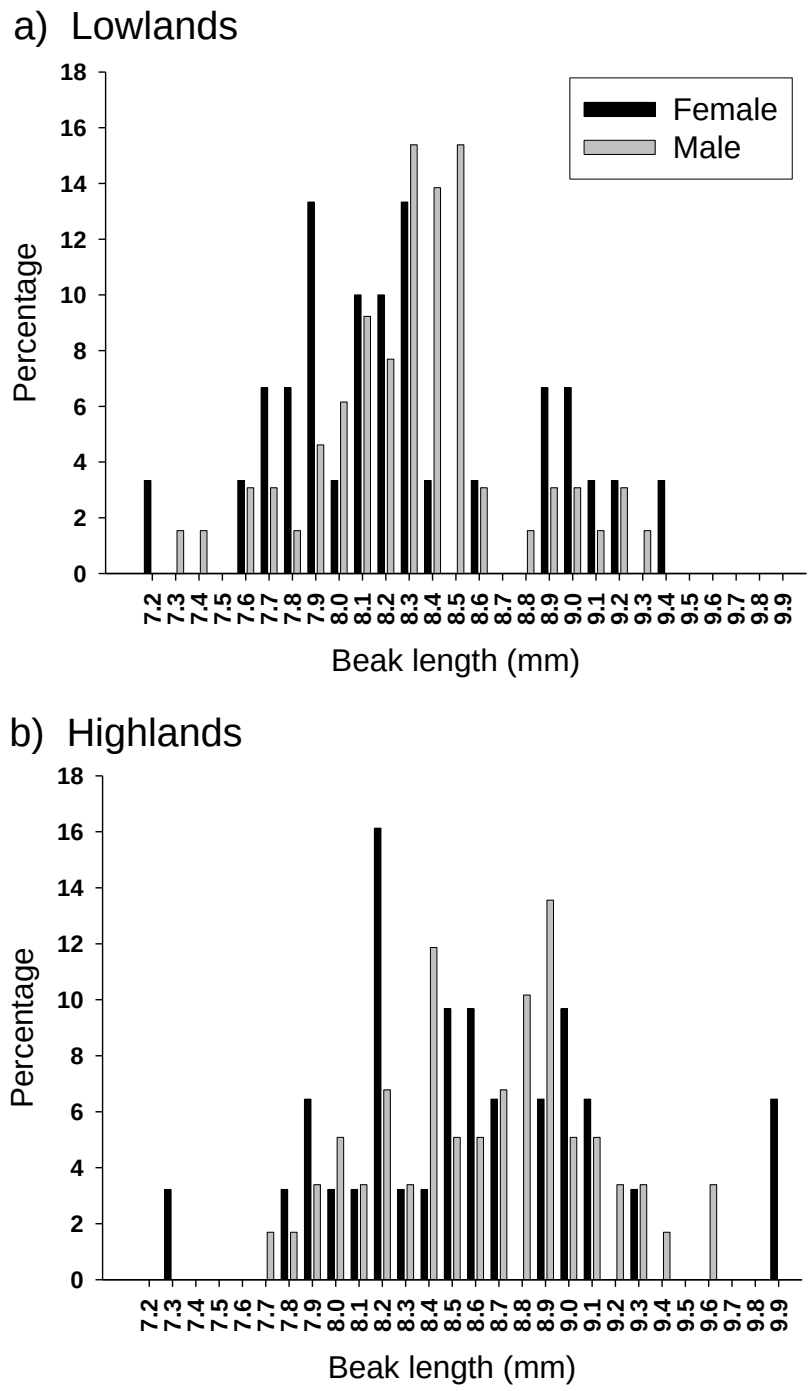


Figure 2: The distribution of beak length (mm) for female and male small ground finch *G. fuliginosa* sampled in the a) arid lowlands and b) humid highlands on the island of Santa Cruz, Galápagos Archipelago (2001-2002). Data is given as the percentage of individuals of each sex within 0.1 mm bins. trait for foraging and song), whereas the highland colonist population did not. We found no evidence that the number of potential mates of similar phenotype was a limiting factor and therefore reject this explanation for the observed loss of assortment in the highlands.

support this prediction. On the island of Santa Cruz, the lowland source population of *G. fuliginosa* showed assortative pairing for beak length (an ecologically-significant.

The loss of assortative mating/pairing may alternatively evolve via a breakdown of mate choice cues or direct selection for disassortative mating/pairing. Here, we argue that neither alternative explanation is likely in the present study.

Assortative pairing may be lost via a breakdown of cues used for detecting and assessing assortment. This breakdown of cues may occur when the transmission or detection of cues are modified in the new environment (Seehausen et al 1997). In Darwin's finches, females use male morphology and song in mate choice (Grant 1999). Experiments have shown that ground finches (*Geospiza* spp.) can discriminate conspecifics based on visual assessment of overall beak and/or body dimensions, and auditory assessment of overall song characteristics (Grant 1999). Song characteristics (trill rate, frequency bandwidth) can reliably indicate beak morphology in Darwin's finches (Podos 2001; Christensen *et al.* 2006). As a result, mate choice is expected to occur in two stages: first, males use song to attract females over longer distances; and second, males use morphology to attract females over shorter distances (Grant 1999). While the more complex vegetation structure of the highland forest may impede the detection of song (Slabbekoorn and Smith 2002), it is not expected to impede visual cues at short distances. From a mate choice perspective, it is likely that homotypic and heterotypic phenotypes are discriminated by females in the same way, and that there has been no notable change in mate choice cues in the novel habitat.

In the Introduction we described the "overexploited resources" and "novel habitat" scenarios that would favour selection for disassortative mating and relaxed selection on mate choice respectively. Direct selection for disassortative mating is not the likely cause of the loss of assortative pairing in this system. Direct selection for disassortative mating in a novel habitat would require the existence of markedly different resources and niches to those in the original habitat. In this case, source populations would have a homotypic advantage and colonist populations would have a heterotypic advantage. While we acknowledge that such a pattern is possible (given the right selection pressures), we think that a shift from assortative mating to disassortative mating during the colonisation process that does not involve an intermediate stage of relaxed mate choice is highly improbable. As previously stated, colonisation of a novel habitat requires colonists with pre-adaptations (Mayr 1965; Tonnis *et al.* 2005). These pre-adaptations may arise intrinsically, through mutation

or recombination, or extrinsically, through changes in the environment, which facilitate colonisation. In the present system, anthropogenic alteration to the highland zone has facilitated the invasion of mostly exotic small-seeding plants, which may have facilitated the expansion of *G. fuliginosa*, a species pre-adapted to foraging on small seeds. A diet shift in highland *G. fuliginosa* towards increased insectivory is linked with an abundance of invertebrate prey (Tebbich *et al.* 2002) and the local extinction of the insectivorous sharp-beaked finch in the highlands (*G. difficilis*; discussed in Kleindorfer *et al.* 2006, Kleindorfer and Mitchell 2009). However, seeds remain an important component in the diet of highland *G. fuliginosa* (Kleindorfer *et al.* 2006). Therefore, the maintenance of seed-foraging pre-adaptations in some highland *G. fuliginosa*, alongside the generation of invertebrate-foraging adaptations in other highland *G. fuliginosa*, is favoured. This logic is parsimonious with the loss of assortative mating, and not selection for disassortative mating, in this system in particular and for range expansions into novel habitats in general.

The data support the idea that loss of assortative pairing was caused by relaxed selection for assortative mating in a novel habitat, given that novel habitats offer ecological opportunities and reduced interspecific competition. We suggest that the ecological contrast between the lowland and highland zones on the island of Santa Cruz (and other elevated islands of the Galápagos Archipelago) has intensified the contrasting pattern of assortative and disassortative pairing observed. The arid lowlands represent a "severe" habitat that would favour strict assortative pairing; the humid highlands represent a "benign" habitat that would favour relaxed assortative pairing.

This difference in selection intensity between ecological zones is the product of differences in rainfall and, subsequently, food supply. During the prevailing *La Niña* climatic conditions on the Galápagos Archipelago – *La Niña* periods typically span 2-11 years (Snell and Rea 1999) – annual rainfall in the lowland zone was less than 250 mm on average, defining it as a desert; highland rainfall was never less than 650 mm (based on records over the past decade). *La Niña* periods are interspersed by *El Niño* periods, spanning 1-2 years, which bring high rainfall to both lowlands and highlands (Snell and Rea 1999). While we did not measure differences in food supply between ecological zones, Tebbich *et al.* (2002) showed a higher diversity and abundance of invertebrate prey in the highlands than the lowlands. Invertebrate prey constitutes a large portion of the diet in *G. fuliginosa* (Kleindorfer *et al.* 2006).

As a consequence of the ecological contrast between source and colonist population in our study, selection pressure driven by food supply is expected to be more intense in the lowlands than in the highlands. Therefore, the maintenance of tested foraging adaptations (a specific beak length) via assortative mating would be favoured in lowland *G. fuliginosa*, not only because most niches in the original habitat are expected to be filled, but because food supply is limited. In contrast, the greater number and diversity of prey and available niches in the highlands could favour deviations from tested foraging adaptations, and thereby tolerate mating between dissimilar phenotypes.Divergence in beak length between lowland and highland *G. fuliginosa* previously observed in this system (Kleindorfer *et al.* 2006) raises the question: how can a loss of assortative pairing result in apparent directional selection for longer beaks in the highlands? A possible answer is: directional selection for longer beaks is in fact not occurring in the highlands, rather only directional selection for shorter beaks is occurring in the lowlands. In other words, beak length has not become longer in the highlands, but shorter in the lowlands. This idea fits with that of greater selection intensity in the lowlands compared to the highlands.

Studies that have examined beak size bimodality in populations of medium ground finch (*Geospiza fortis*) in the lowlands of the island of Santa Cruz indirectly add support to our findings. At the location *El Garrapatero*, strong disruptive selection (Hendry *et al.* 2009) and assortative pairing (Huber *et al.* 2007) maintains divergence between small and large beak morphs. Interestingly, there was a trend for stronger assortment in years with low rainfall than years with high rainfall (Huber *et al.* 2007), which is analogous to the arid lowlands and humid highlands in our study, respectively. Such a trend suggests that assortative pairing in Darwin's finches can be plastic and has a propensity to relax with selection pressure (as suggested here). Further, at a second location on the island of Santa Cruz – Academy Bay – where the environment has changed substantially as a result of anthropogenic modification, historic beak size bimodality, and presumed assortative mating in *G. fortis*, has been lost (Hendry *et al.* 2006). Hendry *et al.* (2006) suggest that beak size bimodality (and assortative mating) may have been lost via relaxed selection in a "more benign" environment created by humans (exhibiting a diversity of seeding plants, permanent water, direct and indirect hand-feeding, etc.). Therefore, the loss of assortative

pairing in highland *G. fuliginosa* that we observed appears to be a repeatable phenomenon in finch populations that experience relaxed selection.

We acknowledge that our present study suffered from a small sample and no replication in space (sample sites) or time (sample periods). Given that assortative pairing may be a plastic response to variation in selection pressures, the patterns observed in this study therefore may not be representative of greater patterns in space and time. However, we are confident that in this study lowland *G. fuliginosa* paired assortatively (power analysis = 0.99), which was significantly different to the pattern of pairing in highland *G. fuliginosa* (power analysis = 0.94).

ACKNOWLEDGEMNETS

We thank the Galápagos National Park; Charles Darwin Research Station; TAME airlines; Max Planck Institute for Ornithology; American Bird Conservancy; Conservation International; Galápagos Conservation Fund; Winifred Violet Scott Trust; Andrew Hendry; Rachael Dudaniec; Rebekah Christensen; Jeremy Robertson; Santiago Torres; and Carlos Vinueza. This research was conducted under the ethics approval granted by Flinders University (E189).

REFERENCES

Arnquist, G., Rowe, L., Krupa, J.J. and Sih, A. 1996. Assortative mating by size: a meta-analysis of mating patterns in water striders. *Evol. Ecol.*, **10**: 265-284.

Bateson, P. 1983. *Mate Choice*. Cambridge, UK: Cambridge University Press.

Boag, P.T. 1983. The heritability of external morphology in Darwin's ground finches (*Geospiza*) on Isla Daphne Major, Galápagos. *Evolution*, **37**: 877-894.

Boag, P.T. and Grant, P.R. 1978. The heritability of external morphology in Darwin's finches. *Nature*, **274**:793-794.

Bolnick, D.I., Yang, L.H., Fordyce, J.A., Davis, J.M. and Svanbäck, R. 2002. Measuring individual-level resource specialization. *Ecology*, **83**: 2936-2941.

Bowman, R.I. 1961. Morphological differentiation and adaptation in the Galapagos finches. *Univ. Calif. Publ. Zool.*, **58**: 1-302.

Cohen, J. 1988. *Statistical Power Analysis for the Behavioural Sciences* 2nd edn. Hillsdale, NJ: Lawerance Erlbaum Associates. **Christensen, R. and Kleindorfer, S. 2007.** Assortative pairing and divergent evolution in Darwin's small tree finch, *Camarhynchus parvulus*. *J. Ornith.,* **148**: 463-470.

Christensen, R., Kleindorfer, S. and Robertson, J. 2006. Song is a reliable signal of beak morphology in Darwin's small tree finch *Camarhynchus parvulus*, and vocal performance predicts male pairing success. *J. Avian Biol.,* **6**: 617-624.

Crespi, B.J. 1989. Causes of assortative mating in arthropods. *Anim. Behav.,* **38**: 980-1000.

Grant, B.R. and Grant, P.R. 1989. *Evolutionary dynamics of a natural population: the large cactus finch of the Galápagos*. Chicago, IL: University of Chicago Press.

Grant, B.R. and Grant, P.R. 1996. High survival of Darwin's finch hybrids: effects of beak morphology and diets. *Ecology,* **77**: 500-509.

Grant, P.R. 1999. *Ecology and Evolution of Darwin's finches,* 2nd edn. Princeton, NJ: Princeton University Press.

Grant, P.R. and Grant, B.R. 1994. Phenotypic and genetic-effects of hybridization in Darwin's finches. *Evolution,* **48**: 297-316.

Grant, P.R. and Grant, B.R. 2008. Pedigrees, assortative mating and speciation in Darwin's finches. *Proc. R. Soc. Lond. B.,* **275**: 661-668.

Hendry, A.P. Huber, S.K., De León, L.F., Herrel, A. and Podos, J. 2009. Disruptive selection in a bimodal population of Darwin's finches. *Proc. R. Soc. Lond. B.,* **276**: 753-759.

Hendry, A.P., Grant, P.R., Grant, B.R., Ford, H.A., Brewer, M.J., and Podos, J. 2006. Possible human impacts on adaptive radiation: beak size bimodality in Darwin's finches. *Proc. R. Soc. Lond. B.,* **273**: 1887-1894.

Huber, S.K., De León, L.F., Hendry, A.P., Bermingham, E. and Podos, J. 2007. Reproductive isolation of sympatric morphs in a population of Darwin's finches. *Proc. R. Soc. Lond. B.,* **274**: 1709-1714.

Huber, S.K. and Podos, J. 2006. Morphology and song features covary in a population of Darwin's finches (*Geospiza fortis*). *Biol. J. Linn. Soc.,* **88**: 489-498.

Keller, L.F., Grant, P.R., Grant, B.R. and Petren, K. 2001. Heritability of morphological traits in Darwin's Finches: misidentified paternity and maternal effects. *Heredity*, **87**: 325-336.

