The ecology of adaptive radiation in Darwin's finches

by

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Dedication

A mis padres:

Audelia Reyna y Fernando De León

Abstract

The ecological theory of adaptive radiation postulates that divergent natural selection between populations occupying contrasting environments, or using different resources, can drive adaptive divergence and thereby cause speciation. My thesis investigates this hypothesis in Darwin's finches of the Galápagos Islands. I use field observations, genetic tools, and statistical analyses to study patterns of sympatric coexistence, niche use, adaptive divergence, and gene flow within and among four closely-related species of Ground finches (*Geospiza* spp.). I also quantify possible impacts of human disturbances on this adaptive radiation. My first major finding is that the coexistence of sympatric, closely related species is facilitated by partial differences in diets (imperfect generalism), promoted by high spatial and temporal environmental heterogeneity. My second major finding represents a demonstration that a single generalist species (*Geospiza fortis*) might be composed of a number of individual specialists. This individual specialization might be important in promoting the initial stages of adaptive divergence. My third major finding is of strong reproductive isolation between coexisting large versus small beak size morphs with *G. fortis*. This result suggests the possibility that adaptive divergence drives genetic divergence in the face of initially high gene flow for sympatric populations on a single island. My final major finding is that human activities appear to be negatively impacting adaptive divergence between the small and large beak size morphs of *G. fortis*. I specifically show how the diet and performance (bite force) differences that characterize the two morphs at an undisturbed site where the morphs remain distinct have degraded at a human-disturbed site where the morphs are no longer distinct. These findings confirm some of the major predictions of the ecological theory of adaptive radiation by revealing how resource (diet) differences that cause adaptive (beak) divergence promote ongoing ecological speciation in Darwin's finches. Overall my thesis illustrates how the study of ecological interactions is central to understanding the factors that promote and maintain the process of adaptive radiation.

Résumé

La théorie écologique des radiations adaptatives propose que la sélection naturelle divergente entre des populations qui occupent des environnements différents, ou utilisent des ressources différentes, peut promouvoir la divergence adaptative et ainsi initier la spéciation. Ma thèse examine cette hypothèse dans le contexte des Pinsons de Darwin des îles Galápagos. J'utilise des observations écologiques, des outils génétiques, et des analyses statistiques pour étudier la coexistence en sympatrie, l'utilisation des niches, la divergence adaptative, et le flux génique entre et au sein de quatre espèces de pinsons étroitement reliées (*Geospiza* spp.). J'examine également les impacts potentiels de perturbations humaines sur cette radiation évolutive. Ma première conclusion importante est que la coexistence d'espèces étroitement reliées vivant en sympatrie est facilitée par des petites différences dans leur régime alimentaire (le généralisme incomplet), aidé par un niveau élevé d'hétérogénéité spatiale et temporelle. Ma deuxième conclusion est qu'une seule espèce généraliste (*Geospiza fortis*) est peut-être composée de plusieurs individus spécialisés. Cette spécialisation au niveau de l'individu pourrait être importante pour faciliter les premières étapes de la divergence adaptative. Ma troisième conclusion est qu'il existe une forte isolation reproductive entre deux formes de *G. fortis* qui sont divergentes dans la morphologie de leurs becs, mais qui coexistent dans le même milieu. Ce résultat suggère que la divergence adaptative peut entrainer la divergence génétique même avec un haut niveau initial de flux génique dans des populations vivant en sympatrie sur une même île. Ma dernière conclusion est que les activités humaines sont peut-être en train de restreindre la divergence adaptative entre ces deux formes de *G. fortis*. Plus précisément, je démontre comment les différences reliées au régime alimentaire et à la performance qui caractérisent les deux formes dans un site non-perturbé, où les deux formes demeurent distinctes, se sont réduites dans un site perturbé par les humains, où les formes ne sont maintenant plus distinctes. Ensembles, ces résultats confirment plusieurs des prédictions les plus importantes de la théorie écologique des radiations adaptatives, en démontrant comment des différences en ressources alimentaires peuvent entrainer la divergence adaptative dans la morphologie des becs, qui promeut ensuite la spéciation écologique continue dans les Pinsons de Darwin. En général, ma thèse illustre comment l'étude des interactions écologiques est essential pour bien comprendre les facteurs qui promeuvent et maintiennent le processus de la radiation adaptative.

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Contribution of authors

I am the main author of all the chapters included in this thesis. This means that I organized and executed the experiments, carried out the analyses, and wrote most of the text. Professors Andrew Hendry, Jeffrey Podos and Eldredge Bermingham are coauthors on all chapters. They have contributed to all phases of the development of this project, including development of research questions, experimental design, data collection, analysis and writing.

Other co-authors have also contributed in different phases of this project. Anthony Herrel (Chapter 2, 5) contributed with equipment, collection of data on bite force and seed hardness and writing. Joost Raeymaekers and (Chapter 3, 5) and Tariq Gardezi (Chapter 2) contributed to the data analysis. All of the co-authors have agreed to be included on the papers and chapters included in this thesis

Animal ethics

This work was conducted in accordance with Animal Use Protocols approved by the University of Massachusetts Amherst, U.S.A

Table of Contents

List of tables

List of appendices

List of figures

CHAPTER 1:

General introduction

1.1 Introduction

Adaptive radiation occurs when a single ancestral species diversifies into an array of new descendent species that are adapted to different resources (Lack 1947; Simpson 1953; Grant 1999; Schluter 2000). Understanding this process is a central goal of evolutionary biology (Darwin 1859; Mayr 1963; Schluter 2000). Toward this goal, "the ecological theory of adaptive radiation has become the major synthesis of ideas concerning the origin of ecological diversity" (Schluter 2000, p.1). Indeed, this theory is now widely believed to explain the origin of much of the observed patterns of biological diversity (Lack 1947; Simpson 1953; Grant 1999; Schluter 2000; Rundle & Nosil 2005). My thesis investigated the ecological basis of an adaptive radiation in Darwin's finches of the Galápagos. My study revealed new insights into the mechanisms involved in both promoting and constraining the initial stages of divergence within natural populations and permitting the coexistence of young divergent species within an adaptive radiation

The ecological theory of adaptive radiation has its foundation in divergent natural selection between populations occupying different environments or using different resources. Divergent selection is important because it "pulls" populations toward different adaptive peaks caused by different environments/resources and thereby "pushes" them apart from each other. The resulting adaptive population divergence should then trigger the evolution of reproductive isolation as an incidental byproduct (Schluter 2000), as will be explained further below. Competition for shared resources can also be important because it helps to maintain divergent selection (Dieckmann & Doebeli 1999;

Rueffler et al. 2006), and also because it can lead to character displacement that increases morphological divergence between species (Brown & Wilson 1956; Grant 1972; Bulmer 1974; Schluter *et al.* 1985; Grant & Grant 2006).

Interactions between divergent natural selection, ecology, and phenotypic divergence can be depicted by using fitness (Wright 1931) and adaptive (Simpson 1953) landscapes (Fig. 1.1). The axes of *individual fitness landscapes* represent possible phenotypic trait values (x-axis) and the expected fitness (y-axis) of an individual having those phenotypes (Wright 1931). The different peaks on such a landscape correspond to trait combinations that are well suited for use of a particular environment/resource (e.g., food type). Adaptive radiation is then conceptualized by converting these individual fitness landscapes to *adaptive landscapes* at the population level (Simpson 1953). On adaptive landscapes, the axes now represent mean phenotypes (x-axis) of hypothetical populations and the expected mean fitness (y-axis) of populations having those mean values. Population divergence then proceeds when divergent natural selection displaces different populations toward different peaks on a rugged adaptive landscape (Schluter 2000). Once phenotypic divergence has been triggered and populations enjoy a fitness advantage on alternative adaptive peaks, intermediate phenotypes (e.g., hybrids) will fall into the low fitness valleys between peaks.

Once populations diverge toward adaptive peaks, several factors can cause reproductive isolation between them. First, individuals with phenotypes adapted to a given peak will have low fitness if they attempt to use resources characteristic of a different peak (selection against migrants, Hendry 2004)

Second, hybrids between populations occupying different peaks should have low fitness because they will often fall into the valleys between peaks (Schluter 2000). Third, populations adapted to different peaks might avoid mating with each other if phenotypes that undergo adaptive divergence also influence mate choice (Schluter 2000). All of these factors should reduce the exchange of genes between populations and thereby allow/drive the accumulation of genetic differences (Korol *et al.* 2000; Schneider 2000).

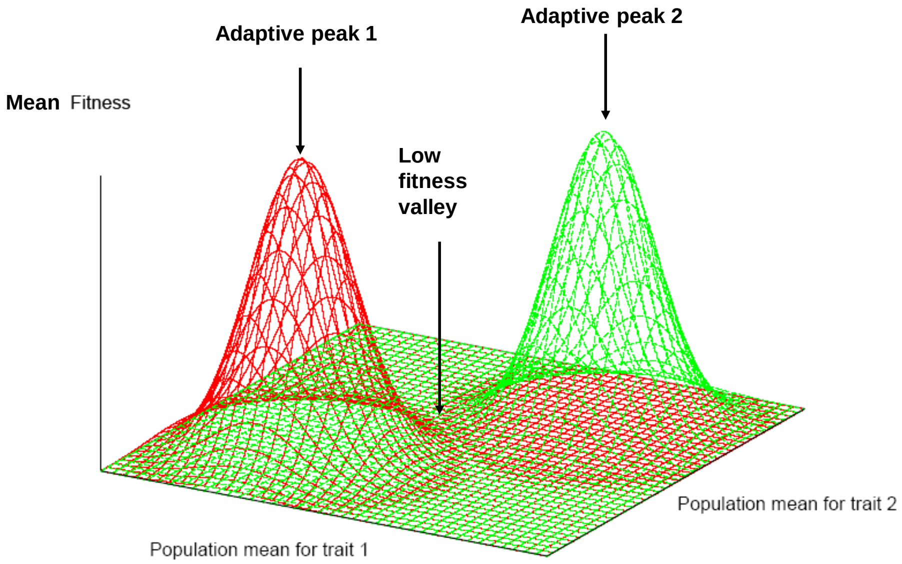

Figure 1.1 A fitness surface showing the interaction between phenotypic trait values and fitness. The peaks represent local maxima that can be occupied by different populations. (Courtesy of Xavier Thibert-Plante).

The ecological theory of adaptive radiation has been tested largely through intensive studies of a few classic systems. Examples include the African haplochromine cichlids (Nagl *et al.* 1998; Sturmbauer 1998), *Heliconius* butterflies (Jiggins *et al.* 2001); *Timema* walking sticks (Nosil *et al.* 2002), Darwin's finches (Lack 1947; Grant 1999), *Anolis* lizards (Roughgarden 1995), and threespine stickleback (Schluter 2000). A great deal of information on ecology and adaptation has been collected for these systems, but inferences are often limited because critical scenarios, such as sympatric populations that show only partial divergence (e.g., bimodal populations which still have intermediates) are rare. The passage of time has therefore obscured the forces that initially drove the radiation. One way to observe these forces is to examine populations that are in the early stages of diverging in their use of different resources. Such populations are rare indeed, but we have found one in Darwin's finches of the Galápagos (see below). The main objective of my thesis was to use these populations to understand how ecological differences drive adaptive divergence and reproductive isolation in Darwin's finches. This study was carried out on Santa Cruz Island, the second largest island in the Galápagos Archipelago, Ecuador (Fig. 1.2).