Kleindorfer, S., Chapman, T.W., Winkler, H. and Sulloway, F.J. 2006. Adaptive divergence in contiguous populations of Darwin's small ground finch (*Geospiza fuliginosa*). *Evol. Ecol. Res.,* **8**: 357-372.

Kleindorfer, S. 2007. The ecology of clutch size variation in Darwin's small ground finch, *Geospiza fuliginosa*: comparison between lowland and highland ecological zones. *Ibis,* **149**: 730-741.

Kleindorfer, S. and Mitchell, J.G. 2009. Biological Networks: Rainforests, Coral Reefs, and the Galapagos Islands. In: *The Network Challenge: Strategy, Profit, and Risk in an Interlinked World.* pp. 85-105. Eds.: Kleindorfer, P.R. and Wind, Y. Upper Saddle River, NJ: Wharton School Publishing.

Kleindorfer, S. and Dudaniec, R.Y. 2009a. Love thy neighbour? Social nesting pattern, host mass, and nest size affect ectoparasite intensity in Darwin's tree finches. *Behav. Eco. Sociobiol,* **63**: 731–739.

Kleindorfer, S., Sulloway, F.J. and O'Connor, J. 2009b. Mixed species nesting associations in Darwin's tree finches: Nesting pattern predicts breeding performance. *Biol. J. Linn. Soc.,* **98**: 313–324.

Mayr, E. 1965. The nature of colonisation in birds. In *The Genetics of Colonising species* (H.G. Baker and G.L. Stebbins, eds.), pp. 29-47. New York, NY and London, UK: Academic press.

Pallant, J. 2007. *SPSS Survival Guide*. Maidenhead, Berkshire: Open University Press/McGraw-Hill HousePetren, K,, Grant, R.B. and Grant, P.R. 1999. Low extrapair paternity in the Cactus Finch (Geospiza scandens). *Auk* **116:** 252–256

Podos, J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* **409**: 185_188.

Snell, H. and Rea, S. 1999. The 1997-98 El Niño in Galapagos: Can 34 years of data estimate 120 years of pattern? *Not. Galap.,* **60**: 11-20.

Seehausen, O., van Alphen, J.J.M. and Witte, F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science,* **277**: 1808-1811.

Slabbekoorn, H. and Smith, T.B. 2002. Bird song, ecology and speciation. *Phil. Trans. R. Soc. Lond. B.,* **357**, 493-503.

PAWS 18.0 2009. Chicago: SPSS Inc., an IBM Company.

Tebbich, S., Taborsky, M., Fessl, B. and Dvorak, M. 2002. The ecology of tool-use in the woodpecker finch (*Cactospiza pallida*). *Ecol. Letters,* **5**: 656-664.

Tonnis, B., Grant, P.R., Grant B.R. and Petren, K. 2005. Habitat selection and ecological speciation in Galápagos warbler finches (*Certhidea olivacea* and *Certhidea fusca*). *Proc. R. Soc. Lond. B,* **272**: 819-826.

CHAPTER FIVE

Differential response to local and foreign song following colonisation of a new environment by Darwin's small ground finch, *Geospiza fuliginosa*

Toby H. Galligan and Sonia Kleindorfer

BMC Evolutionary Biology (in preparation)

ABSTRACT

Range expansion can lead to reproductive isolation between source and colonist populations via adaptive and non-adaptive divergence. In many species of bird, divergence in song following range expansion can directly restrict gene flow when foreign song is not recognised; however, not all species show differences in song or song discrimination among geographically separated populations. In this study, we examine the role of novel environments for divergence in song and song discrimination following range expansion. Our study system is a population of Darwin's small ground finch *Geospiza fuliginosa* on the island of Santa Cruz, Galápagos Archipelago, which has relatively recently expanded its range from the arid coastal lowlands into the humid central highlands. We compare differences in song characteristics and response to playback of local and foreign song within and between ecological zones. Despite short and approximately equivalent geographical distances between localities sampled, we found differences in song and song discrimination between the lowlands and highlands, but not within either ecological zone. Specifically, lowland *G. fuliginosa* sang longer songs than highland *G. fuliginosa*; and lowland *G. fuliginosa* gave a greater response to local than foreign song, whereas highland *G. fuliginosa* responded no different to either local or foreign song. We discuss two alternative explanations for song discrimination in lowland individuals: foreign song was not recognised; or foreign song was recognised but not considered worthy of response. The former would suggest dispersal between ecological zones is unidirectional; the latter would suggest intrasexual competition is stronger in the more 'severe' lowlands. In addition, restricted gene flow is predicted by the former, but not the latter.

Keywords: range expansion, song divergence, song discrimination, playback experiment, adaptive divergence, barrier to gene flow, reproductive isolation.

INTRODUCTION

Range expansion is the critical first step towards allopatric and parapatric speciation (Mayr 1947). Following range expansion, gene flow can be progressively reduced via various geographical, ecological, social, and biological mechanisms acting between source and colonist populations until populations are reproductively isolated (Dobzhansky 1937; Mayr 1942, 1963; Schluter 2000; Coyne and Orr 2004; Nosil, *et al.* 2005). One such isolating mechanism is divergence in courtship signals, where failure to recognise divergent signals prevents individuals mating.

Song is an important courtship signal in many bird species. Various natural and sexual selective pressures, in conjunction with genetic and cultural drift, shape song within populations (Catchpole and Slater 1995; Price 2008). Accordingly, differences in selection and drift between populations can lead to divergence in song. Among Passeriformes, song differences can delineate both species and populations; and may promote barriers to gene flow (reviewed in Slabbekoorn and Smith 2002). Song divergence often precedes other signal divergence (Grant and Grant 2008). Following range expansion, populations should diverge in song; and such divergence is predicted to be accompanied by discrimination of local conspecific song. While many species show geographical variation in song (e.g. Baptista 1975; Bitterbaum and Baptista 1979; Nelson 1998; Irwin 2000) and differential discrimination of local and foreign song (e.g. Petrinovich and Patternson 1981; Barker 1983; Thielcke and Wustenberg 1985; Dingle *et al.* 2010), there are species that do not (e.g. Martens 1975). The question therefore arises: under what conditions of range expansion are divergence in song and song discrimination likely?

Darwin's small ground finch *Geospiza fuliginosa* on the island of Santa Cruz in the Galápagos Archipelago has recently expanded its breeding range from the coastal lowlands to the central highlands. We have two lines of evidence that support a recent range expansion: (1) *G. fuliginosa* was not recorded breeding in the highlands up until the 1960s, but since 2000 breeds regularly in the highlands (Lack 1947; Bowman 1961; Curio 1969; Kleindorfer 2007); and (2) *G. fuliginosa* has taken advantage of two ecological opportunities that have resulted from anthropogenic destruction and degradation of the highlands: namely, the invasion of exotic small-seeding plants (Chapter 4) – the preferred prey of *G. fuliginosa* (Grant 1999;

Kleindorfer, *et al.* 2006); and the extinction of the sharp-beaked ground finch *Geospiza difficilis* (Lack 1947; Bowman 1961; Sulloway 1982) a known competitor (Schluter and Grant 1982).

The lowlands and highlands represent ecologically contrasting arid and humid habitats, respectively. The lowlands support dry-deciduous open forest; the highlands support evergreen closed forest. In addition, the food supply and interspecific competition differ between zones. Specifically, food supply is predicted to be more frequently renewed in the highlands due to regular precipitation throughout the year; and competition with the medium ground finch *Geospiza fortis* is predicted to be greater in the lowlands, where it is more abundant than in the highlands. Further, the ecological contrast is strengthened during low rainfall *La Niña* periods, which are the typical climatic condition in the Galápagos (spanning 2-11 years). Therefore, lowland-source and highland-colonist populations of *G. fuliginosa* are subjected to divergent selection regimes.

Adaptive divergence has been observed in this system (Kleindorfer *et al.* 2006; Sulloway and Kleindorfer in review) despite presumably high gene flow between zones (Chapter 2 and 3). Importantly, highland *G. fuliginosa* had longer beaks and more often gleaned insects from understory foliage whereas lowland *G. fuliginosa* had shorter beaks and more often picked seed from the ground (Kleindorfer *et al.* 2006). Adaptive divergence was maintained over a six year low rainfall period (2000 to 2005): the typical climatic condition for the Galápagos (Kleindorfer *et al.* 2006; Sulloway and Kleindorfer in review). However, in the high rainfall year of 2008, morphological divergence was no longer measurable (Chapter 3). The most likely reason for this observation was relaxed selection against ill-adapted immigrants in that year and non-adaptive convergence in mean trait values (Chapter 3). However, an absence of morphological divergence is expected to be only brief, like the high rainfall periods that cause them (Chapter 3).

In addition, differential mating strategies have been observed in this system (Chapter 4). Specifically, while lowland *G. fuliginosa* had assortative pairing for beak length, highland *G. fuliginosa* did not (Chapter 4). Loss of assortative pairing is a predicted response following range expansion as selection on mate choice is relaxed in response to ecological opportunities. The "benignity" of the highlands (i.e., increased food supply) is expected to further facilitate a loss of assortative pairing (Chapter 4).

In this study, we test the hypothesis that range expansion into a novel environment, despite short geographical separation, can lead to divergence in song and song discrimination in *G. fuliginosa*. We compare variation between ecological zones (between-zone variation) with variation between localities within the same ecological zone (within-zone variation). Geographical distance is largely controlled for by sampling sites of similar proximities (Fig. 1). If the ecological contrast between zones has primarily influenced divergence we predict greater differences in song and greater song discrimination between-zones than within-zones.

To test the role of song as a premating barrier, we used playback experiments of lowland and highland song in each habitat. If song divergence between locations contributes to gene flow or assortative pairing, we predict a stronger response to playback recordings of local song than foreign song – that is, highland males respond more strongly to highland song than lowland song, and vice versa. Male response is a standard proxy for female mate recognition in a wild population of birds (Irwin 2000; Slabbekoorn and Smith 2002), particularly Darwin's finches (Grant and Grant 2002a, b; Podos 2007, 2010), given males that can recognise their competitors cues of attraction would have a selective advantage.

METHODS

Sampling period and sites

Playback stimuli were recorded between January and March in 2000 and 2001. Playback experiments were conducted within the first two weeks of February 2008. We collected additional song recordings between March and May in 2008. Song characteristics were examined in both 2000 and 2001, and 2008.

Playback stimuli were recorded at the localities *El Garrapatero* and *Los Gemelos* (four sites in total; see Christensen *et al.* 2006). For playback experiments, we sampled three lowland and three highland sites; and for analysis of song characteristics, we sampled four lowland and three highland sites (Figure 1; Table 1). Dry-deciduous forest in the lowlands is dominated by *Bursera graveolens*, which is regularly spaced and forms an open canopy. Common plant species in the understorey include *Opuntia echios gigantean, Croton scouleri, Scutia pauciflora,*

Figure 1: The island of Santa Cruz, Galápagos Archipelago, showing the proximity of the four localities and eight sites sampled in this study (see also Table 1).

Table 1: Details of the four lowland and four highland sites sampled: locality; co-ordinates (UTM; zone = 15); altitude (m asl); and sample size in 2008 for our song analysis (n_{sa}) and playback experiment (n_{pe}).

	Locality	N, E	Alt.	n_{sa}	n_{pe}
Lowlands	Academy Bay	0797380, 9918815	65	10	10
	Academy Bay	0800270, 9917844	9	11	-
	El Garrapatero	0809269, 9923213	5	12	9
	El Garrapatero	0808374, 9925013	66	10	10
Highlands	*Media Luna*	0797223, 9929170	702	13	-
	Media Luna	0797450, 9927147	605	-	11
	Los Gemelos	0791254, 9931119	617	12	12
	Los Gemelos	0790140, 9929998	569	15	12

Acacia spp., and *Parkinsonia aculeata*. Evergreen forest in the highlands is dominated by *Scalesia pedunculata* which is densely spaced and forms a closed canopy. The understorey in the highland is sparse but includes *Zanthoxylum fagara*. The substrate in the lowlands is littered with bare volcanic rocks, whereas in the highlands a good layer of soil and herbaceous plants exists.

Song in G. fuliginosa

Male Darwin's finches use song to attract mates and deter rivals (Lack 1947; Bowman 1983; Grant 1999). In the ground finches *Geospiza* spp., song has a simple structure and typically consists of one to three note types repeated up to ten times (Fig. 2; see also Bowman 1983). Considerable variation in song type exists within species; however, each male typically sings a single song type that he learns from his father (Grant 1999). For *G. fuliginosa* on Santa Cruz, song type variation is not habitat-dependent: in fact, greater variation exists within than between ecological zones (Fig. 2).

Analysis of song characteristics

Song recordings were made along one transect per site to avoid resampling of individuals. Individuals were recorded at an estimated vertical distance between 3-5 m and an estimated horizontal distance between 1-3 m. Multiple recordings of each individual's song were made (song exemplers). We used a Sound Devices 722 Digital Recorder with a Telinga Twin Science Parabolic Microphone, and saved recording as 24-bit 48 kHz .wav files.

All individuals that were recorded in this study sang a single song type; however, analysis of song type was avoided because clear distinction between song types is difficult and subjective (Podos 2007). Rather, we used Raven version 1.4 to generate spectrograms with a 256 fast Fourier transform length and a Hanning Window and measured nine song characteristics. Four characteristics were temporal: (1) song duration (s); (2) number of notes (continuous trace on spectrogram); (3) note duration (s); and (4) trill rate (notes/s). The other four characteristics were spectral: (5) minimum frequency (kHz); (6) maximum frequency (kHz); (7) frequency bandwidth (= min. - max. frequencies; kHz); and (8) peak frequency (frequency measured at the maximum amplitude). We then calculated mean individual values for each characteristic from multiple song exemplers. The ninth characteristic was

vocal deviation, an inverse measure of vocal performance, from the relationship between trill rate and frequency bandwidth (Podos 1997). We examined differences in song within and between zones using PASW version 18 (SPSS Inc. 2009). Probabilities for all multiple comparisons were corrected using the Bonferroni sequential method.

Analysis of playback response

We opportunistically tested solitary males along one transect per site to avoid resampling and confounding stimuli (i.e., presence of other males). Each test consisted of a series of four randomly ordered stimuli: (1) the song of a lowland *G. fuliginosa*; (2) the song of a highland *G. fuliginosa*; (3) the song of a Cassin's finch, *Carpodacus cassini* (experiment control); and (4) silence (experimenter control). *Geospiza fuliginosa* recordings used as stimuli shared song types with recordings collected in 2008 (see Fig. 2). Five replicates of each stimulus were used to generate 10 unique series. Each stimulus itself consisted of an individual song recording repeated three times at 10 seconds intervals, followed by 1 minute of silence; hence, each series was 6 minutes in duration. Stimuli and series were number-coded to avoid experimenter bias when recording test subject's response.

Geospiza fuliginosa recordings were collected by S. Kleindorfer in 2000-2001 using a Sony WMD6 Cassette Recorder and a Sennheiser ME 80 'Shotgun' Microphone; and digitalised using a MOTU MIDI. *Carpodacus Cassini* recordings were obtained from Cornell Lab of Ornithology Macaulay Library. We used *C. Cassini* recordings as a control because the song of this species: shares characteristics with Darwin's finches; it is not natural heard in the Galápagos archipelago; and has been used in playback experiments before (Grant and Grant 2002b, a). All recordings were saved as 24-bit 48 kHz .wav files, standardised for amplitude, and filtered to reduce background noise.