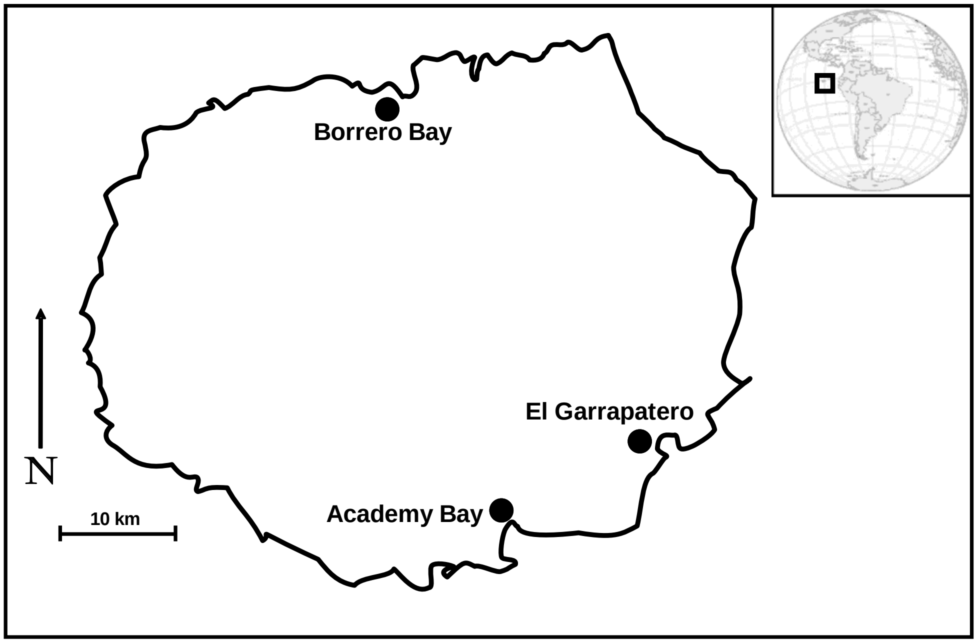

Figure 1.2 Study sites on Santa Cruz Island, Galápagos Archipelago, Ecuador.

1.2 Darwin's finches as a model system to study adaptive radiation

Darwin's finches of the Galápagos have provided strong material for the study of adaptive radiation (Lack 1947; Bowman 1961; Grant 1999; Petren *et al*. 1999; Sato *et al*. 1999; Schluter 2000). Fourteen species are currently recognized (Fig. 1.3), all having evolved from a single colonizing species approximately 2.3 million years ago (Sato *et al.* 2001). Most of the radiation is thought to be the result of sequential adaptation to empty feeding niches on different islands, followed by back-colonization of distinct forms that can now coexist with their ancestors (Lack 1947; Schluter & Grant 1984; Grant 1999). After secondary contact, divergence was then enhanced by competition, assortative mating and selection against hybrids (Lack 1947; Schluter & Grant 1984; Grant & Grant 1993; Grant 1999). Reflecting this divergence in feeding niches, the different species have developed distinctive anatomy and behaviors appropriate for exploiting their chosen food types (Bowman 1961; Grant 1999). In particular, beak sizes and shapes have diversified for crushing seeds of different sizes, pecking at wood, catching insects, eating leaves, or probing nectar (Grant 1999; Fig. 1.3).

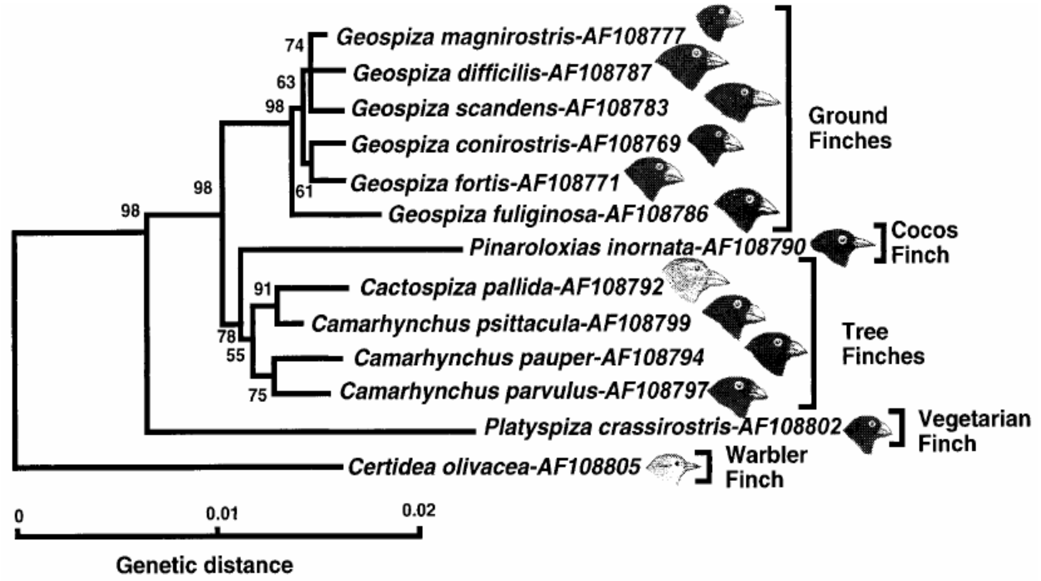

Figure 1.3 Darwin's finches phylogeny based on neighbor-joining (NJ) tree of combined cytochrome *b* (*cyb)* and control region (*cr)* sequences. (*Source:* Sato *et al*. 1999).

The focus of my thesis falls on the four, very closely related, ground finch species. The small ground finch (*Geospiza fuliginosa*) has a small beak size and commonly feeds on small, soft seeds. The medium ground finch (*Geospiza fortis*) has a medium beak size and commonly feeds on seeds of intermediate size and hardness. The large-beaked ground finch (*Geospiza magnirostris*) has a large beak size and commonly feeds on large, hard seeds. The small cactus finch (*Geospiza scandens*) has a pointed beak and commonly feeds on the seeds and nectar of cacti. Despite dramatic differences in morphology and diet between these species, they can hybridize in nature and show no genetic incompatibilities (Grant & Grant 1996; Grant *et al.* 2004; Grant *et al.* 2005). These species are therefore thought to maintain their distinctiveness based primarily on ecological selection against hybrids that fall between parental fitness peaks (Grant & Grant 1996), as well as assortative mating based on song (Grant 1999; Podos 2001).

Not only do differences in beak sizes (and shapes) characterize the Darwin's finch radiation as a whole, but beak sizes are also known to evolve adaptively on very short time scales. For example, drought periods have imposed strong selection on beak size of *Geospiza fortis* on the small island of Daphne Major (Boag & Grant 1981). Because beak size is highly heritable (Keller *et al.* 2001), subsequent generations showed expected changes in beak sizes (Grant & Grant 1995; Grant 1999; Grant & Grant 2002). Selection on beak size within this species probably differs among locations, given that the beak size of *G. fortis* differs among islands and even among locations on some of the islands (Grant 1999, p. 77-79). This fine-scale spatial and temporal variation suggests that we might be able to study ecological speciation in action, particularly since we have identified one population, *G. fortis* on Santa Cruz Island, which appears to be in the early stages of divergence, as we outline below.

G. fortis in this population show 1) strong bimodality in beak size distributions with small and large beak morphs coexisting in sympatry with relatively few intermediates (Hendry, *et al*. 2006, Figs. 1.4 and 1.5), 2) strong divergence in song types between the morphs (Podos 2001; Huber & Podos 2006; Podos 2010), and 3) strong divergence in bite force (a functional performance measure related to foraging ability) between the morphs (Herrel *et al.* 2005a, b; Herrel *et al.* 2010). These findings suggest that this *G. fortis* population at this site may be splitting along the same beak size axis that characterizes differences among the seed-eating ground finch species (*G. fuliginosa*, *G. fortis*, and *G. magnirostris*).

This parallelism of divergence within and between species is useful because it suggests that the two levels of diversification might be driven by the same ecological forces: i.e., adaptation to different seed sizes. If so, studying diversification between beak size morphs within *G. fortis* could be an excellent way to reveal the evolutionary forces that drove the adaptive radiation as a whole. On the other hand, studying the interactions among sympatric species on a single island could reveal the factors that allow coexistence of closely related species at the early stages of an adaptive radiation. Additionally, in other locations on this Island, finch populations are in close contact with human populations, which provides an interesting scenario to test for factors which might constrain adaptive radiation, particularly those related to human disturbances.

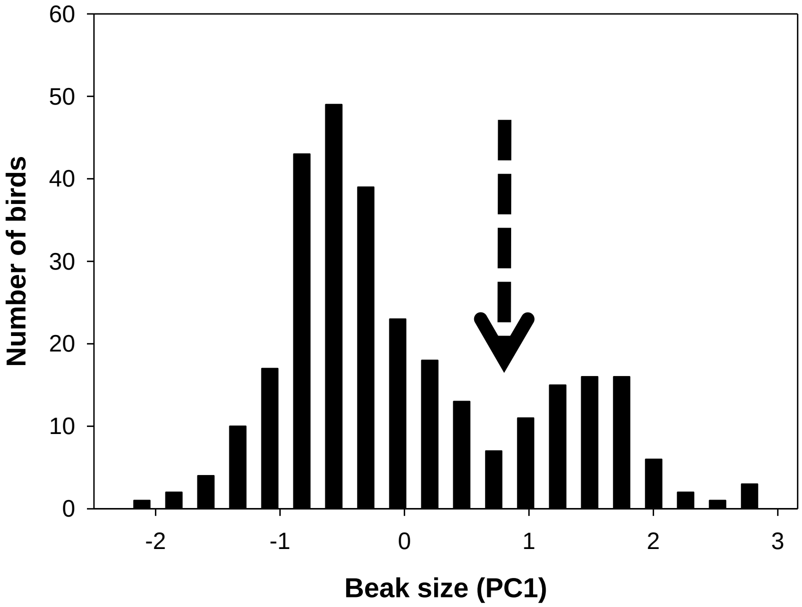

Figure 1.4 Distribution of beak sizes in *G. fortis* at El Garrapatero, Santa Cruz Island. The arrow indicates the separation between the two beak morphs as inferred statistically (data from Hendry *et al*. 2006).

Figure 1.5 Small (left) and large (right) beak size morphs of *G. fortis*. These two birds were caught in the same mist net, at the same place and time (from Hendry *et al*. 2006)

Considering these *G. fortis* beak size morphs in the context of the ecological theory of adaptive radiation, we can make several predictions for the El Garrapatero population. First, large- and small-beaked *G. fortis* should specialize on distinct resources (e.g., seeds of different sizes). This hypothesis has not been tested and is the focus of one chapter of my thesis. Second, divergent natural selection should be acting on the two morphs. We have confirmed this in a previously published paper (Hendry *et al.* 2009) that showed a strong disruptive selection against intermediates birds, however this is not included in my thesis. Third, at least some reproductive isolation should be present. We previously confirmed that the two morphs do pair assortatively by beak size and show some genetic differences (Huber *et al*. 2007 – not included in the thesis), but I will test the latter more rigorously here. Confirmation of these predictions – and their gradation into between-species differences (also considered in my thesis) – would provide evidence for ongoing incipient ecological speciation, and would thereby provision a means to study processes occurring during the early stages of an adaptive radiation. Deviations from these predictions will also be informative in the context of determining what limits ecological speciation.