On locating a suitable test subject we approached no closer than 6 m and waited at least 60 s before commencing playback. We used an Apple iPod and mono speaker belt attached to an experimenter to broadcast our stimuli. We expected negligible effect among sites because all trial locations are highly frequented by humans, males tested held territories close to human-made paths, and *G. fuliginosa* has a confiding nature around humans in general.

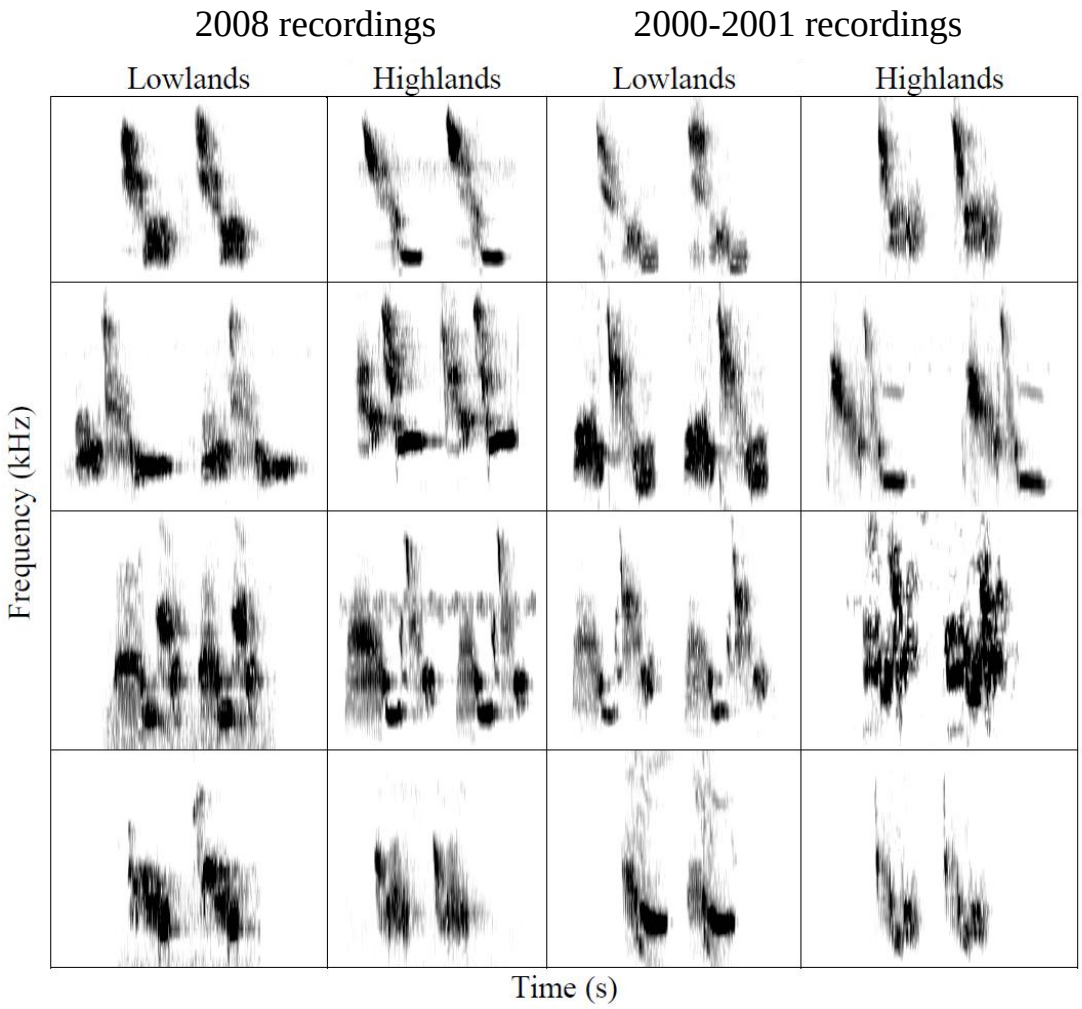


Figure 2: A sample of spectrograms of *Geospiza fuliginosa* song type variation between ecological zones. The first two columns are recordings from 2008 used in our analysis of song divergence. The second two columns are recordings from 2000-2001 used in as stimuli in our playback experiments. Variation among song type is greater within (columns) than among (rows) ecological zones and time periods.

We recorded: (1) latency to respond (either song or approach; s); (2) number of songs; (3) number of approaches; (4) minimal vertical distance of approach (m); and (5) minimal horizontal distance of approach (m). Vocal responses were recorded as 24-bit 48 kHz .wav files using a digital recorder and parabolic microphone (details above), and measured after the experiment. No response to a given stimuli was recorded as 120 s latency. Distances of approach (responses 4 and 5) were recorded to a maximum of 10 m. Only test subjects that remained in our sight during the whole playback series were included in analyses.

We avoided pseudoreplication by following the procedure suggested by Kroodsma (1989, 2001); specifically, we used the number of unique series as our sample size (i.e., n = 10). Accordingly, our data points were the mean response for individuals that were presented with the same series. For our analysis within ecological zones, we used the mean response from sites of the same locality (see Table 1). We compared *El Garrapatero* against *El Mirador*; and *Media Luna* against *Los Gemelos*. For our analysis between ecological zones, we used the mean response from sites of the same zone (see Table 1). We used PASW version 18 (SPSS Inc. 2009) to examine differences in song discrimination between lowland and highland *G. fuliginosa*. Again, probabilities for all multiple comparisons were corrected using the Bonferroni sequential method.

RESULTS

Song characteristics between locations

In 2008, we obtained song recordings from 43 lowland males and 40 highland males (see Table 1 for sample sizes per site; and Table 2 for variable means). For each individual we obtained 4.02 ± 1.36 song exemplers. We examined variation in our 2008 data separately and in combination with our 10 lowland and 10 highland song recordings from 2000-2001. The 2008 data alone represents the pattern of variation in song at the time of the playback study; the combined data represents the variation between song at the time of the playback study and those songs used as stimuli in our playback study.

We used principal component analysis (PCA) with a promax rotation (Kappa = 4) to reduce our song characteristics into three principle components (PC1-PC3) that explained 87.5 % of the observed variance (Table 3). PC1 had high loadings for vocal deviation, frequency bandwidth, and high and low frequency; PC2 had high loadings for number of notes, note duration, and trill rate; and PC3 had high loadings for the song duration, and low loadings for peak frequency, number of notes, and low frequency (Table 3). Because song duration alone was loaded highly to PC3, we analysed this variable in addition to our principal components.

In the lowlands, we found a significant difference in song duration and PC3 between recording periods (song duration t = -3.14, p = 0.003; PC3 t = 4.43, p <

0.001; n = 53): songs were longer in 2008. No other song characteristics was significant different between recording periods in either lowlands (PC1 t = -0.61, p = 0.545; PC2 t = 1.51, p = 0.161; n = 53) or in highlands (PC1 t = -1.10, p = 0.277; PC2 t =0.66, p = 0.036; PC3 t = 1.21, p = 0.253; song duration t = -0.66, p = 0.513; n = 50).

We found no significant difference in song characteristics between ecological zones for sampling periods combined (PC1 t = 0.60, p = 0.548; PC2 t = 0.63, p =0.528; PC3 t = -1.71, p = 0.090; song duration t = 2.05, p = 0.043; n = 103). When separated by sampling period, we found a significant difference in song duration in 2008 (t = 2.56, p = 0.012, n = 83); but not for any other combination of sampling period and song characteristic (2000-2001 [PC1 t = 0.78, p = 0.445; PC2 t = 0.27, p = 0.789; PC3 t = 0.22, p = 0.831; song duration t = -0.49, p = 0.631; n = 20]; 2008 [PC1 t = 0.31, p = 0.756; PC2 t = 0.68, p = 0.498; PC3 t = -2.34, p = 0.022; n = 83]).

We found no significant difference between all sites sampled (PC1 $F_{6, 82}$ =

Table 2: Mean (± standard deviation) values for song characteristics measured in male lowland and highland *Geospiza fuliginosa* used in our analysis of song divergence (variation data [2008 recordings]) and analysis of song discrimination (playback stimuli [2000-2001 recordings]). Sample sizes (lowlands:highlands) were 43:40 variation data and 10:10 playback stimuli.

	Variation data (2008 recordings)		*Playback stimuli (2000-2001 recordings)*	
	Lowland	Highland	Lowland	Highland
Song duration (s)	1.04 ± 0.23	0.91 ± 0.23	0.81± 0.11	0.86 ± 0.30
# notes	2.81 ± 0.76	2.55 ± 0.78	3.20 ± 1.31	2.90 ± 0.57
Note duration (s)	0.32 ± 0.09	0.32 ± 0.12	0.24 ± 0.14	0.25 ± 0.10
Trill rate (notes/s)	2.75 ± 0.69	2.91 ± 0.90	4.19 ± 2.24	4.20 ± 3.06
Min. frequency (kHz)	1.79 ± 0.27	1.76 ± 0.29	2.13 ± 0.11	2.10 ± 0.40
Max. frequency (kHz)	6.76 ± 1.03	6.63 ± 1.07	6.90 ± 0.99	6.52 ± 0.97
Freq. Bwidth (kHz)	4.96 ± 1.05	4.88 ± 1.25	4.77 ± 0.96	4.42 ± 0.97
Peak frequency (kHz)	3.98 ± 0.65	4.14 ± 0.94	4.43 ± 1.03	4.33 ± 1.01
Vocal deviation	4.92 ± 1.04	4.83 ± 1.24	4.72 ± 0.95	4.38 ± 0.96

1.60, $p = 0.165$; PC2 $F_{6, 82} = 0.51$, $p = 0.801$; PC3 $F_{6, 82} = 1.95$, $p = 0.083$), except for song duration between a one lowland (Academy Bay) and one highland (*Los Gemelos*) site ($F_{6, 82} = 2.71$, $p = 0.019$). Importantly, we found no difference between localities within the lowlands (PC1 $t = 1.10$, $p = 0.284$; PC2 $t = 1.02$, $p = 0.314$; PC3 $t = 1.87$, $p = 0.069$; song duration $t = -1.37$, $p = 0.18$; $n = 53$) and the highlands (PC1 $t = -0.05$, $p = 0.962$; PC2 $t = 0.78$, $p = 0.441$; PC3 $t = 1.83$, $p = 0.073$; song duration $t = -2.01$, $p = 0.05$; $n = 50$).

Playback response

We obtained data from 29 out of 30 experiments on individual finches in the lowland zone and 35 out of 37 experiments on individual finches in the highland zone (see Table 1). Failed trials were the result of test subject moving away. Each series was

Table 3: Principal component analysis loadings for 2 sets of variables: song characteristics (PC1-PC3) and playback responses (PC4). Each set was calculated separately using a promax rotation method. Only loadings above 0.35 are shown.

Characteristic	PC1	PC2	PC3	PC4
Vocal deviation	1.00			
Frequency bandwidth (kHz)	1.00			
Highest frequency (kHz)	0.95			
Lowest frequency (kHz)	-0.43		0.44	
# Notes		0.89		
Note duration (s)		-0.94		
Trill rate (notes/s)		0.87	0.56	
Song duration (s)			-0.88	
Peak frequency (kHz)			0.50	
Latency (s)				0.91
# flights				0.87
Min. horizontal distance (m)				0.87
Min. vertical distance (m)				0.81
# songs				0.68

tested 2-5 times.

We examined variation in song characteristics between our 2000-2001 recordings and 2008 (split by ecological zone) to assess the accuracy of our playback stimuli simulating contemporary song (see Table 2 for variable means). To do this we calculated three composite variables from standardised measurements (*z*-scores) with 2008 and 2000-2001 recordings entered, which were based on the three principal components generated for the 2008 data set alone (see above). We did not use PCA directly because preliminary analysis showed that the 2000-2001 recordings did not fit the three components found for the 2008 recordings. We considered this discrepancy a result of the small sample size for 2000-2001 recordings given the accuracy of PCA increases with sample size. We found no *z*-score composite that differed significantly between recording periods in the lowland zone (ZC1 t = -1.29, p = 0.204; ZC2 t = -1.26, p = 0.239; ZC3 t = 0.929, p = 0.357; n = 53) or in the highland (ZC1 t = -0.34, p = 0.737; ZC2 t = -1.17, p = 0.269; ZC3 t = 0.78, p = 0.442; n = 50). We found a nonsignificant trend for a difference in song duration (ZC3 t = 2.11, p = 0.037, n = 101) and no difference in frequency (ZC1 t = 0.62, p = 0.537, n = 101) and trill (ZC2 t = 0.87, p = 0.385, n = 101).

Numerous "no responses" (e.g. # songs = 0) meant that three of our five response to playback variables did not fit a normal distribution (i.e., Kolmogorov-Smirnov test, $p < 0.05$). Standard methods of variable transformation did not resolve this issue. Log-linear modelling using a Poisson distribution was not an appropriate alternative because our data points were means and therefore included non-integers. Consequently, we used nonparametric analyses to examine response data.

Response data for all response variables were highly correlated (Spearman's *Rho* > 0.36-0.83); therefore, we used PCA (as above) to reduce our response variables into a single response component (PC4) that explained 69.1 % of the observed variance (Table 3). Prior to PCA, we transformed variables 3-5 using the formula: new variable = absolute value of the (old variable – greatest value); so that the direction of all responses was the same.

For each stimuli, we found no difference in PC4 between localities in the lowlands (lowland song Z = -1.81, p = 0.075, n = 20; highland song Z = -2.12, p = 0.035, n = 20; Cassin's finch Z = -1.36, p = 0.190, n = 20; silence Z = -0.49, p = 0.631, n = 20 [adjusted alpha = 0.0125]) and the highlands (lowland song Z = -1.31,

p = 0.211, n = 20; highland song Z = -0.42, p = 0.705, n = 20; Cassin's finch Z = -1.52, p = 0.143, n = 20; silence Z = -1.07, p = 0.315, n = 20 [adjusted alpha = 0.0125])

Between ecological zones, we found no clear distinction in test subject response (PC4) to local and foreign song (i.e., test subject's did not respond solely to local song; Table 4). However, we found a significant difference in PC4 among stimuli for test subjects in both the lowland zone (χ^2 = 22.8, df = 3, p < 0.001) and the highland (χ^2 = 27.6, df = 3, p < 0.001; Fig. 3). The response (PC4) of lowland males to lowland songs was significantly greater than to highland songs and control stimuli (Table 5; Fig. 3). In contrast, highland males showed no difference in response (PC4) to lowland and highland songs; but did show a significantly greater response (PC4) to either *G. fuliginosa* songs than to the controls (Table 5; Fig. 3). No single stimuli series elicited a greater or lesser response (PC4) than the others in either our lowland (Kruskal-Wallis: χ^2 = 6.19, df = 9, p = 0.720) and highland (χ^2 = 3.62, df = 9, p = 0.935) subjects.

DISCUSSION

We found a single significant difference in song between lowland-source and highland-colonist *G. fuliginosa*: in 2008, song duration was longer in the lowlands. Between localities within each zone we found no difference in song characteristics. Across sampling periods lowland songs were significant longer in 2008 than in 2000-2001; however, we found no difference in highland songs across sampling periods. In our playback experiment, lowland males gave a greater overall response to local song than to foreign song; whereas, highland males did not respond differently to local or foreign song. Nonetheless, between localities within each zone, neither lowland nor highland males responded differently to local or foreign song. Therefore, despite a significant difference in song duration in the lowlands between sampling periods, lowland males gave a greater response to lowland than highland song, suggesting the potential for a partial barrier to gene flow between zones. Together, these findings support the hypothesis that range expansion into a novel environment.

The difference in song duration between 2000-2001 and 2008 in lowland *G. fuliginosa* likely caused the difference in song duration between ecological zones

observed in 2008 when 2000-2001 and 2008 data were combined. Interestingly, 2000-2001 fell within a decade-long low rainfall *La Niña* period on Santa Cruz, which ended in the high rainfall *El Niño* year of 2008. During this *La Niña* period, selection intensity is expected to have increased in the lowlands; but not in the highlands where rainfall remained relatively high (see Chapter 3 [Fig. 1]). Therefore, the difference in song duration in lowland *G. fuliginosa* may be the result of increasing selection intensity over time, if longer songs provided directly or indirectly a fitness benefit to lowland *G. fuliginosa*. Changing demography of lowland *G. fuliginosa* across sampling periods may explain the differences in song duration observed. Unfortunately, we lack data from intervening years to explore this possibility further.

That said, if song divergence is driven by ecological differences we would predict the greatest difference in song between zones to occur at the end of the low rainfall period (i.e., in 2008) because then the compounded product of years of divergent selection would be greatest. In this way, adaptation to temporal changes within zones, such as an increase in interspecific competition for diminishing resources in the lowlands (*sensu* Grant and Grant 2010), may influence song variation in *G. fuliginosa* more than adaptation to more static ecological differences between zones, such as habitat structure, interspecific competition, and food types (the latter directly effecting beak morphology and thereby song characteristics). In support of this idea, *G. fortis* and *G. fuliginosa* are known to share song types (Ratcliffe 1981; Bowman 1983), distinguishable by mean values for song characteristics; and published song durations for lowland *G. fortis* (Podos 2007 [Table 1]) on Santa Cruz are shorter than song durations we report for *G. fuliginosa.* Therefore, longer songs in lowland *G. fuliginosa* may represent an adaptation to shorter songs in *G. fortis*, which is more abundant in the lowlands than the highlands and an aggressive competitor for signal space. However, on this point we only

Table 4: Response given by lowland and highland *Geospiza fuliginosa* to playback of four stimuli: (1) highland *G. fuliginosa*; (2) lowland *G. fuliginosa*; (3) Cassin's finch *Carpodacus cassini*; and (4) silence. The five responses measured were: (1) number of songs (# Songs); (2) number of flights (# flights); (3) minimum vertical distance (MVD); (4) minimum horizontal distance (MHD); and (5) latency to respond (Latency).

	Stimuli	*# songs*	*# flights*	*MVD* (m)	*MHD* (m)	*Latency* (s)
Lowland	*Highland*	1.67 ± 0.69	1.08 ± 0.91	3.63 ± 2.27	5.20 ± 2.07	65.35 ± 44.14
	Lowland	2.88 ± 1.04	1.52 ± 0.66	2.68 ± 1.79	3.59 ± 1.35	26.22 ± 24.47
	Cassin's	1.23 ± 0.80	0.28 ± 0.21	5.54 ± 1.46	7.20 ± 1.93	106.98 ± 14.96
	Silence	0.96 ± 0.65	0.23 ± 0.63	5.40 ± 2.76	6.89 ± 3.21	112.38 ± 17.83
Highland	*Highland*	2.87 ± 1.08	1.07 ± 0.91	3.89 ± 1.72	5.96 ± 2.07	44.37 ± 21.82
	Lowland	2.79 ± 0.71	1.47 ± 0.87	4.32 ± 1.49	4.98 ± 2.19	29.27 ± 18.39
	Cassin's	1.35 ± 1.02	0.36 ± 0.40	6.55 ± 1.88	7.99 ± 1.77	94.78 ± 23.02
	Silence	1.16 ± 1.04	0.00 ± 0.00	7.25 ± 1.85	8.81 ± 1.40	114.51 ± 10.69

Table 5: Pairwise differences (Mann-Whitney *Z* values) in overall response to playback stimuli given by lowland and highland *Geospiza fuliginosa*. Values above the diagonal are from experiments conducted in the highland and values below the diagonal are from experiments conducted in the lowland zone. Statistical significance levels are shown.

	Lowland	Highland	Cassin's	Silence
Lowland	-	-0.75	-3.55***	-3.78***
Highland	-2.19*	-	-3.33***	-3.78***
Cassin's	-3.78***	-2.87**	-	-1.29
Silence	-3.48***	-2.42*	-0.91	-

*0.05, **0.01, ***0.001

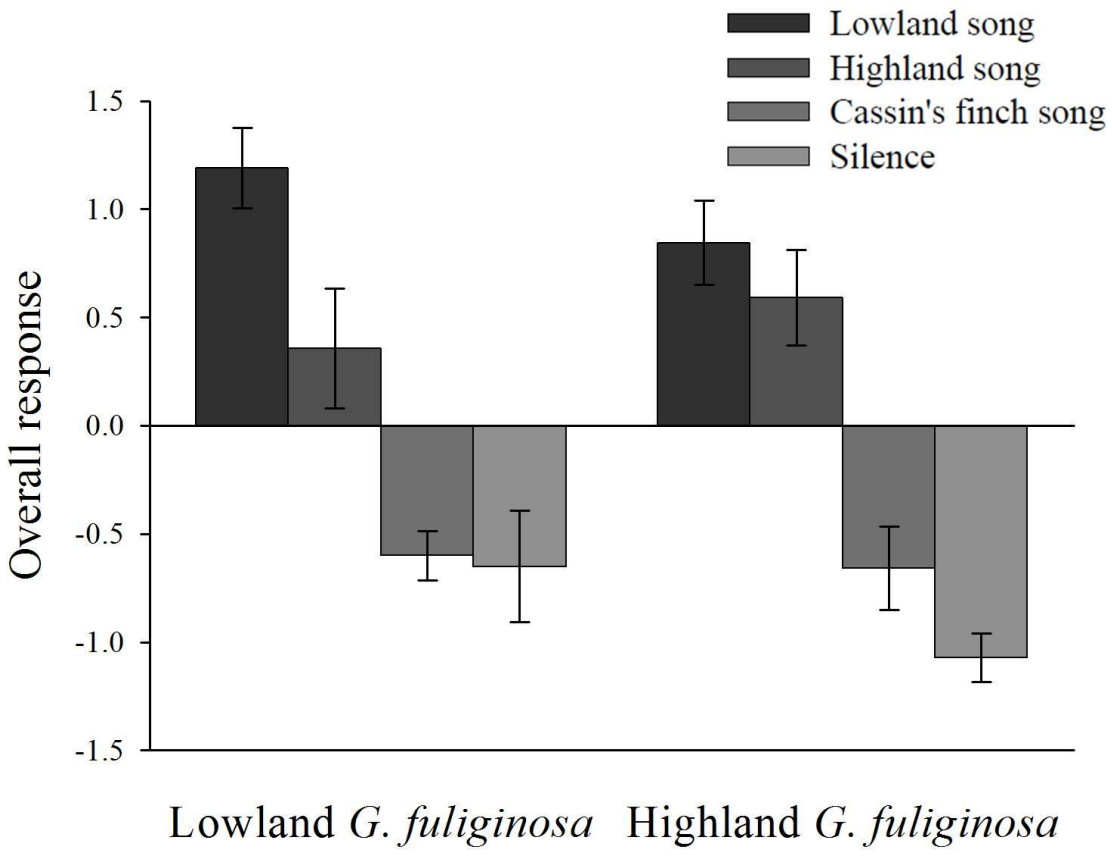


Figure 3: Difference in overall response (PC4; Mean ± SD) given by lowland zone and highland *Geospiza fuliginosa* to playback of four stimuli: (1) highland *G. fuliginosa*; (2) lowland zone *G. fuliginosa*; (3) Cassin's finch *Carpodacus cassini*; and (4) silence. Overall response was a composite derived from five quantitative measurements using principle component analysis.

speculate, as the examination of the mechanisms behind song variation in *G. fuliginosa* is beyond our present data set.

An animal's response to discriminatory experiments, such as song playback experiments, reflects two confounding processes: (1) its capacity to discriminate between different stimuli; and (2) its inclination to respond differently to different stimuli. As a result, no difference in response to local and foreign songs may indicate either a failure to discriminate or successful discrimination, but a failure to react differently. Consequently, we cannot say with certain that highland *G. fuliginosa* do not discriminate between local and foreign songs; hence, we concentrate the discussion of our playback study on the differential response detected in lowland *G. fuliginosa*. (It is less likely, although possible, that lowland males did not discriminate between local and foreign songs, but chose to respond significantly greater to lowland song than highland song; this would invoke errors in the experimental design).

Despite little evidence of song divergence between zones, lowland males discriminated between local and foreign song. Further, lowland males responded greater to local song despite a significant difference in song duration between playback tracks (2000-2001 recordings) and contemporary song (2008 recordings). Therefore, lowland males did not perceive the song of highland males as that of a potential rival. If all conspecific males are considered potential rivals, then lowland males may not have recognised highland song as that of a conspecific. Alternatively, if only conspecific males of similar adaptations or social status are considered potential rivals, then lowland males may recognise highland song as that of a conspecific, but one that is subordinate, dominant, or morphologically dissimilar, and therefore not a worthy rival. This latter point bears on the threat level hypothesis used to explain the nasty neighbour phenomenon where neighbouring conspecifics interact more aggressively than do strangers (Ferkin 1988; Temeles 1994; Müller and Manser 2007).

To expand, lowland males may not have recognised highland song as that of a conspecific because highland immigrants are rare in the lowlands. Darwin's finches may discriminate conspecific song based on songs heard and memorised (Podos 2007). This exemplar-based mechanism of song discrimination best explains how variation within zones can be greater than between zones in this study and others (see Fig. 2; Podos 2007). *Geospiza fuliginosa* is expected to disperse between zones

(Chapter 2 and 3). Differential movement of lowland and highland males between zones may thereby explain the discrepancy in song discrimination between zones. In turn, our present data may provide insight on the finer details of dispersal, which has proven difficult to obtain thus far (Chapter 2 and 3). If males in both zones gave a greater response to local than foreign song, dispersal between zones is probably negligible. Alternatively, if males in both zones did not discriminate between local and foreign song, dispersal between zones could be bidirectional. Our data suggests dispersal is unidirectional: from the lowlands to the highlands. Dispersal is expected (although not always) to improve an individual's habitat (reviewed in Chapter 3): movement from the "severe" pressures of the lowlands to the "benign" pressures of the highlands (i.e., differences in food supply and interspecific competition) is likely to achieve this (Chapter 4). Note, this explanation requires that highland males fail to discriminate local and foreign song, which, as discussed above, we cannot be certain of.

Alternatively, lowland males may not have recognised highland song as that of a morphologically similar or socially equivalent conspecific because an aspect of the song portrayed otherwise. As an alternative to exemplar-based discrimination, Darwin's finches may discriminate conspecific song based on specific aspects of the song (Ratcliffe and Grant 1985; Grant 1999). In 2000-2001, when the playback tracks were recorded, highland *G. fuliginosa* had longer beaks than lowland *G. fuliginosa*; and more often gleaned invertebrates from foliage (Kleindorfer *et al.* 2006). In the lowlands, invertebrates are less abundant than in the highlands (Tebbich *et al.* 2002); therefore, highland males are expected to be ill-adapted in the lowlands. Such ill-adapted individuals are likely to lose competitive intersexual interactions, possess low quality territories, and fail to attract high quality mates. Beak length is known to influence song production in some species of Darwin's finches (Podos 2001; Christensen *et al.* 2006; Huber and Podos 2006) and may do so in *G. fuliginosa* as well; thus, lowland males may detect subtle differences in highland song that are associated with ill-adapted beaks and respond less aggressively. Certainly in the lowlands, females choose males assortatively for beak length (Chapter 4); therefore lowland males are expected to give greater response to other males with similar beak morphology and presumably similar song. In the highlands, non-assortative pairing for beak morphology was observed (Chapter 4); which may explain why highland males give equal responses to lowland and

highland songs. Similar, lowland males may detect subtle differences in highland song that are considered socially unequivocal (i.e., either subordinate or dominant); however, such cues must be zone-specific for highland males to respond equally to lowland and highland song.

An important distinction between the two possibilities outlined above is the functional significance of each. If lowland males recognise highland song, then lowland females are expected to also; similarly, if lowland males do not recognise highland song, then lowland females are not expect to either (Searcy 1988; Ratcliffe and Otter 1996). Thus, each possibility has different implications for gene flow: only if highland song is not recognised in the lowlands will gene flow between ecological zones be restricted. Even so, this barrier is unidirectional (highland females are likely to recognise lowland song, because highland males can). A bidirectional barrier is required for reproductive isolation. Whether such a barrier could initiate among *G. fuliginosa* on Santa Cruz, given time, remains to be seen. Complete reproductive isolation between lowland and highland *G. fuliginosa* is not likely (Coyne and Price 2000); however, within-island song divergence may facilitate reproduction isolation among populations inhabiting other islands following differential dispersal of lowland and highland individuals (Kleindorfer *et al.* 2006).

Our study is not the first to show song discrimination within an island population of Darwin's finch (Podos 2007, 2010), nor is it the first to show song divergence along an elevation gradient (Bowman 1979; Ratcliffe 1981). However, it is the first, to show (partial) song discrimination between subpopulations inhabiting different ecological zones within an island. Further, we have shown no difference in song discrimination between localities within ecological zones, which are separated by similar geographic distances as localities between ecological zones. Therefore, our study provides an important insight: range expansion into a novel environment can initiate barriers to gene flow in birds even across very short geographical distances. Our study also adds to growing evidence that substantial adaptive divergence can arise within islands (Kleindorfer *et al.* 2006; Ryan *et al.* 2007; de Leon *et al.* 2010; Mila *et al.* 2010).

ACKNOWLEDGEMENTS

We thank the following: the Galápagos National Park and the Charles Darwin Research Station for the opportunity to conduct this research; Max Planck Institute for Ornithology, American Bird Conservancy, Conservation International, Galápagos Conservation Fund, and Flinders University for their generous financial support; TAME airlines for assistance with reduced airfare to and from the archipelago; Zonnetje Auburn, Noni Allen, and Mari Cruz for their assistance in the field; and Jeremy Robertson, Rebekah Christensen, Angela McGuire, and Jody O'Connor for their assistance in the lab. This research was conducted under the ethics approval granted by Flinders University (E189).

REFERENCES

Baptista, L. F. 1975. Song dialects and demes in sedentary populations of the white-crowned sparrow. *University of California Publications in Zoology* **105**:1-52.

Barker, M. C. 1983. The behavioural response of female Nuttall's white crowed sparrows to male song of natal and alien dialects. *Behavioural Ecology and Sociobiology* **12**: 309-315.

Bitterbaum, E., and Baptista, L. F. 1979. Geographical variation in songs of California house finch (*Carpodacus mexicanus*). *Auk* **96**: 462-474.

Bowman, R. I. 1961. Morphological differentiation and adaptation in the Galapagos finches. *University of California Publications in Zoology* **58**: 1-302.