1.3 Logical order of the thesis

My first chapter will investigate the partitioning of food resources within and among the four ground finch species. Here I use a large multi-year data-set on feeding observations to determine the types of food consumed by each species. This analysis of resource partitioning is combined with data on the availability of food types across different sites on Santa Cruz Island. My goal is to use these data to study how variation in niche overlap and resource partitioning might help to maintain closely related sympatric species within an adaptive radiation. My second chapter uses a similar multi-year data set of feeding observations, this time of individually-banded birds, to look at the patterns of resource use (diet) at the individual level (individual specialization) and between the two beak morphs. My goal here is to examine the potential for individual specialization to promote adaptive radiation. My third chapter returns specifically to the predictions outlined above by quantifying the degree of reproductive isolation between beak size morphs through genetic analyses using microsatellite DNA markers. With this method, I estimate gene flow between the beak size morphs and among ground finch species, both between and within sites on Santa Cruz Island. The last chapter of my thesis attempts to identify any possible effects of human disturbances on this adaptive radiation. Here I will contrast the strength of the morphological-performance-environmental correlation between beak morphs at a human-disturbed versus an undisturbed site.

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Linking statement between Chapter 1 and Chapter 2

Chapter 1 provided a general synthesis of adaptive radiation, and touched on some of the theoretical and empirical considerations related to the study of this process. It also showed how Darwin's finches have become an iconic study system for advancing our knowledge about the process of diversification. I explained the sympatric coexistence of closely related species at the early stages of an adaptive radiation and the high morphological variation found in heterogeneous environments. In the next chapter I study the ecological factors that allow the coexistence of sympatric, closely related species. Specifically, I examine the temporal and spatial patterns of resource partitioning among ground finches on the island of Santa Cruz.

CHAPTER 2

The sympatric co-existence of imperfect generalists: temporal and spatial variation in diet and niche overlap of Darwin's finches

2.1 Abstract

The coexistence of closely related sympatric species that share similar resources may have important consequences for the phenotypic diversification of the interacting species. Two schools of thought have surrounded this topic. On the one hand, sympatric coexistence of related species involves adaptive divergence to use different resources; on the other hand, coexistence of sympatric species can be maintained without the need of adaptive differences. We inform this discussion by looking at the temporal and spatial variation in niche partitioning among four coexisting species of Darwin's ground finches on Santa Cruz Island, Galápagos, Ecuador. We found that niche overlap varies across time and space, reflecting differences in resource diversity at different sites and in different years. In some sites and years, species showed considerable niche conservationism; whereas in others they were quasi-generalist in their resource use. These analyses reveal that ground finches can be regarded as imperfect generalists, using a diversity of partially overlapping resources, but still retaining a series of private resources on which they are better adapted. Our results thus suggest that the dichotomy between adaptive and non-adaptive views of radiation and sympatric coexistence is a false one. Many of the patterns and processes argued on both sides seem to play out for Darwin's finches – and they can be reconciled together.

Key words: Darwin's finches, resource use, adaptive radiation, ecological speciation, competition, available resources.

2.2 Introduction

When different groups (e.g., populations or new species) in an ongoing radiation come into contact, several outcomes are possible (Barton & Hewitt 1981; Butlin 1987; Sanderson 1989; Liou & Price 1994; Young *et al.* 2009). One is the continued coexistence of both groups in roughly their original form, which is expected when they rarely interbreed and show adaptive differences that reduce competition and gene flow. A second possibility is that one group will exclude the other, which is expected when they are reproductively isolated but compete for the same resources (Gause 1932; Hardin 1960; Macarthur & Levins 1967). A third possibility is that the two groups fuse together into a hybrid swarm or a new hybrid species (e.g., Frakes & Johnson 1982; Seehausen 2004; Streelman *et al.* 2004), which is expected when they are not reproductively isolated. A fourth possibility is that the two groups may diverge further, which is expected in the case of ecological character displacement to reduce competition, or reproductive character displacement to reduce maladaptive mating (Brown & Wilson 1956; Bulmer 1974; Abrams 1986; Grant 1999; Schluter 2000; Grant & Grant 2006).

Considering the above alternatives together, the upshot of all these options is that the theory of adaptive radiation predicts that closely-related sympatric species will be adaptively divergent for the use of different niches/resources/environments/habitats (Lack 1947; Simpson 1953; Schluter 2000; Ackerly *et al.* 2006; Grant & Grant 2008a).

Two opposing schools of thought have coalesced around the above adaptive hypothesis for sympatric coexistence. On the positive side, three forms of evidence have been advanced. First, closely related species often show ecological and reproductive character displacement in sympatry relative to allopatry (Schluter *et al.* 1985; Schluter & McPhail 1992; Grant & Grant 2006; Rice *et al.* 2009). Second, speciation events in some groups are clearly linked to niche shifts (e.g., Losos *et al.* 2003; Graham *et al.* 2004; Knouft *et al.* 2006; Lovette & Hochachka 2006; Broennimann *et al.* 2007), although cause and effect are not always certain (Rundell & Price 2009). Third, related species in at least some communities partition resources (and differ in traits) in ways that seem to reduce competition (Voigts 1973; Wiens 1992; Jones & Barmuta 2000; Peterson & Holt 2003; Garcia & Arroyo 2005; Mason *et al.* 2008). These results support the idea that sympatric coexistence of related species involves adaptive divergence to use different niches/resources/environments/habitats.

On the negative side, three other forms of evidence have been advanced. First, closely-related species sometimes occupy very *similar* niches: i.e., "niche conservatism" (Wiens 2004; Wiens & Graham 2005; Knouft *et al.* 2006; Kozak *et al.* 2006; Lovette & Hochachka 2006). Second, patterns of species coexistence in at least some communities can be explained without recourse to adaptive differences: i.e., neutral theory (Bell 2001; Hubbell 2001). Third, even species that compete strongly could theoretically persist for long periods of time simply through stochastic processes (Grossman *et al.* 1982; Tilman 2004; Cadotte 2007). These results argue against the idea that sympatric coexistence of related species involves adaptive divergence to use different niches/resources/environments/habitats.

We hope to inform this debate by examining niche overlap among young species in a putative adaptive radiation. Our particular focus is on niche variation in space and time. This variation might be important for several reasons. In a temporal context, many species might feed on the same foods until those foods become limited, at which time they might specialize on foods to which they are differentially adapted (Grant *et al.* 1976a; Schoener 1982; Smith 1991; Bolnick 2001; Grant & Grant 2002b). Failing to document rare but critical episodes of adaptive specialization might lead investigators to miss the niche differences that allow coexistence. For instance, when some putative specialist species turn out to be widely generalist ("Liem's paradox; Liem 1980). In a spatial context, dispersal from source areas of strong niche partitioning might maintain species coexistence in areas of weak niche partitioning. This possibility reminds us of the idea of hot and cold spots in a geographical mosaic of co-evolution and co-existence (Thompson 1997; Benkman 1999; Gomulkiewicz *et al.* 2000). Another reason for encompassing both temporal and spatial contexts refers to non-equilibrium communities (MacArthur & Wilson 1967; Losos & Ricklefs 2009) where high/low niche overlap in the present might cause extinction/divergence in the future. With these motivating possibilities in mind, we examine niche overlap among four closely-related sympatric Darwin’s finches in Galapágos.

2.2.1 Darwin’s finches

Darwin’s finches in the Galápagos are considered a classic example of adaptive radiation, here specifically onto different food types (Lack 1947; Bowman 1961;

Grant 1999). Moreover, competition and niche partitioning are thought to have been very important in their speciation and in their patterns of sympatric coexistence (Lack 1947; Grant 1999; Schluter 2000; Grant & Grant 2008a). At deeper levels of the phylogeny, niche differences are large and relatively consistent through space and time. That is, the ground finches (*Geospiza* spp.) feed often on seeds on the ground, the tree finches (*Camarhynchus* spp.) feed mainly on fruits and insects in trees, the vegetarian finch (*Platyspiza crassirostris*) has a diet mainly of leaves and fruit on the standing vegetation, and the warbler finches (*Certhidia* spp.) concentrate on mainly arthropods (Lack 1947; Bowman 1961; Grant 1999; Grant & Grant 2008a). These diet differences are coupled with differences in foraging traits (beak size and shape) in ways that clearly improve their foraging efficiency on the chosen resource type (Lack 1947; Bowman 1961; Grant 1999; Grant & Grant 2008a). Shallower in the phylogeny, corresponding to more recent speciation events, the picture is less obvious.

We here focus on the *Geospiza* ground finches (Fig. 2.1), all of which are very closely related and show at least some hybridization in sympatry (Grant & Grant 1997; Sato *et al*. 1999; Zink 2002; Grant & Grant 2008b). These finches might therefore inform the importance of niche partitioning when the outcomes of coexistence of diverging groups (see above) are uncertain. What is certain, however, is that the four species show morphologies that should suit them for foraging on different food types (Lack 1947; Bowman 1961; Grant 1999; Herrel *et al.* 2005a; Foster *et al.* 2008; Grant & Grant 2008a). *Geospiza scandens* (the cactus finch) has a long beak often used to probe the fruits and flowers of *Opuntia* cacti. Compared to this species, the other three have a beak that is comparably shorter and deeper, which they often use to crack seeds. Beak size, and the size and hardness of the seeds that can be cracked, increases dramatically from *Geospiza fuliginosa* (the small ground finch) to *Geospiza fortis* (the medium ground finch) to *Geospiza magnirostris* (the large ground finch). Despite these seemingly clear adaptive differences; dietary niche overlap between the species is considerable, particularly in the wet season (Smith *et al.* 1978; Schluter 1982; Boag & Grant 1984). This is also interesting because diet niche is thought to be the main ecological dimension separating these species (Lack 1947; Grant 1999).