Bowman, R. I. 1979. Adaptive morphology of song dialects in Darwin's finches. *Journal fu¨r Ornithologie* **120**: 353-389.

Bowman, R. I. 1983. The evolution of song in Darwin's finches. In: Patterns of Evolution in Galapagos Organisms. Bowman, R. I., Berson, M. and Leviton, A. E. (eds). American Association for the Advancement of Science, Pacific Division: San Francisco, CA

Catchpole, C. K. and Slater, P. J. B. 1995. *Bird Song: Biological Themes and Variation*. Cambridge University Press: Cambridge, U. K.

Christensen, R., Kleindorfer, S. and Robertson, J. 2006. Song is a reliable signal of beak morphology in Darwin's small tree finch *Camarhynchus parvulus*,

and vocal performance predicts male pairing success. *J. Avian Biol.*, **6**: 617-624.

Coyne, J. A. and Orr, H. A. 2004. *Speciation*. Sinauer Associates: Sunderland, M. A.

Coyne, J. A. and Price, T. D. 2000. Little evidence for sympatric speciation in island birds. *Evolution* **54**: 2166-2171.

Curio, E. 1969. Funktionsweise und Stammesgeschichte des Flugfeinderkennens einiger Darwinfinken (Geospizinae). *Z. Tierpsych.* **26**: 394-487.

de Leon, L. F., Bermingham, E., Podos, J. and Hendry, A. P. 2010. Divergence with gene flow as facilitated by ecological differences: within-island variation in Darwin's finches. *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**: 1041-1052.

Dingle, C., Polestra, J. W., Halfwerk, W., Brinkhuizen, D. M. and Slabbekoorn, H. 2010. Asymmetric response patterns to subspecies-specific song differences in allopatry and paraptry in the grey-brested wood-wren. *Evolution* **64**: 3537-3548.

Dobzhansky, T. 1937. *Genetics and the Origin of Species*. Columbia University Press: New York, N. Y.

Ferkin, M. H. 1988. The effect of familiarity on social interactions in medow voles, *Microtus pennsylvanicus*: a laboratory and field study. *Anim. Behav.* **36**: 1816-1822.

Grant, B. R. and Grant, P. R. 2002a. Lack of premating isolation at the base of a phylogenetic tree. *American Naturalist* **160**: 1-19.

Grant, B. R. and Grant, P. R. 2002b. Simulating secondary contact in allopatric speciation: an empirical test of premating isolation. *Biological Journal of the Linnean Society* **76**: 545-556.

Grant, B. R. and Grant, P. R. 2010. Songs of Darwin's finches diverge when a new species enters the community. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 20156-20163.

Grant, P. R. 1999. *Ecology and Evolution of Darwin's Finches*. Princeton University Press: Princeton, N. J.

Grant, P. R. and Grant, B. R. 2008. *How and Why Species Multiple: the Radiation of Darwin's Finches* Princeton University Press: Princeton, N. J.

Huber, S. K. and Podos, J. 2006. Beak morphology and song features covary in a population of Darwin's finches (Geospiza fortis). *Biological Journal of the Linnean Society* **88**: 489-498.

Irwin, D. E. 2000. Song variation in an avian ring species. *Evolution* **54**: 998-1010.

Kleindorfer, S. 2007. The ecology of clutch size variation in Darwin's Small Ground Finch Geospiza fuliginosa: comparison between lowland and highland habitats. *Ibis* **149**: 730-741.

Kleindorfer, S., Chapman, T. W., Winkler, H. and Sulloway, F. J. 2006. Adaptive divergence in contiguous populations of Darwin's Small Ground Finch (Geospiza fuliginosa). *Evolutionary Ecology Research* **8**: 357-372.

Lack, D. 1947. *Darwin's Finches*. Cambridge University Press: Cambridge, U. K.

Martens, J. 1975. Akustische Differenzierung verwaldtschaftlicher Beziehungen in der*Parus Periparus* - Gruppe nach Untersuchungen im Nepal-Himalaya. *Journal fur Ornithologie* **116**: 369-433.

Mayr, E. 1942. *Systematics and the Origin of Species*. Columbia University Press: New York, N. Y.

Mayr, E. 1947. Ecological factors in speciation. *Evolution* 1: 263-288.

Mayr, E. 1963. *Animal Species and Evolution* Belknap Press: Cambridge, M. A.

Mila, B., Warren, B., Heeb, P. and Thebaud, C. 2010. The geographic scale of diversification on islands: genetic and morphological divergence at a very small spatial scale in the Mascarene grey white-eye (Aves: Zosterops borbonicus). *Bmc Evolutionary Biology* **10**: 158-171.

Müller, C. A., Manser, M. B. 2007. 'Nasty neighbours' rather than 'dear enemies' in a social carnivore. *Proc. Roy. Soc. Lond. B* **274**:959-965.

Nelson, D. A. 1998. Geographical variation in the song of Gambel's white crowed sparrow. *Behaviour* **135**: 321-342.

Nosil, P., Vines, T. H. and Funk, D. J. 2005. Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* **59**: 705-719.

Petrinovich, L. and Patternson, T. L. 1981. The response of white crowned sparrows. *Zietschrift fur Tierpsychologie* **57**: 1-14.

Podos, J. 1997. A performance constraint on the evolution of trilled vocalisations in a songbird family (Passeriformes: Emberizidae). *Evolution* **51**: 537-551.

Podos, J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* **409**: 185-188.

Podos, J. 2007. Discrimination of geographical song variants by Darwin's finches. *Animal Behaviour* **73:** 833-844.

Podos, J. 2010. Acoustic discrimination of sympatric morphs in Darwin's finches: a behavioural mechanism for assortative mating? *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**: 1031-1039.

Price, T. 2008. *Speciation in Birds*. Roberts and Company: Greenwood Village, C.O.

Ratcliffe, L. M. 1981. Species recognition in Darwin's ground finches (Geospiza Gould). Ph.D. thesis, McGill University.

Ratcliffe, L. M. and Grant, P. R. 1985. Species recognition in Darwin's finches (Geospiza Gould). III. Male responses to playback of different song types, dialects, and heterospecific songs. *Animal Behaviour* **33**: 290-307.

Ratcliffe, L. M. and Otter, K. 1996. Sex differences in song recognition. In: Ecology and evolution of acoustic communication in birds (Eds: Kroodsma, D. E. and Miller, E. H.) Cornell University Press: Ithaca, N. Y.

Ryan, P. G., Bloomer, P., Moloney, C. L., Grant, T. J. and Delport, W. 2007. Ecological speciation in South Atlantic island finches. *Science* **315**: 1420-1423.

Searcy, W. A. and Brenowitz, E. A. 1988. Sexual differences in species recognition of avian song. *Nature* **332**: 152-154.

Slabbekoorn, H. and Smith, T. B. 2002. Bird song, ecology and speciation. *Philosophical Transactions of the Royal Society B-Biological Sciences* **357**: 493-503.

Schluter, D. 2000. *The Ecology of Adaptive radiations*. Oxford University Press: Oxford, U. K.

Schluter, D. and Grant, P. R. 1982. The distribution of *Geospiza difficilis* in relation to *Geospiza fuliginosa* in the Galapagos islands - test of three hypotheses *Evolution* **36**: 1213-1226.

SPSS Inc., a. I. C. 2009. *PAWS 18.0*. Chicago, IL.

Sulloway, F. J. 1982. The Beagle collections of Darwin's finches (Geospizinae). *Bulletin of the British Museum of Natural History (Zoology)* **43**: 2246-2253.

Tebbich, S., Taborsky, M., Fessl, B. and Dvorak, M. 2002. The ecology of tool-use in the woodpecker finch (*Cactospiza pallida*). *Ecol. Letters*, **5**: 656-664.

Temeles, E. J. 1994. The role of neighbours in territorial systems: when are they 'dear enemies'? *Anim. Behav.* **47**:339-350.

Thielcke, G. and Wustenberg, K. 1985. Experiments on the origins of dialects in the short-toed tree-creeper (*Certhia brachydactyla*). *Behavioural Ecology and Sociobiology* **16**: 195-201.

CHAPTER SIX

Naris and beak malformation caused by the parasitic fly *Philornis downsi* in Darwin's small ground finch, *Geospiza fuliginosa*

Toby H. Galligan and Sonia Kleindorfer

Biological Journal of the Linnean Society (2009) 98: 577-585

ABSTRACT

Parasites induce phenotypic modifications in their hosts, which can compromise host fitness. For example, the parasitic fly *Philornis downsi*, which was recently introduced to the Galápagos Islands, causes severe naris and beak malformation in Darwin's finches. The fly larvae feed on tissues from the nares of developing finch nestlings, thereby permanently altering the size and shape of the nares and beak. While the parasitism is age-specific (adult finches are not parasitised), naris and beak malformations persist into adulthood as parasite-induced morbidity. We systematically examined adult populations of Darwin's small ground finch, *Geospiza fuliginosa,* on the island of Santa Cruz for *P.downsi*-induced malformation. We found that malformed birds had significantly longer nares, and shorter, shallower beaks than birds considered to be normal (that is, with no nares or beak malformation). In addition, normal birds showed an isometry between naris length and beak dimensions (beak length feather and beak depth), which was not found in malformed birds. These differences suggest that beak morphology was influenced by *P. downsi* parasitism. Interestingly, we did not find any evidence of developmental impairment (smaller body size) or reduced foraging efficiency (lower body condition) between normal and malformed birds. Our findings of *P. downsi*-induced malformation raise new questions about the evolutionary trajectory and conservation status for this group of birds.

Keywords: invasive parasite – age-specific parasitism – parasite-induced – deformation – phenotypic variation – developmental instability – phenodeviants – bill length

INTRODUCTION

Parasites reduce host fitness through the acquisition of resources (Price, 1980; Loye & Zuk, 1991; Clayton & Moore, 1997). Long term observational and experimental studies have shown the effects on host longevity and fecundity due to permanent parasitism (for example: Hudson, Newborn & Dobson, 1992; Hudson, Dobson & Newborn, 1998) and temporary parasitism (for example: Møller, 1990; Richner, Christe & Oppliger, 1995; Brown, Brown & Rannala, 1995). These fitness estimates have been largely derived from examples of recurrent parasitism. In contrast, age-specific parasitism, where the host-parasite interaction is confined to a stage of the host's life-cycle (for example during immaturity), has rarely been analysed in terms of future host fitness. Yet age-specific parasitism is important for many species, especially species that produce nidicolous young that are often hosts for nest-based ectoparasites (Marshall, 1981). It is possible that future fitness costs of age-specific parasitism are under-reported in the literature because the finite duration of the interaction erroneously implies a finite duration of total host fitness costs. Based on this misunderstanding, combined with a lack of data on age-specific parasitism, researchers may preferentially report on direct parasite-induced mortality and neglect the fitness costs for hosts that survive age-specific parasitism (Hudson and Dobson, 1997). Failure to consider post-parasitism fitness costs of age-specific parasitism, therefore, can have serious conservation implications for many species.

Survivors of age-specific parasitism often exhibit phenotypic modifications that are the pathological consequence of past host-parasite interactions (Poulin & Thomas, 1999; Møller, 2006). For mature hosts, phenotypic modifications arise solely from parasite resource acquisition. However, for immature hosts, tissue damage (that is, deformation) can be combined with parasite-induced developmental instability (that is, malformation) to generate phenotypic modifications (Møller, 2006). Notably, while deformation can vary in persistence from short-term to permanent, malformation is almost always permanent. Further, parasite-induced malformation has the potential to completely compromise host fitness as it occurs before the first reproductive event in the host (Møller, 1997; Møller & Swaddle, 1997). For these reasons, phenotypic modification is particularly important for age-specific parasitism where the host is immature. Hereafter, we will focus on parasite-induced malformation.

Parasite-induced malformation is expected to impair an individual's overall development, increasing its vulnerability to competitors, predators, and parasites and thereby decreasing its longevity and reproductive output (Møller, 1997). A number of studies have shown that malformation caused by parasites can alter host development; but of these, only a few have shown reduced fitness in as a consequence (Møller, 1992, 1996; Polak, 1993; Potti, 2008). For example, a recent study by Potti (2008) showed delayed effects of nestling parasitism (that is, post-parasitism): specifically, female pied flycatchers, *Ficedula hypoleuca*, that were parasitised as nestlings by the blowfly *Protocalliphora azurea* had consistently smaller egg size as adults.

The nestlings of Darwin's finches (Passeriformes: Emberizidae; Gould) on the Galápagos Islands are novel hosts to the parasitic larvae of an introduced fly, *Philornis downsi* (Diptera: Muscidae; Dodge and Aitken). Adult finches are not parasitised; therefore, the interaction is age-specific. *P. downsi* larvae reside by day in the base of finch nests and feed by night on the blood and tissues of the nidiculous young. The preferred feeding site for the first larval instar is the nestling's nares, a behaviour that can cause malformation of the surrounding tissue and keratin, and result in permanent enlargement of the nares (Fessl, Sinclair & Kleindorfer, 2006a) and a shape-change in naris from teardrop-shaped to circular. Second and third larval instars can further increase malformation by using the nares to access internal feeding sites, which causes repeated ulceration and bone-reabsorption (O'Connor, Robertson & Kleindorfer, in review). In addition, malformation of the beak can also occur. Grooves, cracks, and gouges in the beak keratin radiating from the nares are obvious evidence of *P. downsi*-induced beak malformation, but an overall reduction in beak size due to developmental instability is also expected. Accordingly, naris and beak malformation observed in adult Darwin finches are considered symptoms of past *P. downsi* parasitism.

Philornis downsi is identified as the most significant threat to Darwin's finches (Causton *et al.*, 2006). Originating from the northern Neotropics (reviewed in Dudaniec & Kleindorfer, 2006), *P. downsi* larvae were discovered in the nests of Galápagos birds in 1997 (Fessl, Couri & Tebbich, 2001) although adult specimens were collected from the islands in 1964 (see Causton *et al.*, 2006). Presently, *P. downsi* is known to affect 9 of the 13 species of Darwin's finch (Fessl *et al.*, 2001; Fessl & Tebbich, 2002; O'Connor *et al.*, unpublished data; B. Fessl pers. com) on 12

of the 18 major islands of the Galápagos (Wiedenfeld *et al.*, 2007; pers. comm. Peter Grant). Previous studies have shown a complete bombardment of *P. downsi* parasitism on Darwin's finches: 100% parasite prevalence in nests (Dudaniec, Fessl & Kleindorfer, 2007); up to 6 infestation events per nest (Dudaniec *et al.*, 2009); up to 64 larvae per nestling (Fessl & Tebbich, 2002); and 95% nestling mortality due to parasitism in some years (Fessl *et al.*, 2006a). To date there is no data on parasite-induced malformation or associated fitness consequences among surviving fledglings and adult finches.

In this paper, we examine the extent and consequences of malformation caused by *P. downsi* in an adult population of Darwin's small ground finch (*Geospiza fuliginosa*). We predict that malformed birds will have larger nares (specifically, longer naris length), and smaller beak size (specifically, smaller overall size, shorter beak length, and shallower beak depth) than birds considered normal with no obvious malformation. We also predict that malformed birds will have smaller body size and lower body condition than their normal counterparts due to developmental impairment and its effect on foraging efficiency and competitiveness.