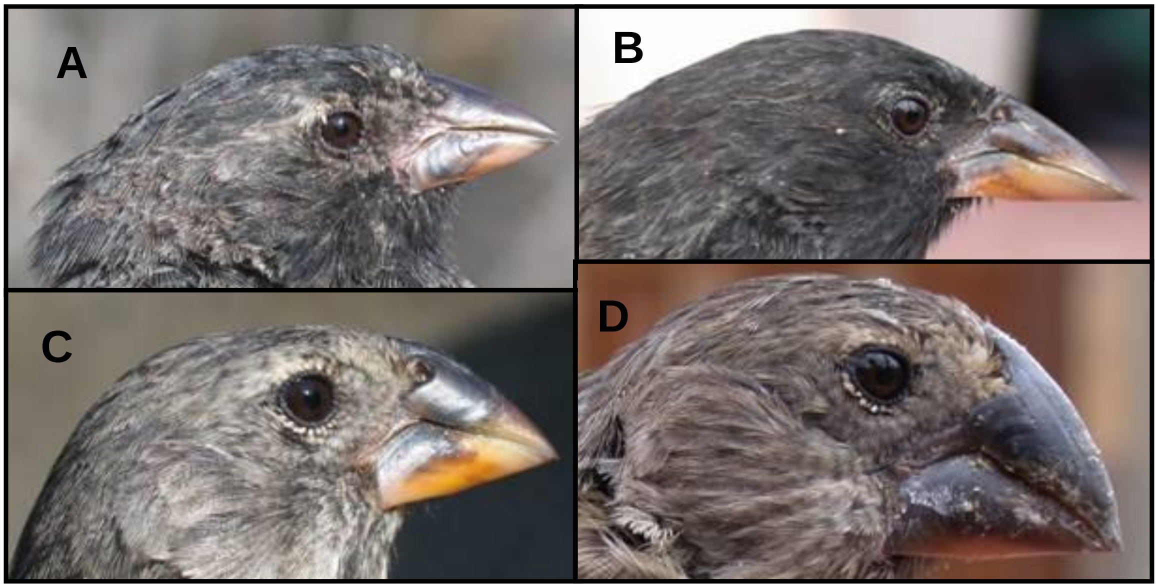

Figure 2.1 Characteristic beak morphology of Darwin's ground finches from Santa Cruz Island, Galapágos, Ecuador. The species are *Geospiza fuliginosa* (A), *Geospiza scandens* (B), *Geospiza fortis* (C) and *Geospiza magnirostris* (D).

We examine diet and niche overlap in the four *Geospiza* species over several years at several sites on Santa Cruz Island. This island is one of the largest, highest, and most ecologically diverse in Galapágos (Wiggins & Porter 1971; Parent *et al.* 2008). As a result, spatial variation in plants and finch diets is considerable (Wiggins & Porter 1971; Grant *et al.* 1976; Abbott *et al.* 1977; Smith *et al.* 1978). Like the other Galapágos Islands, Santa Cruz is characterized by dramatic seasonality, with most plants producing seeds during the wet season (Wiggins & Porter 1971; Grant 1999). The wetness of these "wet" seasons, is, however, quite variable, with dramatic differences among years in the amount of rain and therefore plant reproduction (Grant 1999; Grant & Grant 2006; Hendry *et al.* 2009). This dramatic variation across the island, between seasons and among years suggests the potential for competition and niche overlap in the *Geospiza* to also vary dramatically in space and time. The goal of our study was to quantify this variation, and interpret it in the context of the sympatric coexistence of closely related species.

2.3 Material and methods

2.3.1 Study sites

We studied the four ground finches at three sites (Academy Bay, El Garrapatero, Borrero Bay) on Santa Cruz Island, Galápagos, Ecuador (Fig. 1.2). Academy Bay is located at the southern edge of the island, where the coast is bordered by a dense belt of coastal vegetation, dominated by *Cryptocarpus pyriformes* and *Scutia spicata*. Our data collection took place along trails in this coastal zone. El

Garrapatero is located at the eastern edge of the island about 10 km from Academy Bay. A band of coastal vegetation is also present here, but our data collection took place 50-250 m inland from this band. Where our sampling took place, the dominant large plants are the trees *Bursera graveolens* and *Cordia lutea,* as well as the cactus *Opuntia echios*. Below these, the ground is relatively open and dominated, during wet conditions, by herbaceous plants and grasses. Borreo Bay is located at the northern edge of the island and has only a narrow and patchy band of coastal vegetation. Here, our data collection took place 10-250 m inland of the coastal vegetation in extensive areas of bare lava interspersed with red-soil areas dominated by *Bursera graveolens* and shrubs of *Scalesia* and *Hibiscus*.

2.3.2 Feeding observations

Feeding observations were based on morning and (sometimes) late afternoon walks that covered the entire survey area (approximately 0.25 km^2 at each site). Once a bird was encountered, we followed it and with the use of binoculars we identified the species and determined what it was eating – if anything. After this, we immediately moved on to search for another bird. Some of these observations (1200) were done on individual banded birds and will be part of my Chapter 3). The rest of the observation were done in non-banded birds. Our data thus represent counts of discrete feeding observations by particular species on particular food types. We adopted this point-observation procedure, rather than following individual birds for longer periods of time (Abbott *et al.* 1977; Smith *et* *al.* 1978), because we sought to increase independence among the observations. Results should nevertheless be qualitatively comparable between the two methods because both estimate the proportion of time each species feeds on each food type.

In many cases, the food item that a bird was eating could be easily identified to a specific plant species and part: flower, fruit, or seed. This precision was possible because Darwin's finches are very tame and can be easily observed through binoculars at short distances (2-5 m) while they engage in normal feeding behavior (Lack 1947; Grant 1999). In some cases, however, we had to recover remnants of the food item to confirm its identity. Plant and seed identification was made by reference to Wiggins and Porter (1971) and by comparison to collections at the Charles Darwin Research Station. When the food item was not an identifiable plant, it was classified into several other categories. "Insect" referred to situations where the bird was feeding on an insect or was searching for insects (an obvious behavior). "Ground" meant that the bird was feeding on very small seeds on the ground but the specific species of seed could not be identified. "Grass" was similar to "ground," except that the foraging took place on or immediately below dried grasses.

All data collection took place from 2003 to 2007 during the Galapágos "wet" season (January to April). The quotes around "wet" are because very little rain fell during the wet seasons of 2003-2006, effectively making them an extended dry season (Grant & Grant 2006; Hendry *et al.* 2009). We obtained data from at least three of these years for each of the three sites, and sample sizes were generally very large (Table 2.1).

2.3.3 Available foods

We surveyed the different food types available at each site by extending the methods of Abbott *et al*. (1977). We first used randomly-generated GPS coordinates within each site to position 50 different 1-m^2 plots. Within each plot, we first identified and counted the seeds, flowers, and fruits attached to standing vegetation for each plant species. We then identified and counted the seeds of each plant species on the ground. This was done for a 10 cm^2 subplot randomly positioned within each plot: both on the surface of the subplot in the field, and in a superficial soil sample of approximately 45 g that was sorted under a stereoscope in the laboratory. We sampled the same plots (with new subplots) in each of three years (2005, 2006, and 2007) at Academy Bay and El Garrapatero, and in each of two years (2005 and 2007) at Borrero Bay – although a few of the plots could not be found in the later years. We did not record insects in the plots because these were extremely rare. We used the Shannon-Weiner diversity index and Hurlbert´s (1971) evenness index to quantify the diversity of food items in various combinations of site and year.

For each of the 38 most common seed types consumed by finches, we measured 10 intact individual seeds collected from the ground. For each seed, we recorded length, width, and depth. Hardness was estimated by cracking individual seeds with a Kistler force transducer attached to a handheld Kistler charge amplifier (Kistler Inc., Winterthur, Switzerland). Following Abbott *et al.* (1977b), we estimated an index of seed hardness (H*i)* as a combined measure of the average seed depth (D) and hardness (H) for each species ($Hi = \sqrt{HD}$). This index was used because both larger and harder seeds should be more difficult for small-beaked birds to crack (Abbott *et al.* 1977; Grant 1999). For some plat species we were not able to estimate seed hardness in which case we used estimates reported by Abbott *et al*. (1970).

Table 2.1. Feeding observations from Darwin's finches recorded at different sites and in different years on Santa Cruz Island, Galápagos, Ecuador.

Site	Year	*G. fortis*	*G. fuliginosa*	*G. magnirostris*	*G. scandens*	Total
Academy Bay	2003	870	364	65	129	1428
	2004	707	201	66	98	1072
	2005	511	198	31	84	824
	2006	525	136	43	24	728
	2007	51	13	6	12	82
Sub-total		2664	912	211	347	4134
Borrero Bay	2004	60	5			65
	2005	154	26		3	183
	2007	201	50	2	5	258
Sub-total		415	81	2	8	506
El Garrapatero	2003	342	148		21	511
	2004	244	23	2	23	292
	2005	618	98	18	23	757
	2006	811	161	26	33	1031
	2007	89	39	5	9	142
Sub-total		2104	469	51	109	2733
Grand Total		5183	1462	264	464	7373
%		70.30	19.83	3.58	6.29	

2.3.4 Partitioning the variance in diet

Here we test how the feeding of ground finches varies according to available resources (food types), and as a function of year and site. First, we log-transformed the data when necessary to improve normality. Second, we used CANOCO (version 3.1) to perform an unconstrained ordination (PCA) on all the feeding observations. This allowed us to infer the degree of diet partitioning among species. Third, we also used CANOCO to perform a redundancy analysis (RDA). This analysis allowed us to partition the variance in species-specific diets according to that explained by year, site, and the interaction between year and site (Borcard *et al.* 1992). The significance of these results was assessed with the unbiased variance partitioning estimator developed by Peres-Neto *et al.* (2006). Fourth, we further examined the variance in diet by performing a non-parametric multivariate analysis of variance. This allowed us to infer diet differences among species based on count data. This analysis was based on Bray-Curtis distance matrices of proportionally scaled diets using the Adonis function (Anderson 2001) in the software R (R Development Core Team 2007). Statistical significance was obtained after 1,000 permutations of the raw data.

2.3.5 Niche overlap

Using data for finches feeding on specific food items (i.e., plant species and parts), we calculated niche overlap between species at four levels: 1) overall niche overlap across all feeding observations (i.e., pooling all sites and years), 2) niche overlap at a given site (i.e., pooling the different years), 3) niche overlap in a given year (i.e., pooling the different sites), and 4) site- and year-specific niche overlap. In all cases, we used both Pianka's (1973) and Czechanowski's (Feinsinger *et al.* 1981) niche overlap indices as implemented in EcoSim V.7.72

(Gotelli & Entsminger 2009). These indices assume symmetry in the degree of niche overlap, and they range from zero (no resources in common) to unity (perfect overlap in resource use). Indices were calculated with and without correcting for available food resources (Hurlbert 1978) by dividing diet values by the relative abundance of each resource type. These analyses excluded food categories not specifically recorded in the available food surveys (e.g., insects, ground).

EcoSim was also used to generate null models of expected niche overlap (Gotelli & Entsminger 2009). These models allowed us to estimate whether a given species pair showed significantly greater or lesser niche overlap than expected at random under a given randomization algorithm. We specifically used 1000 permutations of the RA3 algorithm (Lawlor 1980), which randomizes the types of resources used by different species but fixes the number of species and their niche breadth. This algorithm is particularly powerful for detecting non-trivial pattern of niche overlap in natural systems (Winemiller & Pianka 1990) – and similar results were obtained with different algorithms (results not shown). In order to assess the general validity of our results we reviewed the literature to collect published estimates of Pianka's (1973) niche overlap indexes in bird populations.

2.4 Results

2.4 .1 Feeding observations

When interpreting these data, it must be remembered that they are based on counts. Because seeds vary dramatically in size and energy content, they do not represent the relative contribution of each plant type to the total energy budget by a bird. In general, however, we are interested in differences among species, sites, and years in the relative contributions of each food type. These sorts of comparisons can reasonably be made based on count data.

We obtained a total of 7373 discrete feeding observations of ground finches (Table 2.1). All four species were observed at all three sites, but the frequency of observations varied considerably: *G. fortis* represented 67% of all feeding observations, followed by *G. fuliginosa* (19%), *G. scandens* (6%), and *G. magnirostris* (3%). The frequency of observations was highest at Academy Bay (57%), intermediate at El Garrapatero (36%), and lowest at Borrero Bay (6%). Interestingly, Academy Bay is adjacent to the human population of Puerto Ayora, where there seems to be a tendency in finches to explore human food items and introduced plants species (Chapter 5). This phenomenon may be altering the availability of feeding resources and consequentially the niche overlap among species. More than 1000 observations were made in each year, except for 2007 (N = 482). The variation in sample size among species and years was partly a result of relative species abundances (e.g., *G. magnirostris* is relatively rare), and partly a result of effort (less time was spent doing feeding observations in 2007 and at Borrero Bay).

We identified 51 different plant species, or other food categories, consumed by ground finches (Appendix 2.1-2.4). At Academy Bay, the most common plants in the diet were *Scutia spicata* (32% of observations), *Portulaca oleracea* (11%), *Cryptocarpus pyriformis* (10%), *Tournefortia psilostachya* (5%), *Cordia lutea* (4%), and *Trianthema portulacastrum* (2%). At El Garrapetero, they were *Scutia spicata* (15%), *Opuntia echios* (3%), *Bursera graveolens* (3%), *Boerhaavia caribaea* (2%), and *Castela galapageia* (2%). At Borrero Bay, they were *Croton scouleri* (17%), *Scutia spicata* (14%), *Cordia leucophlyctis* (4%), *Cryptocarpus pyriformis* (3%), and *Waltheria ovata* (2%). In addition, many observations, particularly at El Garrapatero, were of unidentified small seeds on the "ground." Finally, "insects" represented 3% of the feeding observations in Academy Bay, 34% at El Garrapatero, and 34% at Borrero Bay.

2.4 .2 Available food resources

We here include only plant species from which specific edible parts (seeds, flowers, fruits, buds) were recorded within the surveyed plots. We found 56 plant species (Appendix 2.5) that represented a wide distribution of seed size/hardness – and these distributions differed among the three sites (Fig. 2.2). At Academy Bay, the most common plant foods by frequency were *Cryptocarpus piryformis* (40%), *Tournefortia psilostachya* (20%), *Scutia spicata* (11%), *Cordia lutea* (6%), *Tournefortia pubescens* (4%), and *Portulaca oleracea* (3%). By volume, they were *Cordia lutea* (77%), *Scutia spicata* (4%), *Castela galapageia* (2%), *Lantana peduncularis* (2%), *Passiflora foetida* (2%), *Cordia leucophlyctis* (1%). At El Garrapetero, they were *Cordia leucophlyctis* (14%), *Croton scouleri* (12%), *Waltheria ovata* (9%), *Chamaesyce sp.* (8%), *Lantana peduncularis* (7%), *Vallesia glabra* (5%), *Scutia spicata* (4%), and *Tournefortia psilostachya* (4%). By volume, they were *Cordia lutea* (56%), *Castela galapageia* (8%), *Tribulus cistoides* (7%), *Scutia spicata* (4%), *Cordia leucophlyctis* (2%), *Bursera graveolens* (2%), *Passiflora foetida* (1%). At Borrero Bay, they were *Cryptocarpus piryformis* (80%), *Croton scouleri* (6%), *Tournefortia psilostachya* (4%), *Scutia spicata* (3%), *Maytenus octogona* (2%), and *Waltheria ovata* (2%). By volume, they were *Cordia lutea* (71%), *Scutia spicata* (6%), *Maytenus octogona* (3%), *Bursera graveolens* (2%), *Cordia leucophlyctis* (1%), *Opuntia echios* (1%). Also very common (29%) at El Garrapatero was a more inclusive category of very small "grass" seeds, including *Setarea cetosa, Cyperus confertus*, and *Leptochloa sp.*. By volume however; this category contributed less than 1%.

Shannon-Weaver indices of diversity were 2.02 for Academy Bay, 1.03 for Borrero Bay and 2.43 for El Garrapatero. Hulbert's index of evenness was 0.56 at Academy Bay, 0.73 at Borrero Bay and 0.91 at El Garrapatero. For volume, Shannon-Weaver indices of diversity were 1.93 for Academy Bay, 1.56 for Borrero Bay and 2.14 for El Garrapatero. Hulbert's index of evenness was 0.74 at Academy Bay, 0.62 at Borrero Bay and 0.76 at El Garrapatero. These indices also increase through time (from 2005 to 2007) at each site. For instance, the Shannon-Weaver index increased from 1.5 to 2.5 at Academy Bay, from 0.6 to 1.97 at Borrero Bay and from 2.33 to 2.61at El Garrapatero. The Hulbert's index of evenness increase from 0.65 to 0.89 at Academy Bay, from 0.21 to 0.80 at Borrero Bay and from 0.86 to 0.91 at El Garrapatero.

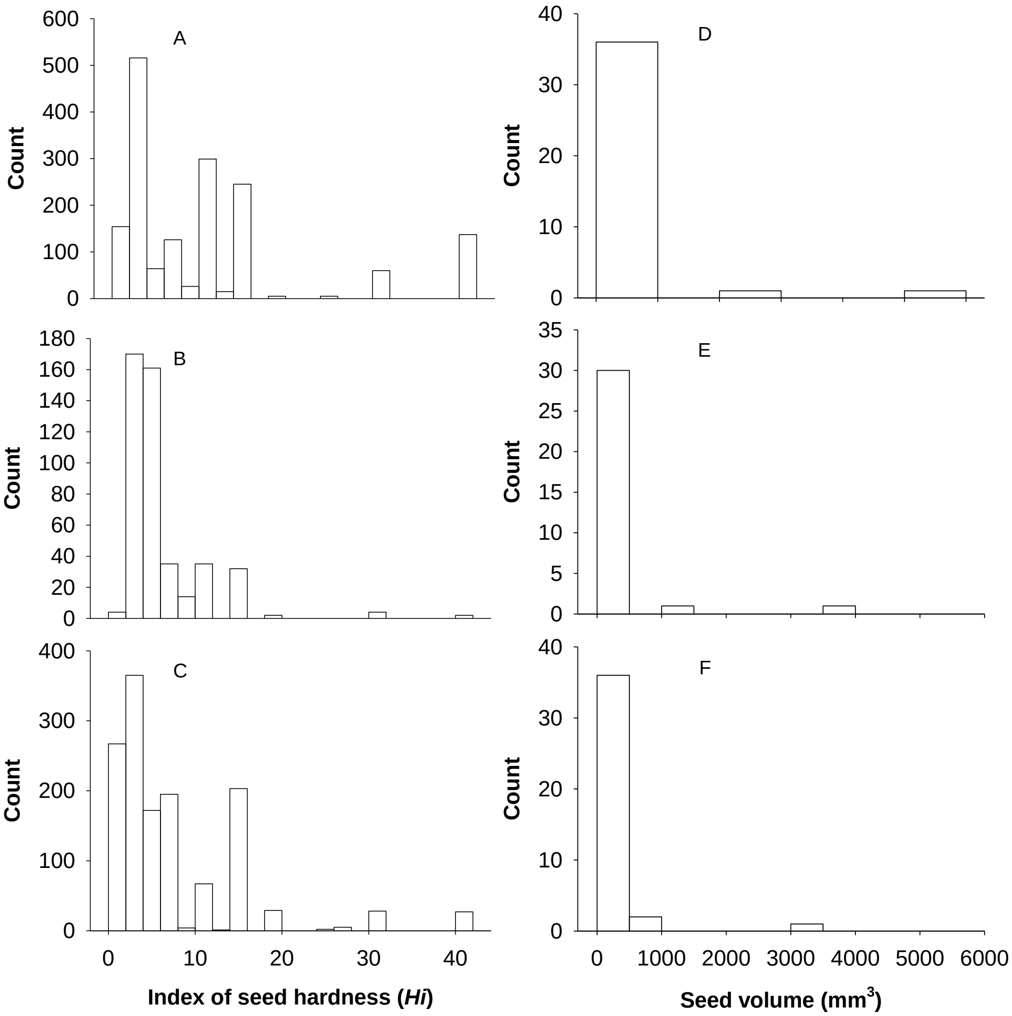

Figure 2.2 Histograms of the properties of available resource at three sites on Santa Cruz Island. Panels A-C represent the index of seed hardness of different seed types commonly consumed by ground finches at Academy Bay (A), Borrero Bay (B) and El Garrapatero (C). Kolmogorov-Smirnov tests indicated that seed distribution was significantly different between pairs of sites: A-B, D=0.358, P < 0.001; A-C, D=0.233, P < 0.001; B-C, D=0.235, P < 0.001. Panels D-F represent the volume (V=3/4πr2) of different seed types commonly consumed by ground finches at Academy Bay (D), Borrero Bay (E) and El Garrapatero (F). Kolmogorov-Smirnov tests indicated that the distribution was not significantly different between pairs of sites: D-E, D=0.1628, P = 0.746; D-F, D=0.2476, P = 0.188; E-F, D=0.131, P = 0.921.

2.4 .3 Explaining diet variation

The different ground finch species differed considerably in their diets (Fig. 2.3; Table 2.2; Appendix 2.1-2.4). The most common plant species and food categories consumed are: for *G. fortis, Scutia spicata* (30%), ground (23%), insect (18%), *Portulaca oleracea* (5%), *Tournefortia psilostachya* (2%), *Cryptocarpus pyriformis* (2%), grass (2%) and *Bursera graveolens* (2%). For *G. fuliginosa*, *Cryptocarpus pyriformis* (23%), ground (20%), *Portulaca oleracea* (14%), insect (10%), grass (8%) and *Scutia spicata* (7%). For *G. magnirostris, Cordia lutea* (37%), *Scutia spicata* (31%), ground (14%), insect (7%), *Bursera graveolens* (2%) and *Cordia leucophlyctis* (2%)., and for *G. scandens, Opuntia* (29%), ground (24%), *Scutia spicata* (15%), *Portulaca oleracea* (8%), insect (8%), *Jasminocereous thouarsii* (3%), *Trianthema portulacastrum* (3%) and *Tournefortia psilostachya* (3%). In general, these preferences held within each of the sites.

Although the different species showed some reasonably consistent differences in diet, as described above, the diet of each species also varied in space and time (Fig. 2.3; Appendix 2.1-2.4). *G. fortis* was the most variable species, as seen in the particularly dramatic differences between sites and years. *G. magnirostris* and *G. scandens* were the least variable, whereas *G. fuliginosa* showed an intermediate level of variation. Formal variance partitioning using RDA in CANOCO showed that 23% of the variation in species specific diets could be attributed to differences among sites (P = 0.01), 15% to differences among years (P = 0.04), and 11% to shared variation between year and site. The strong site effect was seen most clearly for *G. fortis* and *G. fuliginosa*, whose diets sometimes clustered more closely by site than by species.

Comparing the two data sets (feeding observations versus available food resources), revealed that the most common food types in the diet of ground finches were also the most common available foods (Appendix 2.1-2.5). This was also seen when comparing the frequency of feeding to the frequency of available food (Fig. 2.4). This suggests that, in general, ground finches are rather opportunistic in their diets.