METHODS

Study site and species

This study was conducted between January and July 2008. All data were collected from the central Galápagos island of Santa Cruz (986 km^2; 0°37′S, 90°21′W).

Elevated islands of the Galápagos archipelago can be divided into three main ecological zones based on the annual level of precipitation each receives: arid lowlands (mean annual rainfall from 1999-2008 = 288 mm), transitional midlands, and humid highlands (mean annual rainfall from 1999-2008 = 1035 mm). Prevailing southern winds brings more precipitation to lower altitudes on the southern side of the island in comparison to the northern side; as a result the midland and highland zones extend to lower altitudes on the southern slope (that is midlands begin ~100 m and ~600 m above sea level on the southern and northern side, respectively).

We sampled individuals along 3 transects (~15 km) that ran from the lowlands through the midlands to the highlands of Santa Cruz. Transect 1 and 2 were

located on the southern side: T1, *Bahia Academy* (0°44′S, 90°18′W) – *Los Gemelos* (0°37′S, 90°20′W); T2, *El Garrapatero* (0°41′S, 90°13′W) – *Cerro Crocker* (0°38′S, 90°19′W); and Transect 3 was located on the northern side: *Mina Cerro Rojo* (0°37′S, 90°22′W) – *Itabaca Canal* (0°30′S, 90°18′W). We sampled a total of 21 sites: 10 sites in the lowlands; 7 sites in the midlands; and 4 sites in the highlands. Sites were grouped into altitude categories for later analysis on a scale of 1-8, with elevation intervals of 100 m.

Arid lowlands were categorised by dry-deciduous open forest dominated by *Bursera graveolens* (Jackson, 1993; McMullen, 1999). Humid highlands consisted of remnant evergreen *Scalesia* closed forest, *Miconia* shrubland, and fern-sedge pampa (Jackson, 1993; McMullen, 1999). The transitional midlands have been largely modified for agriculture, with the introduction of a variety of exotic trees, shrubs and grasses; however, stands of endemic transitional open forest co-dominated by *Psidium galapageium*, *Pisonia floribunda*, and *Piscidia cathagenensis* persisted in the midlands (Jackson, 1993; McMullen, 1999).

Data collection

Birds were sampled randomly using mist-nets. Only adult birds were processed; we distinguished juveniles based on their prominent yellow gape. We inspected birds for signs of naris and beak malformation caused by *P. downsi*. We categorised individuals as either: normal (no obvious naris or beak malformation; Fig. 1A), malformed (obvious naris or beak malformation; Fig. 1B, C, D, E), or aberrant (presumably genetically caused naris malformation; Fig. 1F). Individuals were considered to have malformed if at least one of the following conditions was met for one naris or both nares: (1) enlarged in size (deep and/or wide); (2) circular in shape; (3) asymmetrical in size or shape; and/or (4) without a septum. In addition, individuals with a malformed beak (grooves, cracks, and gouges in the beak keratin radiating from the naris; see Fig. 1B, C, D) were considered malformed. Most birds considered malformed exhibited three or more of the above criteria. Birds considered normal or aberrant did not exhibit any of the above criteria. Aberrant birds differed from normal birds by the absence or near absence of a naris or both nares.

Despite grossly enlarged nares in some malformed birds, assessment of nares malformation was performed qualitatively as considerable overlap can exist in naris

Figure 1: Variation in nares formation in Darwin's small ground finch, *Geospiza fuliginosa*; a) normal naris and beak; b) enlarged and circular naris with extensive beak malformation (gouges and cracks); c) absence of septum between naris with slight beak malformation (grooves radiating from naris); d) exceptionally enlarged, circular naris with beak malformation (gouges); e) asymmetrical nares as a result of naris malformation; and f) absence of naris (considered non-*Philornis downsi*-induced malformation). These individuals would be categorised as: a) normal; b-e) malformed; and f) aberrant.

length among malformed and normal birds. We did not assume that any one type of disfigurement or combination of disfigurements was more detrimental than another, which was supported by preliminary analysis. Therefore, we grouped all malformed birds together for analyses.

To examine the effect of *P. downsi* parasitism on naris and beak size, we measured naris length and four beak dimensions (mm): beak length feather (length of the culmen); beak length naris (length of the culmen to the anterior edge of the naris); beak depth (at the feather line); and beak width (at the feather line). To examine the possible correlation between body size and naris length, we measured two body size parameters (mm): tarsus length (length of the tarsometatarsus) and wing length (carpal joint to tip of seventh primary). All measurements were taken on the birds' right side using dial callipers to an accuracy of 0.1 mm. We also recorded mass (g), sex, and extent of black plumage in males on a scale of 0-4 as approximation for age (see Grant & Grant, 1989).

Beak length feather, beak length naris, beak depth, and beak width were all positively correlated ($r > 0.3$); as was tarsus and wing length ($r > 0.4$). To avoid multicollinearity in regression analyses, we calculated two principle components using a varimax rotation method with Kaiser normalisation: PC1 (beak size) and PC2 (body size). Together these components explained 54.5 % of the total variance. The strength and direction of the factor loadings for each of the principle component are shown in Table 1.

To assess the effect of malformation on individual survival, we calculated body condition as the residual scores of a least squares linear regression of mass versus the derived body size variable PC2.

Data analysis

All statistical analyses were performed using SPSS version 16 for Windows. The total data set was used to calculate naris shape categories, but aberrant individuals were removed before further analysis.

First, we examined the effect of covariates on naris formation using likelihood ratio and chi-squared analysis. Second, we examined whether naris formation could be predicted by naris length, beak size, body size, or body condition using logistic regression analysis. We calculated the odds ratios (OR) and 95 % confidence interval for the OR, to provide an effect size for the association between malformed and

Table 1: Principal component analysis factor loadings (PC1 and PC2) calculated using a varimax rotation method with Kaiser normalisation.

Variable	PC1	PC2
Beak length feather	0.91	
Beak length naris	0.68	
Beak depth	0.50	
Beak width	0.59	
Tarsus length		0.49
Wing length		0.99

Note: Only loadings above 0.40 are shown.

normal birds. Differences in morphology (ANOVA) and associations between naris length and beak morphology (linear regression analysis) were also tested.

We did not control for body size in the above analyses of nares size and body condition because partial correlation analysis of naris length and beak morphology controlling for tarsus length and wing length found negligible differences in comparison to the zero order correlation ($r < 0.03$).

RESULTS

We collected data from a total of 623 individuals: 65.8% (410/623) were categorised as normal, 36.3% (226/623) as malformed, and 0.3% (2/623) as aberrant.

The number of cases of malformation did not differ across sites ($\chi^2 = 24.45$, df = 20, $p = 0.223$; $n = 621$), altitude categories ($\chi^2 = 11.33$, df = 7, $p = 0.130$; $n = 621$), or ecological zones ($\chi^2 = 4.03$, df = 3, $p = 0.258$; $n = 621$). We also found no significant difference in the frequency of malformation between the southern and northern sides of the island (Fisher's exact test, $p = 0.912$; $n = 621$), the sexes (Fisher's exact test, $p = 0.930$; $n = 621$), or across male age categories ($\chi^2 = 4.08$, df = 4, $p = 0.395$; $n = 406$). In addition, we found no difference in beak length naris

($F_{3, 225}$ = 0.325, df = 3, p = 0.808) nor beak size ($F_{3, 223}$ = 0.183, df = 3, p = 0.908) for malformed finches across ecological zones.

Our logistic regression model correctly classified 72.8 % of individuals as either malformed or normal (Hosmer and Lemeshow Test: χ^2 = 15.58, df = 8, p = 0.49; n = 592). Naris length (Wald statistic = 52.78, β = 4.03, p < 0.001) and beak size (PC1; Wald statistic = 18.35, β = -0.54, p < 0.001) contributed significantly to the overall model. Neither body size (PC2; p = 0.096) nor body condition (p = 0.314) predicted naris formation. A malformed bird was more likely to have a greater naris length (OR = 56.66, CI = 19.07-168.37) and a smaller overall beak size (OR = 0.58, CI = 0.46-0.75) than a normal bird (see Table 2). A significantly shorter beak length naris and beak length feather, as well as smaller beak depth, contributed significantly to a smaller beak size in malformed birds (Table 2).

Naris length for birds with normal nares was positively correlated with beak length feather (r = 0.20, $F_{1, 396}$ = 16.55, p < 0.001; Fig. 2a) and beak width (r = 0.23, $F_{1, 393}$ = 21.00, p < 0.001), and to a lesser extent, beak depth (r = 0.10, $F_{1, 394}$ = 3.87, p = 0.05). We found no association between naris length and beak length naris for normal birds (r = 0.03, $F_{1, 395}$ = 0.45, p = 0.502; Fig. 3a). In contrast, naris length for malformed birds showed a large and significant negative correlation with beak length naris (r = -0.32, $F_{1, 223}$ = 25.40, p < 0.001; Fig. 3b), and, to a lesser extent, a significant positive correlation with beak width (r = 0.16, $F_{1, 223}$ = 5.48, p = 0.020). We found no association between naris length and either beak length feather (r = 0.08, $F_{1, 223}$ = 1.59, p = 0.208; Fig. 2b) or beak depth for malformed birds (r = 0.09, $F_{1, 223}$ = 1.82, p = 0.179). Comparing correlation coefficients between malformed and normal birds, we found a significant difference for naris length and beak length naris only: naris length explained more variance in beak length naris for malformed birds than normal birds (z_{obs} = -3.59).

DISCUSSION

Here, we provide quantitative evidence for naris and beak malformation in adult *G. fuliginosa* as a result of *P. downsi* parasitism. As predicted, malformed birds had

Table 2: Beak length measurements (shown as means ± standard deviation) and results of an ANOVA comparison between beak category (normal, malformed) for Darwin's small ground finch, *Geospiza fuliginosa*, on Santa Cruz, Galápagos Islands.

Variable (mm)	Category	*n*	Mean ± SD	*F*	*P*
Naris Length	Normal	396	1.78 ± 0.16	69.79	<0.001
	Malformed	224	1.92 ± 0.26		
Beak Length Naris	Normal	400	8.39 ± 0.42	50.21	<0.001
	Malformed	226	8.13 ± 0.46		
Beak Length Feather	Normal	401	12.87 ± 0.65	5.47	0.020
	Malformed	226	12.75 ± 0.64		
Beak Depth	Normal	398	7.60 ± 0.35	9.59	0.002
	Malformed	226	7.51 ± 0.31		
Beak Width	Normal	397	6.69 ± 0.38	3.09	0.079
	Malformed	226	6.63 ± 0.36		

greater naris lengths, shorter beak lengths (beak length feathers and beak length naris), and shallower beak depths than birds categorised as normal. Naris length was positively correlated to beak length feather in normal birds; whereas naris length was negatively correlated to beak length naris in malformed birds. Interestingly, malformation had no measurable consequence for adult body size or body condition. Our study also confirmed the wide and apparently comparable distribution of *P. downsi* across ecological zones on Santa Cruz (as described in Dudaniec *et al.*, 2007) and revealed a lack of sex or age bias among malformed individuals affected by *P. downsi* as nestlings.

In sum, our results suggest *P. downsi* parasitism in Darwin's finches: (1) is widespread, well established, and indiscriminate for host sex; (2) has led to measurable naris malformation in adult birds; (3) which is associated with smaller beak dimensions (due to beak malformation); (4) but does not have apparent effects for overall growth and adult body condition (fitness costs).

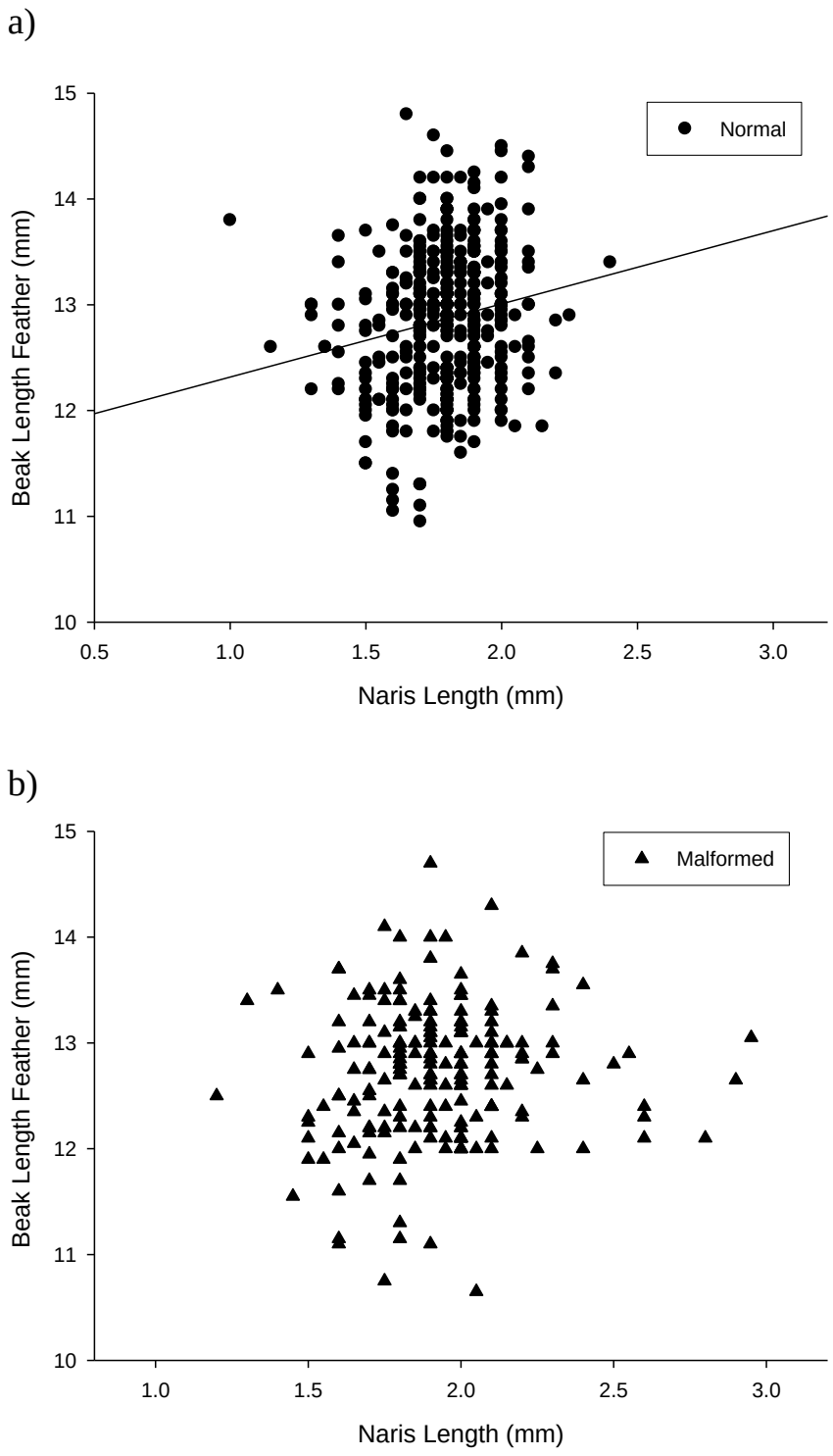


Figure 2: Associations between naris length and beak length feather in Darwin's small ground finch, *Geospiza fuliginosa*, on Santa Cruz, Galápagos Islands: a) the significant positive relationship between naris length and beak length feather in normal birds (r = 0.20, p = 0.010); and b) the nonsignificant relationship between naris length and beak length feather in malformed birds.

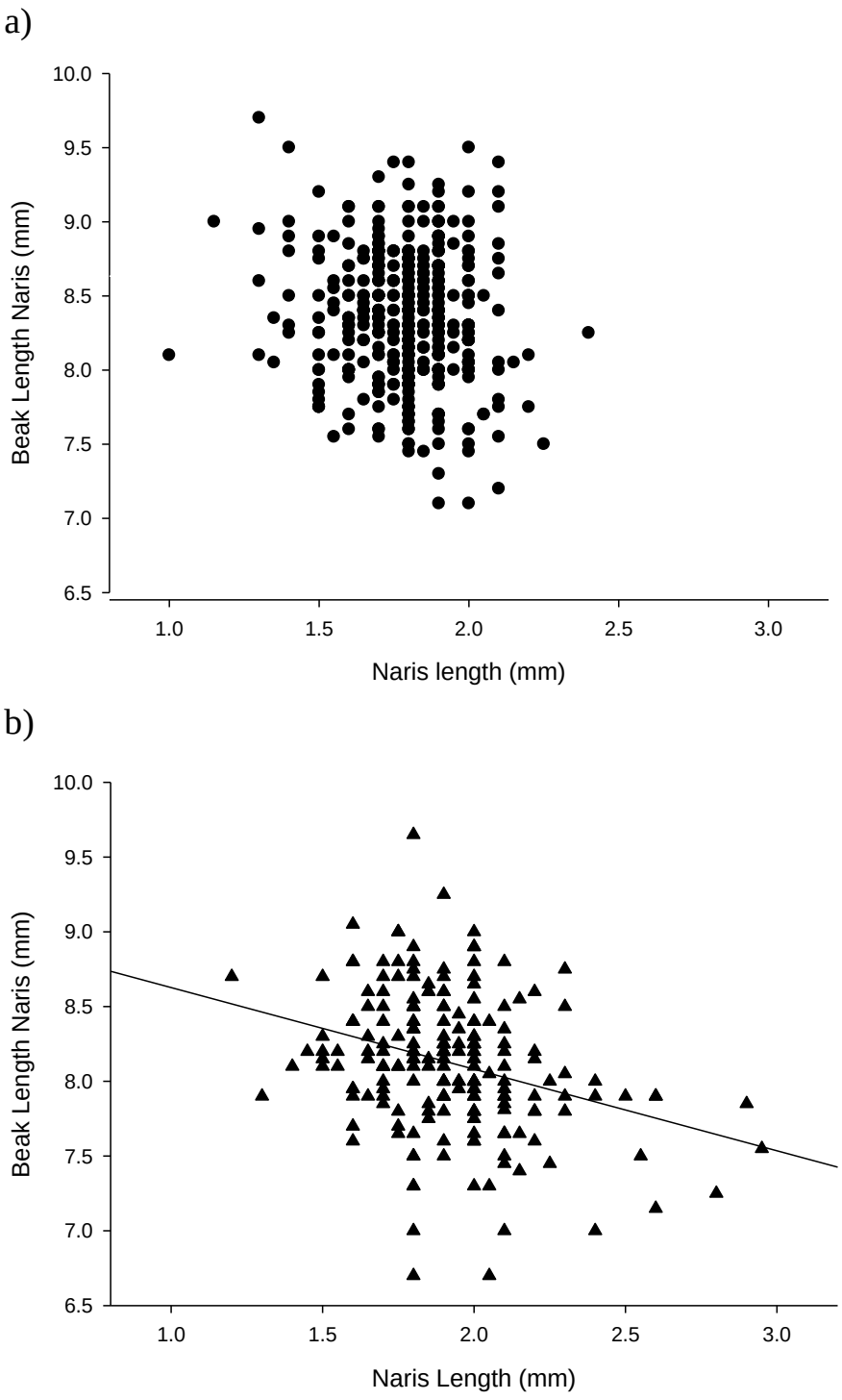


Figure 3: Associations between naris length and beak length feather in Darwin's small ground finch, *Geospiza fuliginosa*, on Santa Cruz, Galápagos Islands: a) the nonsignificant relationship between naris length and beak length naris in normal birds; and b) the significant negative relationship between naris length and beak length naris in malformed birds (r = -0.32, $p < 0.001$).

We found no difference in the number of malformed birds in relation to normal birds across 21 sites of varying vegetation, altitude, and latitude on Santa Cruz. This result concurs with previous studies examining prevalence of *P. downsi* in Darwin's finches (Fessl & Tebbich, 2002; Dudaniec, Fessl & Kleindorfer, 2006; Dudaniec *et al.*, 2007). Currently, we have little knowledge of finch subpopulation movement within and between large islands of the Galápagos Archipelago (but see Chapter 3). However, it is unlikely that all malformed birds that we sampled originated from one or a few locations and dispersed widely across the island. In fact, during our long-term monitoring of Darwin's finch populations on Santa Cruz since 2000 we have never recovered colour banded birds in sites other than the site of banding (see Kleindorfer *et al.*, 2006). The lack of sex and age bias in malformation corresponds with the high prevalence of *P. downsi* parasitism both at the time of its discovery in Darwin's finches in 1997 (Fessl *et al.*, 2001) and since then (Fessl, Kleindorfer & Tebbich, 2006a; Fessl *et al.*, 2006b; Dudaniec & Kleindorfer, 2006; Dudaniec *et al.*, 2007; Huber, 2008; Kleindorfer & Dudaniec, 2009). Further, there is no evidence to suggest differences in parasite vulnerability among male and female nestlings at present. Note: the patterns we report here do not include finches that perish due to *Philornis*-induced parasitism. The positive relationship between naris length and beak length feather in normal birds provides evidence of isometry in these traits. In contrast, the negative correlation between naris length and beak length naris in malformed birds suggests a loss of this isometry as a result of malformation.

We acknowledge that naris length and beak length naris are not independent measurements, and a negative relationship between the two was possible. Thus, we addressed this problem by examining beak length feather in addition to beak length naris. Beak length naris and beak length feather are also dependent measurements, but naris length and beak length feather are not. Because we found beak length feather was also shorter in malformed birds, we conclude that a decrease in total beak length in malformed birds was not caused by the position or length of the naris (as could be expected for effects on beak length naris alone), but was rather explained by malformation of the beak. Further, the correlation between naris length and beak length feather in malformed birds showed no association. These findings provide evidence of a loss of the isometry that exists between beak length naris and beak length feathers in normal birds and support the idea that beak malformation has led to shorter beak length.

Our finding of significant beak malformation is further supported by the only other study that has measured differences in beak dimension between parasitised and nonparasitised birds (Huber 2008). Using an experimental approach in Darwin's medium ground finch, *Geospiza fortis*, Huber (2008) showed that nestlings in nests without *P. downsi* larvae had greater beak depths than nestlings in infested nests. Nestling beak length (equivalent to beak length naris) did not differ in nests with and without parasites (Huber 2008); but importantly, adult beak dimensions in Darwin's finches are not reached until 8-9 weeks post-fledging (Grant 1999). Thus, beak length malformation may only become measurable later in finch development.

The specific criteria used to assess *P. downsi*-induced malformation leaves little doubt that approximately one third of all birds sampled showed evidence of malformation. So, why did the majority of birds sampled have no obvious disfigurement to the nares and/or beak despite the fact that all finch nests on Santa Cruz are likely to have had parasites (100% prevalence: Dudaniec *et al.*, 2007)? One possible explanation for this discrepancy is the extreme variation in intensity of *P. downsi* per nest and per individual, resulting in variable fitness costs and nesting outcomes (Dudaniec *et al.*, 2007). Another possibility is that not all cases of parasitism lead to long-lasting naris or beak disfigurement. In a recent study that analysed within-nest video recordings of interactions between fly larvae and finch nestlings, O'Connor *et al.* (2010) showed a series of factors that can lead to variation in naris and beak malformation in Darwin's finches. These factors can be summarised as: the number of larvae that feed in the nares, the frequency and duration of these feeding events, the nestling's ability to defend itself, and the amount of parental anti-parasite behaviour nestlings obtained. Therefore, variation in adult naris and beak formation is supported by variation in parasite intensity, and the behaviour of both parasite and host.

We predicted that malformed birds would suffer fitness costs as a direct result of reduction in foraging efficiency and competitiveness. The standard indicators of growth (body size) and health (body condition) revealed no significant difference between malformed and normal birds in this respect. In fact, malformed birds were observed as active members of the breeding population: that is, malformed females had brood patches and malformed males held territories. Perhaps birds with the severe beak malformations incur high survival costs and are not recruited into the breeding population, and hence were not measured here. However, numerous

malformed individuals that we sampled were severely disfigured suggesting otherwise (see Fig. 1b, d).

In a study that examined fitness costs incurred by adult Darwin's finches with physical disfigurement caused by avian poxvirus Kleindorfer and Dudaniec (2006) also found no effect on adult body condition. Although, the reduced ability of disfigured males to attract mates indicated a fitness cost (Kleindorfer and Dudaniec, 2006). Similarly, Potti (2008) found no difference in body size or body mass between adult female *F. hypoleuca* that were either parasitised by *P. azurea* as a nestling or not; but a fitness cost evident in the production of smaller eggs. Therefore, fitness costs associated with nares and bill malformation in adult *G. fuliginosa* (and with post-parasitism hosts in general) may be less apparent and more varied than the standard indicators often used to measure fitness costs in hosts presently harbouring parasites.

Further, previous studies that have compared body condition among nestling Darwin's finches in nests with and without *P. downsi* have yielded different results (Fessl *et al.*, 2006a; Huber, 2008). Fessl *et al.* (2006a) found reduced mass gain in parasitised nestlings; whereas, Huber (2008) found no difference in mass gain, nor development of the tarsus or wing between parasitised and non-parasitised nestlings. The relationship between parasitism and body size is complex (reviewed in Møller, 1997).

We believe the reason we found a phenotypic effect of parasitism in nares and beak dimensions is because the nares are the physical location for larval feeding and development (Fessl *et al.*, 2006b), and therefore undergo direct modification as a result of parasitism. In contrast, tarsus and wing length, and mass can be influenced by environmental factors (for example the level of parental care and food quality; see Kruuk *et al.*, 2001).

Beak length naris (often referred to in the literature as *beak length*) and beak depth are standard morphological measurements in ecological, social, and evolutionary studies in Darwin's finches, and other bird species. Our results highlight the role played by an introduced parasite as an agent of change for these key beak variables. These findings are significant given evidence that beak dimensions are important for mate selection in Darwin's finches (Christensen, Kleindorfer & Robertson, 2006; Christensen & Kleindorfer, 2007; Chapter 5). For example, beak dimensions are known to influence the production of song characteristics that are

used to recognise mates and competitors (Podos 2001; Christensen, Kleindorfer & Robertson, 2006). Individuals with nares and beak disfigurement may produce altered and unrecognisable songs, which remains to be tested. Examination of the effects *P. downsi*-induced malformation on song production and mate choice in Darwin's finches may reveal fitness costs to malformed males. Further, as beak dimensions are highly heritable in Darwin's finches (Grant, 1999) and parasitism is known to effect the heritability of traits (Charmantier, Kruuk & Lambrechts, 2004), it is now apparent that, on islands affected by *P. downsi* parasitism, induced beak malformation could set Darwin's finches on a slightly or fundamentally different evolutionary trajectory.

ACKNOWLEDGMENTS

We thank the following: the Galápagos National Park and the Charles Darwin Research Station for the opportunity to conduct this research; Max Planck Institute for Ornithology, American Bird Conservancy, Conservation International, Galápagos Conservation Fund, and Flinders University for their generous financial support; TAME airlines for assistance with reduced airfare to and from the archipelago; Zonnetje Auburn, Mari Cruz Jaramillo, Jody O'Connor, Birgit Fessl, Michael Dvorak, Sarah Huber, Jennifer Koop, Frank Sulloway, and Ryan Buss for their assistance in the field; and Rachael Dudaniec, Jody O'Connor, and two anonymous reviewers for their comments on this manuscript. This research was conducted under the ethics approval granted by Flinders University (E189).

REFERENCES

Brown CR, Brown MB, Rannala B. 1995. Ectoparasites reduce long-term survival of their avian host. *Proceedings: Biological Sciences* **262:** 313-319.

Causton CE, Peck SB, Sinclair BL, Roque-Albelo L, Hodgson CJ, Landry B. 2006. Alien insects: threats and implications for conservation of Galapagos Islands. *Annuals of Entomological Society of America* **99:** 121-143.

Charmantier A, Kruuk LEB, Lambrechts MM. 2004. Parasitism reduces the potential for evolution in a wild bird population. *Evolution* **58**: 203–206.

Christensen R, Kleindorfer S. 2007. Assortative pairing and divergent evolution in Darwin's small tree finch, Camarhynchus parvulus. *Journal of Ornithology* **148:** 463-470.

Christensen R, Kleindorfer S, Robertson J. 2006. Song is a reliable signal of bill morphology in Darwin's small tree finch, *Camarhynchus parvulus*, and vocal performance predicts male pairing success. *Journal of Avian Biology* **37:** 617-624.

Clayton DH, Moore J. 1997. *General Principles and Avian Models.* Oxford: Oxford University Press.

Dudaniec RY, Fessl B, Kleindorfer S. 2007. Interannual and interspecific variation in intensity of the parasitic fly, *Philornis downsi*, in Darwin's finches. *Biological Conservation* **139:** 325-332.

Dudaniec RY, Gardner MG, Kleindorfer, S. 2009. Offspring genetic structure reveals mating and nest infestation behaviour of an invasive parasitic fly (*Philornis downsi*) of Galápagos birds. *Biological Invasions* (online; available at http://www.springerlink.com/content/j6r2125337425t50/)

Dudaniec RY, Kleindorfer S. 2006. The effects of the parasitic flies *Philornis* (Diptera, Muscidae) on birds. *Emu* **106:** 13-20.

Dudaniec RY, Kleindorfer S, Fessl B. 2006. Effects of the introduced ectoparasite Philornis downsi on haemoglobin level and nestling survival in Darwin's small ground finch (*Geospiza fuliginosa*). *Austral Ecology* **31:** 88–94.

Fessl B, Couri MS, Tebbich S. 2001. *Philornis downsi* (Dodge and
Aitken), new to the Galápagos Islands (Diptera, Muscidae). *Studia Dipterologica* **8:** 317–322.

Fessl B, Kleindorfer S, Tebbich S. 2006a. An experimental study on the effects of an introduced parasite in Darwin's finches. *Biological Conservation* **127:** 55-61.

Fessl B, Sinclair BJ, Kleindorfer S. 2006b. The life cycle of *Philornis downsi* (Diptera: Muscidae) parasitizing Darwin's finches and its impacts on nestling survival. *Parasitology* **133:** 739–747.

Fessl B, Tebbich S. 2002. Philornis downsi – a recently discovered parasite on the Galápagos archipelago: a threat for Darwin's finches? *Ibis* **144:** 445–451.

Grant BR, Grant PR. 1989. *Evolutionary Dynamics of a Natural Population: The Large Cactus Finch of the Galápagos.* Chicago: University of Chicago Press.

Grant PR. 1999. *Ecology and Evolution of Darwin's Finches*, 2nd edn. Princeton: Princeton University Press.