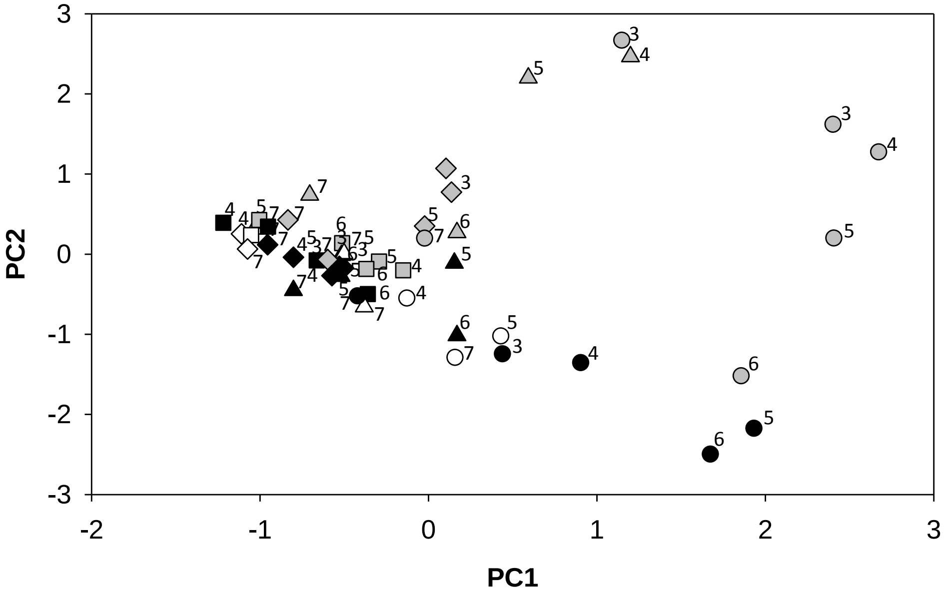

Figure 2.3 Principal component analysis (PCA) on diets of the species of Darwin’s ground finches on Santa Cruz Island, Galápagos. Colors indicate different sites: Academy Bay (gray), Borrero Bay (white) and El Garrapatero (black). Symbols indicate different species: *G. fortis* (circles), *G. magnirostris* (squares), *G. scandens* (rhomboids) and *G. fuliginosa* (triangles) and numbers indicate different sampling years from 2003 - 2007.

Table 2.2 Permutational multivariate analysis of variance using distance matrices. The data represent frequencies of feeding observations of Ground finches on Santa Cruz Island. The test includes foraging frequency by species (*G. fuliginosa, G. fortis, G. magnirostris and G. scandens*), site (Academy Bay, Borrero Bay and El Garrapatero), years (2003-2007) and their interaction. P-values were obtained from 1000 permutations

Test	Df	SS	MS	F	R^2	*P*
Model 1: ANOVA of the foraging frequency at different sites						
a) Species	4	5.23	1.31	8.39	0.37	0.001
b) Site	2	0.96	0.48	3.08	0.07	0.001
c) Species*site	12	3.34	0.28	1.79	0.24	0.001
Residuals	29	4.51	0.16	0.32		
Model 2: ANOVA of the foraging frequency in different years						
a) Species	4	5.23	1.31	6.68	0.37	0.001
b) Year	4	0.67	0.17	0.85	0.05	0.71
c) Species*Year	20	4.43	0.22	1.13	0.31	0.18
Residuals	19	3.72	0.19	0.26		

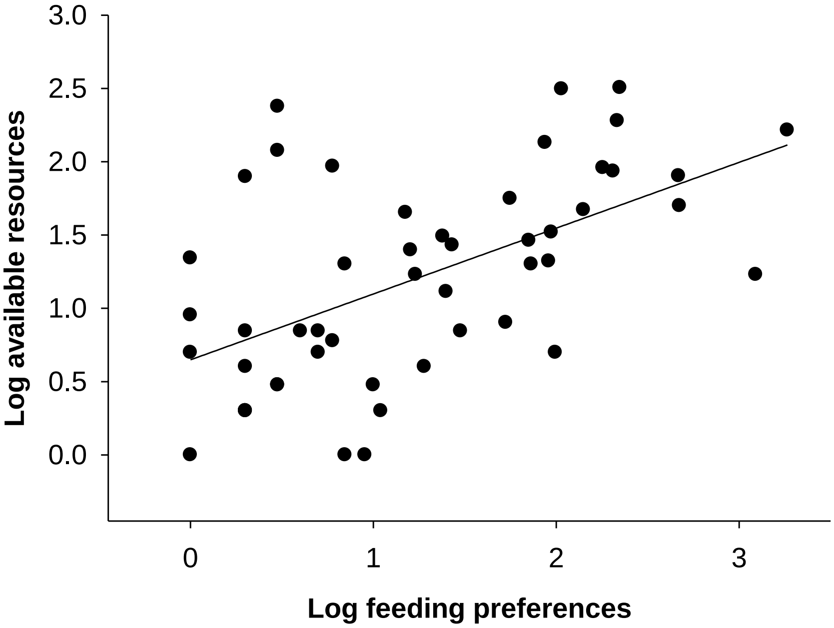

Figure 2.4 The diet in ground finches seems to be strongly influenced by the abundance of available resources (r=0.31, $P < 0.001$).

2.4 .4 Niche overlap

Pianka's niche overlap (combining all sites and years) before correcting for food availability ranged from 0.08 between *G. fuliginosa* and *G. magnirostris* to 0.96 between *G. fortis* and *G. magnirostris* (Fig. 2.5). After correcting for food availability they were much lower, ranging from 0.0001 between *G. fuliginosa* and *G. magnirostris* to 0.76 between *G. fortis* and *G. magnirostris*. The ground finches thus vary in the degree of diet overlap – although randomization analyses in EcoSim suggest that this overlap is nearly always greater than expected by chance when food availability is not corrected for (Fig. 2.5). After correcting for food availability, however, many of the niche overlap indices revealed diets that overlapped significantly less than expected at random, except for *G. fortis* versus *G. fuliginosa* and versus *G. magnirostris* (Fig. 2.5). Niche overlap in general increased from dry to wet years which reflected an increase in the abundance of available resources. A possible bias in these estimates may be that we could not correct for the availability of some of the feeding resources. For instance, insects can represent up to 34% of the finch diet in some years at some of the sites, however we were not able to estimate insect abundance an any of the sites. This could generate high diet overlap among different species. We have notice however that insect are only abundant in the rainy periods where the strength of selection is weakest. Therefore high overlap in this food category seems to be less important in determining the divergence among species. Finally, our estimates of niche overlap among Ground finches largely fitted the 123 estimates of niche overlap indices collected from the published literature (Fig. 2.5). Importantly, we did not survey the abundance of insect at any of the sites

Very large variation in niche overlap was evident among sites, years, and species pairs. To help interpret high variation in niche overlap (Fig. 2.5), we applied a general linear model (species pair, site, year, and all two-way interactions) to the data for Academy Bay and El Garrapatero in 2004-2007 (i.e., niche overlap between each pair of species at each site in each year). These particular sites and years were chosen because they present a balanced structure with high sample sizes. We performed this analysis as an exploratory tool, rather than for hypothesis testing, because the data points are not independent: i.e., the same species contributes to each of three measures of niche overlap – one with each of the other three species. When not correcting for food availability, this analysis suggested that niche overlap differed between species pairs (F = 5.16, P = 0.006) and among years (F = 11.71, P < 0.001) but not between sites (F = 0.13, P = 0.720). A significant interaction was present between species and site (F = 5.16, P = 0.028) but not between species and year (F = 1.46, P = 0.238) or between year and site (F = 1.01, P = 0.54). When correcting for available foods, none of the comparison were significant. For instance, niche overlap did not significantly differ between species pairs (F = 1.16, P = 0.11) and among years (F = 0.052, P < 0.35), between sites (F = 0.035, P = 0.69). An interaction was present between species and site (F = 1.14, P = 0.68) but not between species and year (F = 0.035, P = 0.113) or between year and site (F = 0.279, P = 0.337).

We interpret all of this variation as follows. First, ground finches at Academy Bay and El Garrapatero show similar average niche overlap, whereas those at Borrero Bay seemingly show higher overlap (Fig. 2.5). Second, *G. fortis* shows relatively high niche overlap with the three other species, whereas *G. fuliginosa* and *G. magnirostris* show conspicuously low overlap with each other – especially at Academy Bay. Third, niche overlap varied through time (Fig. 2.5), being lower early in the time series and higher late in the time series.

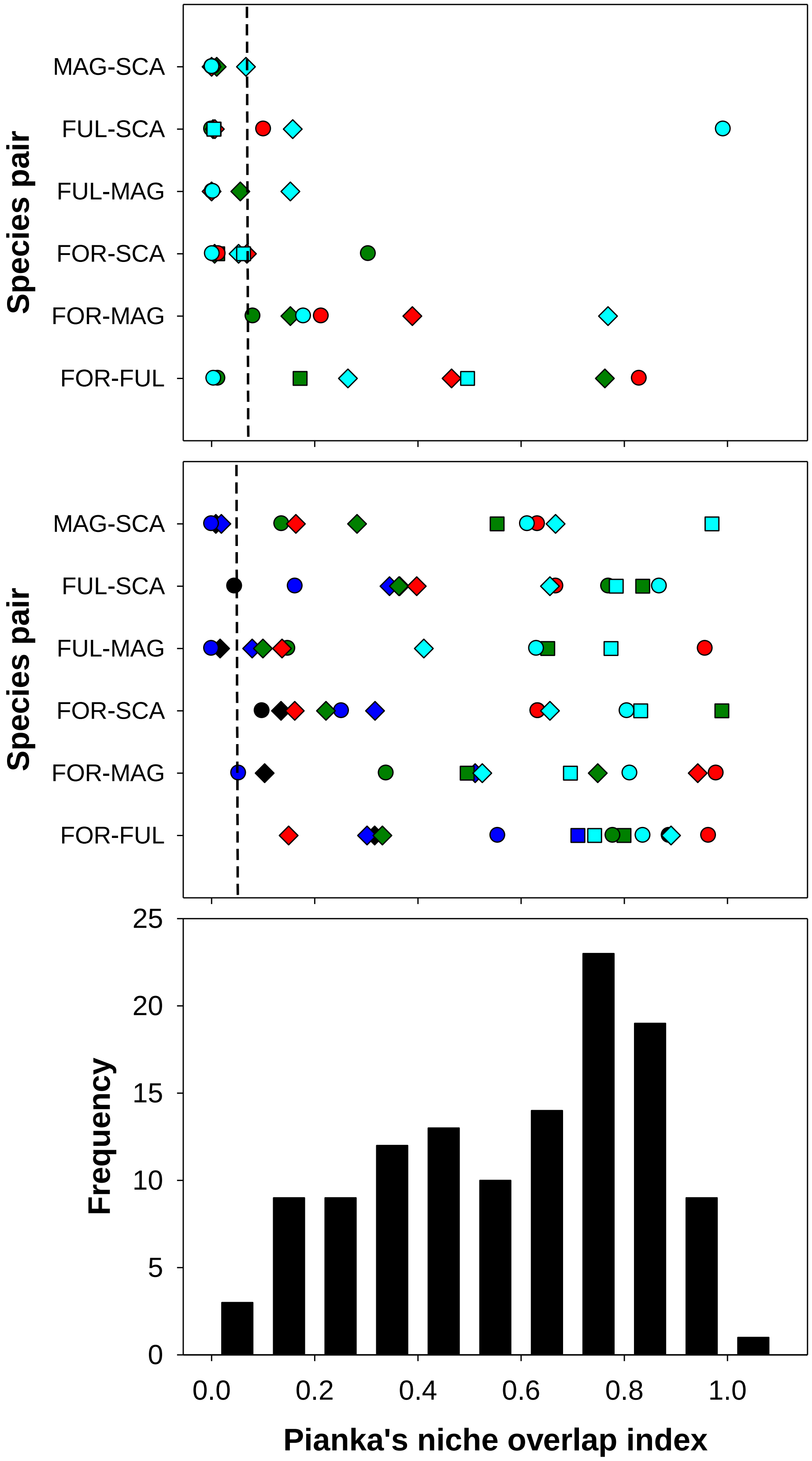
Species pair
MAG-SCA
FUL-SCA
FUL-MAG
FOR-SCA
FOR-MAG
FOR-FUL
Species pair
MAG-SCA
FUL-SCA
FUL-MAG
FOR-SCA
FOR-MAG
FOR-FUL
Frequency
25
20
15
10
5
0
0.0
0.2
0.4
0.6
0.8
1.0
Pianka's niche overlap index