Huber SK. 2008. Effects of the introduced parasite *Philornis downsi* on nestling growth and mortality in the medium ground finch (*Geospiza fortis*). *Biological Conservation* **141:** 601-609.

Hudson PJ, Dobson AP. 1997. Host-parasite processes and demographic consequences. In: Clayton DH, Moore J, eds. *Host-parasite Evolution: General Principles and Avian Models.* Oxford: Oxford University Press, 128-154.

Hudson PJ, Dobson AP, Newborn D. 1998. Prevention of population cycles by parasite removal. *Science* **282:** 2256-2258.

Hudson PJ, Newborn D, Dobson AP. 1992. Regulation and stability of a free-living host-parasite system – *Trichostrongylus tenuis* in red grouse, part 1: monitoring and parasite reduction experiments. *Journal of Animal Ecology* **61:** 477-486.

Jackson MH. 1993. *Galápagos: a Natural History*. Calgary: University of Calgary Press.

Kleindorfer S. 2007. The ecology of clutch size variation in Darwin's small ground finch, *Geospiza fuliginosa*: comparison between lowland and highland habitats. *Ibis* **149:** 730-41

Kleindorfer S, Chapman TW, Winkler H, Sulloway FJ. 2006. Adaptive divergence in contiguous populations of Darwin's small ground finch (*Geospiza fuliginosa). Evolutionary Ecology Research* **8:** 357-372.

Kleindorfer S., Dudaniec RY. 2006. Increasing prevalence of avian poxvirus in Darwin's finches and its effect on male pairing success. *Journal of Avian Biology* **37:** 69-76.

Kleindorfer S, Dudaniec RY. 2009. Love thy neighbour? Social nesting pattern, host mass and nest size affect ectoparasite intensity in Darwin's tree finches. *Behavioural Ecology and Sociobiology* **63:** 731-739.

Kruuk LEB, Merilä J, Sheldon BC. 2001. Phenotypic selection on a heritable size trait revisited. *American Naturalist* **158:** 557-571.

Loye JE, Zuk M. 1991. *Bird-parasite Interactions: Ecology, Evolution, and Behaviour*. Oxford: Oxford University Press.

Marshall, AG. 1981. *The Ecology of Ectoparasitic Insects*.
London: Academic Press.

McMullen CK. 1999. *Flowering Plants of the Galápagos.* Ithaca: Cornell University Press.

Møller AP. 1990. Effects of parasitism by a haematophagous mite on the reproduction in barn swallow. *Ecology* **71:** 2345-2357.

Møller AP. 1992. Parasite differentially increase fluctuating asymmetry in secondary sexual characters. *Journal of Evolutionary Biology* **5:** 691-699.

Møller AP. 1996a. Sexual selection, viability selection, and developmental stability in domestic flies *Musca domestica*. *Evolution* **50:** 746–752.

Møller AP. 1996b. Parasitism and developmental instability of hosts: a review. *Oikos* **77:** 189–196.

Møller AP. 1997. Developmental stability and fitness: a review. *The American Naturalist* **149:** 916-932.

Møller AP. 2006. A review of developmental instability, parasitism and disease. *Infection, Genetics and Evolution* **6:** 133-140.

Møller AP, Swaddle JP. 1997. *Asymmetry, Developmental Stability, and Evolution*. Oxford: Oxford university press.

O'Connor JA, Robertson J, Kleindorfer S. 2010. Video analysis of host-parasite interactions in nests of Darwin's finches. *Onyx* **44:** 588-594.

Podos J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* **409:** 185-188.

Polak M. 1993. Parasites increase fluctuating asymmetry of male *Drosophila nigrospiracula*: implications for sexual selection. *Genetica* **89:** 255–265.

Potti J. 2008. Blowfly infestation at the nestling stage affects egg size in the pied flycatcher *Ficedula hypoleuca. Acta Ornithologica* **43:** 76-82.

Poulin R, Thomas F. 1999. Phenotypic variability induced by parasites: extent and evolutionary implications. *Parasitology Today* **15:** 28-32.

Price PW. 1980. *Evolutionary Biology of Parasites.* Princeton: Princeton University Press.

Richner H, Oppliger A, Christe P. 1993. Effect of an ectoparasite on reproduction in great tits. *Journal of Animal Ecology* **62:** 703-710.

Wiedenfeld DA, Jiménez UGA, Fessl B, Kleindorfer S,Valarezo JC. 2007. Distribution of the introduced parasitic fly, *Philornis downsi*, (Diptera, Muscidae) in the Galápagos Islands. *Pacific Conservation Biology* **13:** 14–19.

CHAPTER SEVEN

Conclusions

Synthesis of findings

Darwin's small ground finch *Geospiza fuliginosa* on the island of Santa Cruz, Galápagos Archipelago, represents one panmictic population (Chapter 2). Gene flow in this system is probably high; and neither restricted by geographical distances, nor ecological differences in the landscape (Chapter 2). Because nonadaptive divergence are unlikely under even low gene flow, phenotypic divergence between *G. fuliginosa* inhabiting different ecological zones on Santa Cruz, shown in previous studies (reviewed in Chapter 2), is a truly adaptive response (Chapter 2). However, I propose that the principal mechanism for phenotypic divergence may not be adaptation in its classic sense, but rather habitat matching choice – that is, phenotype-environment matching via active dispersal (Chapter 2).

This idea is supported by evidence that suggests the breakdown of morphological clines in *G. fuliginosa* in a high rainfall year is dispersal-mediated; certainly, selective mortality and reproduction could not easily explain this pattern (Chapter 3). I speculate that under relaxed selection in such "benign" years, immigrants are able to successful establish territories in habitats they are otherwise ill-adapted to; and they do so in large numbers, effectively reshuffling ecomorphs across the entire island (Chapter 3). It follows that in "severe" low rainfall years, selection against ill-adapted immigrants would be intense, favouring habitat matching in large numbers, and thereby reordering ecomorphs among ecological zones. Overall, plastic dispersal behaviour in response to the alternating climate in the Galápagos Archipelago seems to play a central role in the population dynamics of *G. fuliginosa* on Santa Cruz; and potentially other species of Darwin's finch inhabiting large islands as well.

The colonisation of the highlands by *G. fuliginosa* has had a significant effect on mating behaviour in this species: specifically, a loss of assortative pairing (Chapter 4). This relaxation in mate preferences is likely to be adaptive responses to

ecological opportunity following range expansion; facilitated by the "benign" conditions of the highlands (Chapter 4). Such factors may explain why highland males, and presumably highland females, respond equally to local and foreign song (Chapter 5). However, song recognition may be based on memorised songs that have been heard locally; therefore, our "foreign" songs used to stimulate responses in highland *G. fuliginosa*, may have been considered "local" by our test subjects (Chapter 5); in other words a recognised highland song. As a result dispersal is likely to be largely unidirectional (Chapter 5). This last point suggests dispersal and gene flow (whether in large numbers or not) may primarily move down a selection gradient from the "severe" lowlands to the "benign" highlands; providing finer detail to the analysis of dispersal and gene flow in this system (Chapter 2 and 3).

Philornis downsi-induced post-parasitism morbidity is ubiquitous on Santa Cruz (Chapter 6) – which may provide further evidence of widespread dispersal in *G. fuliginosa*. Parasitism causes deformation and malformation to the nares and beaks of *G. fuliginosa* that persist into adulthood; but does not cause obvious developmental impairment (smaller body size) or reduced foraging efficiency (lower body condition; Chapter 6). The frequency and degree of beak morbidity does not differ among ecological zones, and thereby not expected to have a great effect on morphological clines. That said, the fact that *P. downsi* can effect change in beak dimensions in *G. fuliginosa* suggests implications for effective foraging, mate signalling, and mate recognition; as well as, for future studies examining any of these behaviour in species or any other Darwin's finches (Chapter 6).

My thesis suffers from: low confidence in estimates of contemporary gene flow (Chapters 2 and 3); potential inaccuracies in inter-measurer reliability of morphological traits across sampling periods (Chapter 3); a lack of annual replication (Chapters 3-6); and small sample sizes for some analyses (Chapter 4 and 5). Thus, I have cautious interpreted my findings in relation to these limitations.

In sum, (1) divergent selection between lowland and highland zones, (2) differential selection intensity between those zones (i.e., "severe" and "benign", respectively), and (3) ecological opportunity in the highlands, combine to form strong selection for divergence in morphology, song, and mate preferences. However, the intrinsic aspects of both *G. fuliginosa* (e.g. high mobility) and Santa Cruz (e.g. no physical barriers between zones) suggest dispersal, and possibly gene flow, among zones is high (Chapter 2 and 3). In low rainfall periods, divergent

selection and hence adaptive divergence is predicted to be strongest; whereas, in high rainfall periods divergent selection is weakest and hence immigration of otherwise ill-adapted individuals is high, effectively reshuffling phenotypes among zones. Thus, adaptive divergence in *G. fuliginosa* is frequently halted and perhaps reversed. One piece of evidence – that is, lowland males rarely recognise the song of highland males in a high rainfall year – suggests that barriers to gene flow may have arisen. However, it is only through further research that we might comment on the long-term product of the counter processes in this system.

Future research

I have briefly discussed avenues for future research within each chapter. Here, I provide five of, what I think are, the most important questions arising from my thesis; and propose observational and experimental studies to test .

1) ***Why do a large proportion of adult finches have no obvious morbidity when* Philornis downsi *parasitism is so prevalent?***

   Philornis downsi parasitism is highly prevalent in Darwin's finches and causes incredibly high mortality and morbidity among nestlings (reviewed in Chapter 6). Yet, obvious post-parasitism morbidity can only be seen in one third of the adult population of *G. fuliginosa* (Chapter 6). This discrepancy needs to be addressed. I am confident that post-parasitism morbidity was identified accurately; and the healing of deformations and malformations was not likely. Current research in the BirdLab at Flinders University using video recordings to examine sibling and parent-offspring interactions at parasitized nests, has found evidence of behavioural defence against *P. downsi* that may explain "normal" (i.e., apparently never parasitized) adult finches (O'Connor *et al.* 2010; J. A. O'Connor PhD thesis, in preparation): this work should continue. Monitoring the impact of the prevalence of *P. downsi* parasitism should also continue. While I found no evidence of post-parasitism fitness cost or "morbidity debt" (i.e., an analogous process to extinction debt; Chapter 6), I suggest further examination of the incidence and impact of post-parasitism morbidity in adult finch populations to ensure their conservation. Possible post-parasitism fitness costs, such as feeding inefficacy, mate attraction inefficacy, and social sub-ordinance, may be assessed

experimentally using immature wild finches housed in normal/malformed pairs.

2) ***Is dispersal among ecological zones low or high?***

Despite logically concluding that dispersal, and gene flow, among ecological zones on Santa Cruz is high in *G. fuliginosa* (Chapter 2 and 3), current estimates do not concur (see also Chapter 5). Genetic methods for estimating effective and noneffective dispersal (i.e., that which contributes to gene flow and total dispersal, respectively) cannot (at present) resolve this problem; therefore, accurate estimates must be obtained via mark-recapture-resighting studies. While mark-recapture-resighting data exists for *G. fuliginosa* already, I think that that data can be improved by a dedicated and extensive study (Chapter 3). Mark-recature-resighting studies require considerable effort over a number of years. I suggest a natural experiment to begin in the late dry season when flocks of finches (perhaps in the agricultural zone only) can be netted; large numbers of individuals can be measured and marked; and predictions of individual dispersal can be made based on morphology. The natural experiment can then be completed in the wet (i.e., breeding) season when an extensive survey of all zones can be undertaken using sightings of marked individuals. Effective dispersal for gene flow might be estimated by observations of breeding behaviour of marked individuals. It would be interesting to compare presumed differences in dispersal in low and high rainfall years. Keeping in mind that the primary interest of such work is to answer questions that relate to ecological speciation (e.g. adaptive divergence, matching habitat choice, and immigrant inviability) future studies may need only concentrate efforts across ecological zone boundaries and not large geographical distances.

3) What causes phenotypic divergence among ecological zones – classic adaptive divergence and/or matching habitat choice?

Assuming high gene flow in *G. fuliginosa* on Santa Cruz (Chapter 2 and 3), matching habitat choice is an alternative explanation for evidence provided thus far for classic adaptive divergence in this system (Chapter 2). I expect that both processes are involved in phenotypic variation across ecological

zones (Chapter 2); but to identify the relative contribution of each is important. Distinguishing classic adaptive divergence and matching habitat choice will not be easy; however, Edelaar (2008) provides a framework in which to examine this problem. The next step would be to confirm that selective mortality and reproduction are not the only processes contributing to phenotype variation among ecological zones. This might be achieved by a natural experiment using GPS devices to track the dispersal of immature finches (less than one year old) after the breakup of flocks in the first low rainfall year after a high rainfall year. Individuals would be categorised as arid, transitional, agricultural, or humid ecomorphs based on morphology. I would expect matching habitat choice to be more apparent in immature finches; environment-phenotype matching to be weak in the "benign" high rainfall year (Chapter 3), in which immature finches were born; and the necessity for strong environment-phenotype matching in the "severe" low rainfall year, in which immature finches would then find themselves. The same could be done for immature finches a high rainfall year after a low rainfall year to confirm dispersal-mediated convergence across ecological zones.

4) ***What has led to song divergence between lowland and highland zones?***

Song in *G. fuliginosa* would be influenced by natural and sexual selection both directly and indirectly; plus copying errors and mutations. Determining the relative contribution of different factors is a big job. Further, the relative strength of each factor is likely to change with low and high rainfall years. In preparation of this thesis, I have analysed the relationship between song characteristics and morphological traits for a subset of individuals analysed in Chapter 5. I found a very strong negative relationship between song duration and body mass (r = 0.78). No other beak or body measurement correlated significantly with song. Thus, larger males sang shorter songs with fewer notes than smaller males; but mass, like most other morphological traits, did not differ between zones in 2008 (unpublished data). I think that this strong correlation, in its solitude, warrants further investigation; thus an analysis of the influence of morphology and song may be the first avenue for further research in song divergence in this system. Because body size is correlated

with social dominance in Darwin's finches (Grant and Grant 2010), I suggest a study that combines song, morphological, and behavioural analysis. Further, because *G. fortis* is larger, more dominant, and sings a shorter song than *G. fuliginosa* (see Podos 2007 [Table 1]) thereby such an examination should include *G. fortis*; particularly since the abundance of this species, unlike *G. fuliginosa*, is lower in the lowlands than in the highlands (Incidentally, a low sample size (n = 17) and a single year of sampling were two reasons why I did not continue with this line of investigation for my thesis). The effect of dominance on song duration may be tested experimentally by rearing combinations of small/large bodied (based on parent size) *G. fuliginosa*/*G. fortis* in adjoining cages isolated from all other stimuli. The effect of beak morphology and innate song duration could be controlled in conspecific tests by matching these attributes in the parent finches.

References

Edelaar, P., Siepielski, A. M. and Clobert, J. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. *Evolution* **62**: 2462-2472.

Grant, P. R. and Grant, B. R. 2010. Songs of Darwin's finches diverge when a new species enters the community. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 20156-20163.

O'Connor, J. A., Robertson, J. and Kleindorfer, S. 2010. Video analysis of host-parasite interactions in nests of Darwin's finches. *Oryx* **44**: 588-594.

Podos, J. 2007. Discrimination of geographical song variants by Darwin's finches. *Animal Behaviour* **73**: 833-844.