Figure 2.5 Temporal and spatial variation in niche overlap between pairs of species of Darwin's ground finches from Santa Cruz Island. The upper panel shows values of niche overlap controlling for the abundance of available feeding resources. The middle panel shows values of niche overlap when disregarding the abundance of feeding resources. The data represent 7373 feeding observations recorded at three different sites: Academy Bay (diamonds), Borrero Bay (squares) and El Garrapatero (circles) and different years: 2003 (black), 2004 (dark blue), 2005 (green), 2006 (red) and 2007 (light blue). Statistical significance was tested by comparing observed niche overlaps with pertinent null expectations. For simplicity, the dashed line represents the mean expected niche overlap derived from the null model after 1000 simulations of the raw data. The lower panel shows the frequency distribution of Pianka's (1973) niche overlap indexes in birds. The data represent 123 estimates found in the literature. This index ranges from 0 (no overlap) to 1 (maximum overlap). n=122, x=0.5867, sd=0.25

2.5 Discussion

2.5 .1 Ground finches are imperfect generalists

A first important observation is that the different ground finch species do not have the same diets in sympatry (Appendix 2.1-2.4; Fig. 2.3). For instance, *G. magnirostris*, which has the largest beak and highest bite force, was the only species to feed on the very large/hard seeds of *Cordia lutea* – and it fed on these seeds regularly. *G. scandens*, which has the longest beak, were often seen probing the flowers of *Optuntia* cactus whereas this behavior was rare in the other species. *G. fuliginosa*, which has the smallest beak and lowest bite force, often fed on the very small seeds of *Cryptocarpus piryformis*, whereas this was uncommon for the other species. Such diet differences, and their association with beak size and shape, have been reported previously for Darwin's finches (Lack 1947; Bowman 1961; Abbott *et al.* 1977; Smith *et al.* 1978; Schluter & Grant 1984; Grant 1999). Our data thus conform to the basic idea that the adaptive divergence of Darwin's ground finches has involved divergence along a beak size – bite force – seed size – seed hardness axis.

A second important observation is that, despite the above-described diet differences, the ground finches often overlap more in diet than would be expected by chance (Fig. 2.5). A review of the literature revealed that the range of niche overlap values observed among these species spanned the typical range of niche overlap values in other bird species, which itself spans the entire range from 0 to 1 (Fig. 2.5). When correcting for available foods, niche overlap decreased dramatically to the point where it was often lower than expected by chance, except (as expected) for species closest to each other in beak morphology (Fig. 2.1). That is, *G. fortis* often showed high overlap with *G. fuliginosa* and *G. magnirostris*. Overall, then, some niche conservatism is evident. This also is not surprising given that all of the ground finches do often feed on seeds and, when available, insects. Moreover, our finding that the most common food types consumed at a given site correspond to the most common food types found at that site (Appendix 2.1-2.5; Fig. 2.4), shows that Darwin's finches are somewhat opportunistic in their diets. In short, a number of these food types can be consumed by all of the species and these food types are used when available. This result is also consistent with previous work showing that finches do often overlap in diet – and thereby compete for shared resources (Abbott *et al.* 1977; Smith *et al.* 1978; Grant & Grant 2006).

Based on these observations, we suggest that Darwin's finches correspond to a model of the adaptive radiation of "imperfect generalists" (*sensu* Barrett *et al.* 2005). That is, the different species evolved to use a variety of overlapping resources – but this overlap is not complete. Indeed, recent studies have shown that imperfect generalists are precisely what arise when adaptive radiation proceeds in complex environments (Barrett *et al.* 2005). In this situation, sympatry is maintained by frequency dependent processes, whereby strains that have at least some "private" resources cannot be eliminated by others (Barrett *et al.* 2005). The key to this idea is that there are some situations where the use of private resources is necessary – hence the evolution of differential adaptation to those resources. We sought the potential for such situations by considering spatial and temporal variation on Santa Cruz Island.

2.5.2 Spatio-temporal variation and species coexistence

Temporal variation in diet (Table 2.2) and niche overlap (Figs. 2.5) was very large. For example, the range of diet overlap values spanned almost the entire possible range from zero (no overlap) to unity (complete overlap) and therefore also spanned the entire range of overlap values observed for other bird species (Fig. 2.5). This variation allowed us to consider factors that influence niche variation in space and time.

The most striking observation was that niche overlap between all species increased from 2003 to 2007 (Figs. 2.5). The same pattern was maintained when controlling for available resources (Fig. 2.5). This change is likely related to the increasing precipitation from relatively dry years at the beginning of our study to relatively wet years at the end of our study. Consequently, this also reflected an increasing diversity and abundance of available resources at each site (see results). Previous studies of Darwin's finches have found that niche overlap can (1) increase during rainy periods because of the higher abundance of seeds and insects – when all species converge on easily accessible and nutritious food types, and (2) decrease during dry periods – when these foods become rarer and species diverge onto less profitable resources for which their morphologies are best suited (Abbott *et al.* 1977; Smith *et al.* 1978; Schluter 1982; Boag & Grant 1984; Grant & Grant 2006). These results fit nicely with recent experimental studies in other taxa showing that greater resource limitation leads to greater diet specialization – and that this specialization involves individuals increasingly focusing on foods for which their morphology is well suited (Svanbäck & Bolnick 2007a). Darwin's finches thus conform to the idea that sympatric coexistence of closely related imperfect generalists might be facilitated by episodic periods of resource limitation where diets diverge onto those foods for which specific species are best suited (Schoener 1982; Smith 1991).

Spatial variation was also quite strong, with ground finch diets often clustering as strongly by site (Academy Bay, El Garrapatero, and Borrero Bay) as by species or year (Fig. 2.3). The reason is that the different sites have different available foods (Appendix 2.5; Abbott *et al*. 1977; Smith *et al*. 1978) with different size/hardness properties (Fig. 2.2). This is was reflected by the differences in diversity and abundance of available resources at each site (see results). From the perspective of species coexistence, these ideas become most interesting when considered in combination with the above-described temporal variation. In particular, Darwin's finches at each site seem to converge on the most abundant and highest quality foods, such as insects in certain years at El Garrapatero and *Scutia spicata* especially at Academy Bay, when those resources are readily available. When resources become more limited, however, finches apparently begin to retreat to the private resources for which they are differentially adapted, and these resources differ in their abundance across the different sites. When this happens, we would expect the relative success of different species to differ among sites. When resources flourish again, and population sizes increase, dispersal among sites can then help to maintain system-wide sympatry. In short, we suggest that dispersal among sites with different resources can maintain the coexistence of closely related imperfect generalists in a meta-community dynamic.

2.5 .3 Summary and conclusion

What then of the dichotomous schools of thought outlined in the introduction: essentially the adaptive and non-adaptive views of species radiation and coexistence? On the one hand, Darwin's ground finches do show some niche conservatism in that they have diets that sometimes overlap substantially. This makes sense given that they are at the tips of the radiation (Petren et al. 1999) and so must all be starting from a relatively recent common ancestor. Adaptation in the ancestral species would almost necessarily lead to reasonably similar adaptations in the recently-derived species. On the other hand, however, the ground finches do not have identical diets, instead feeding more often on food types for which their morphologies are best suited. That is, the ground finches are imperfect generalists, using a diversity of partially overlapping resources, but still retaining a series of private resources on which they are superior competitors. These conditions should promote adaptive radiation and sympatric coexistence (Barrett *et al.* 2005). In short, niche conservatism should be present in new species that coexist in sympatry even if adaptive divergence is the very reason for their diversification and coexistence.

Spatio-temporal variation might also be very important. When conditions are good (i.e., high rainfall), all species converge on the best resources – often insects. When conditions worsen, species will still prefer any abundant resources that remain, such as the fruits of *Scutia spicata*. When conditions are very bad (i.e., drought), species will increase their use of those resources for which their morphologies make them superior competitors: small seeds for *G. fuliginosa*, medium seeds for *G. fortis*, large seeds for *G. magnirostris*, and cacti for *G. scandens* (Fig. 2.5; Abbott *et al.* 1977; Smith *et al.* 1978; Schluter 1982; Boag & Grant 1984; Grant & Grant 2006). Thus, sympatric coexistence of species with overlapping niches is facilitated by episodic periods during which conditions necessitate specialization on those resources for which species are superior competitors (Grant & Grant 2002). Spatial variation will probably also be important – because available foods and diets differed dramatically among sites on the same island. This might mean that no single species can be the superior competitor for the island as a whole – and ongoing dispersal will maintain meta-communities of species with partially overlapping niches.

Our results thus suggest that both adaptive and non-adaprive processes could potentially play a central role in the coexistence of sympatric closely related species in Darwin's finches. These processes could also be important for other instances of adaptive radiation and ecological speciation.

2.6 Acknowledgments

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2.8 Appendix

Appendix 2.1 Diet of the medium ground finch (*Geospiza fortis*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003		2004			2005			2006		2007			Total
		AB	EG	AB	BB	EG	AB	BB	EG	AB	EG	AB	BB	EG	
Abutilon depauperatum	na	2		3											5
Alternanthera echinocephala	flower	2		3											5
Amaranthus dubius	na	4		1											5
Blainvillea dichomata	seed							1						1	2
Boerhaavia caribaea	bud										4				4
	seed	2	9	1		4			5		3				24
Boerhaavia erecta	green fruit				3										3
Bursera graveolens	green seed	1		2		1			3						7
	seed	3	8	1		1									13
	bud							1		5	7				13
	fruit		9	5		7	1		2						24
	seed	5	10	11		5	6		5						42
Castela galapageia	bud					4			1						5
	seed			1		5	1		1		1				9
	fruit	4	34			1									39
Chamaesyce sp.	na						3								3
Commicarpus tuberosus	flower									4					4
	bud									1	1	2			4
	seed	19		9		2	8		7	2					47

Appendix 2.1 (continuation). Diet of the medium ground finch (*Geospiza fortis*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003		2004			2005			2006		2007			Total
		AB	EG	AB	BB	EG	AB	BB	EG	AB	EG	AB	BB	EG	
Cordia leucophlyctis	bud										1				1
	fruit							1							1
	fruit	3		1			10		5			1			20
	green seed											3	18	8	29
Cordia lutea	bud									2					2
	seed							1	2	2					5
	flower	3					1		3						7
	fruit	7		1											8
	na	22									1				23
	green seed			4											4
Croton scouleri	fruit											1			1
	seed												2		2
	na				3		1					1	12	1	18
	green seed				5							3	55	4	67
Cryptocarpus pyriformis	seed			1											1
	flower						1								1
	green seed				1			1		1					3
	bud	5		17			3								25
		38					2								40
	leaf	18		9	4	1	9			8		1	3		53

Appendix 2.1 (continuation). Diet of the medium ground finch (*Geospiza fortis*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003		2004			2005			2006		2007			Total
		AB	EG	AB	BB	EG	AB	BB	EG	AB	EG	AB	BB	EG	
grass	seed		88	0	0	4	1	0	3	0	0	0	10	1	107
ground	na	230	80	186		94	45	53	203	84	194	3	11	3	1186
Heliotropium angiospermum	seed			1											1
human food	human food			6			12			29		1			48
insect	insect	8		3	8	11	11	5	29	2	150		21	139	227
Lantana peduncularis	fruit												1		1
	flower									1					1
	seed			12										1	13
Lycium minimum	leaf	3							3		18				13
Maytenus octagona	seed				2										2
	fruit	11							2						13
Opuntia echios	fruit						1								1
	seed	4	3	1		1			1						10
	flower					3	2		1	1	3				10
	na		1	3		2	9		1	15	1				32
Parkinsonia aculeata	bud									1					1
	flower						1		2						3
Passiflora foetida	green seed	3													3
Portulaca oleracea	bud	92		53			76					9		1	231
Prosopis juliflora	flower										5				3

Appendix 2.1 (continuation). Diet of the medium ground finch (*Geospiza fortis*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003		2004			2005			2006		2007			Total
		AB	EG	AB	BB	EG	AB	BB	EG	AB	EG	AB	BB	EG	
	bud						3		1						4
Rhynchosia minima	green seed	1	11			1									13
Sarcostemma angustissima	flower										1				1
Scutia spicata	green fruit				13			7				2	15		37
	flower								4						4
	green fruit						2		5	3		1	1		12
	green seed						42	1	10	44	15	2			114
	fruit			1			21		15	48	47	1	2	4	139
	dry seed	3		116		26	91		70	209	33				548
	seed	260	4	220	1	37	205	22	110	250	93	1	0	0	1203
Sida ciliaris	na	14		4					1						19
Tournefortia psilostachya	green seed			1											1
	bud								2	1					3
	seed			32						1	1				34
	na	63	1	33		1	14								112
Tournefortia pubescens	fruit										2				1
	green seed									1					1
	seed			6			2		1	6	1				16
	na	24	1				1		1						27

Appendix 2.1 (continuation). Diet of the medium ground finch (*Geospiza fortis*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003		2004			2005			2006		2007			Total
		AB	EG	AB	BB	EG	AB	BB	EG	AB	EG	AB	BB	EG	
	bud						3		1						4
Trianthema portulacastrum	seed			21											21
	bud	8		29			2		1						40
Tribulus cistoides	seed									1					1
	green seed			2			2		1	1					6
Vallesia glabra	bud							1							1
	green seed				1								1		2
	fruit			2				2	1						5
	seed			1			2	2	6	3	5	1			19
Waltheria ovata	bud							2							2
	flower							2							2
	na					1		5							6
Total		870	300	707	60	244	511	154	618	525	811	50	201	89	5140

Appendix 2.2 Diet of the small ground finch (*Geospiza fuliginosa*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003		2004			2005			2006		2007			Total
		AB	EG	AB	BB	EG	AB	BB	EG	AB	EG	AB	BB	EG	
Alternanthera echinocephala	flower	3		17			2			1					23
Alternanthera filifolia	flower	1		1											2
Amaranthus dubius	na	2													2
Blainvillea dichomata	seed													1	1
Boerhaavia caribaea	bud										4				4
	seed	4	26			2			2		3				37
Bursera graveolens	fruit			1											1
	leaf						3								3
	bud							1		11	7				19
Castela galapageia	fruit	3													3
	seed		4			2					1				7
Chamaesyce sp.	na						5								5
Commicarpus tuberosus	fruit									1					1
	seed	11		9		3	5		1	2	1		1		33
Cordia leucophlyctis	fruit												1		1
	flower										1				1
	green seed											1	2	11	14
Croton scouleri	na												2		2
	seed												5		2
	green seed												3		3

Appendix 2.2 (continuation). Diet of the small ground finch (*Geospiza fuliginosa*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003		2004			2005			2006		2007			Total
		AB	EG	AB	BB	EG	AB	BB	EG	AB	EG	AB	BB	EG	
Cryptocarpus pyriformis	leaf	1		1											2
	flower						3		2						5
	seed			19											19
	green seed				2			3		16					21
	bud									38					38
	fruit	78		6			5			3		1			93
	bud	45		50			43		8						146
grass	seed	0	80	0	0	1	0	0	1	0	0	0	11	7	100
ground	na	83	32	26		11	17	9	54	12	51	2	3	2	302
human food	human food									6					6
Insect	insect	2		3	1	1	6		3	1	28		2	2	49
Lycium minimum	leaf										1				1
Maytenus octogona	fruit	2													2
Opuntia	flower										2			2	4
	na						1			2					3
Parkinsonia aculeata	flower								2						2
Portulaca oleracea	bud	71		24			96					6			197
Prosopis juliflora	flower									1					1
	bud			2											2
Rhynchosia minima	green seed	1													1

Appendix 2.2 (continuation). Diet of the small ground finch (*Geospiza fuliginosa*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003		2004			2005			2006		2007			Total
		AB	EG	AB	BB	EG	AB	BB	EG	AB	EG	AB	BB	EG	
Scutia spicata	green fruit												2		2
	green seed						1				1				2
	seed	1		7					1	2					11
	seed	15	0	10	0	0	0	2	2	4	3	0	0	0	36
	fruit	2		1		1	6		6	25	12		1		54
Sida ciliaris	na	2					1								3
Tournefortia psilostachya	bud						1								1
	green seed			1											1
	seed			5											5
	na	31		5											36
Tournefortia pubescens	seed			2											2
	na	1	1	1											3
Trianthema portulacastrum	seed			4											4
	bud	1		8											9
Waltheria ovata	flower									1					1
	bud										3				2
	na	1	4					2	1						8
Total		364	148	201	5	23	198	26	98	136	161	13	50	39	1462

Appendix 2.3 Diet of the large ground finch (*Geospiza magnirostris*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003	2004		2005		2006		2007			
		AB	AB	EG	AB	EG	AB	EG	AB	BB	EG	Total
Alternanthera echinocephala	flower		1									1
Bursera graveolens	fruit		1									1
	seed		3			1						4
Castela galapageia	fruit	1										1
	bud			1								1
Cordia leucophlyctis	seed										1	1
	fruit	1			1							2
	green seed								2			2
Cordia lutea	fruit		1									1
	flower		4		1	1						6
	na	4	8	1								13
	seed	39	17		7	8	6					77
Croton scouleri	na				1							1
ground	na	10	13		4	1	2	3		1	1	35
insect	insect							3			1	4
Maytenus octogona	fruit	2										2
Parkinsonia aculeata	green seed						2					2
Passiflora foetida	green seed		3									3
Portulaca oleracea	bud	1			1				1		1	4

Appendix 2.3 (continuation). Diet of the large ground finch (*Geospiza magnirostris*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003	2004		2005		2006		2007			
		AB	AB	EG	AB	EG	AB	EG	AB	BB	EG	Total
Scutia spicata	green seed				1			2	2			5
	green fruit				1		4					5
	fruit				1	1	5	4				11
	seed		7		2	3	24	2				38
	seed	4	11	0	12	5	24	3	0	0	0	59
Tournefortia psilostachya	na	2										2
	seed		2									2
Vallesia glabra	bud	1										1
	seed		1									1
Total		65	66	2	31	18	43	26	6	2	5	264

Appendix 2.4 Diet of the cactus finch (*Geospiza scandens*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003		2004		2005			2006		2007			
		AB	EG	AB	EG	AB	BB	EG	AB	EG	AB	BB	EG	Total
Abutilon depauperatum	na	1												1
Boerhaavia caribaea	seed		1			2								3
	fruit		1											1
Bursera graveolens	seed		2											2
Castela galapageia	seed				1									1
	fruit		3											3
Commicarpus tuberosus	seed			1									1	2
Cordia leucophlyctis	green seed										3		2	5
Croton scouleri	green seed											1		1
Cryptocarpus pyriformis	fruit	4												4
grass	seed	0	1	0	0	0	0	0	0	0	0	0	0	1
ground	na	40	13	29	3	10		9	10	6			7	121
human food	human food					2								2
insect	insect	1			1			2	1		1			6
Jasminocereous thouarsii	seed	10				7								17
Lantana peduncularis	seed	1												1
Opuntia echios	fruit	1		1	1		1		3	1				8
	seed	19	17		3	3		1						43
	flower	10	22	10	12	14		2	2	12	1		1	86
	na					6		1		1				8

Appendix 2.4 (continuation). Diet of the cactus finch (*Geospiza scandens*) at three different sites on Santa Cruz Island: Academy Bay (AB), Borrero Bay (BB) and El Garrapatero (EG). The data represent number of foraging observations on both plant species and plant items of individual birds during a study of five years period (2003-2007).

Plant species	Food item	2003		2004		2005			2006		2007			
		AB	EG	AB	EG	AB	BB	EG	AB	EG	AB	BB	EG	Total
Portulaca oleracea	bud	11		14		11					2			38
Scutia spicata	green seed					1								1
	seed			9					1					10
	fruit			2		18		5	5	3				33
	seed	21	0	12	0	6	1	0	1	0	0	0	0	41
Sida ciliaris	na	2		1										3
Tournefortia psilostachya	seed			5										5
	na	6		5										11
Tournefortia pubescens	na	1												1
Trianthema portulacastrum	seed			8										8
	bud			9										9
Total		129	62	98	23	84	3	23	24	33	12	5	9	505

Appendix 2.5 Available food resources for Darwin's finches on Santa Cruz Island, Galápagos, Ecuador. The total represent number of food item per species found at different sites. Percentages are given in parenthesis.

	Academy Bay			Total	El Garrapatero			Total	Grand Total
Plant species	2005	2006	2007		2005	2006	2007		
Acacia nilotica		13	24	37 (0.09)		14	13	27 (0.15)	64 (0.11)
Acacia rorudeana	210	273	248	731 (1.73)	172	160	237	569 (3.2)	1300 (2.16)
Alternanthera echinocephala	58	12	77	147 (0.35)					147 (0.24)
Amaranthus sp.		6	11	17 (0.04)	6	20	12	38 (0.21)	55 (0.09)
Bastardia viscosa	12	10	42	64 (0.15)	3		31	34 (0.19)	98 (0.16)
Blainvillea dichotoma	1	1		2 (0)	197	291	298	786 (4.42)	788 (1.31)
Boerhaavia caribaea	1	21	188	210 (0.5)	12	42	55	109 (0.61)	319 (0.53)
Bursera graveolens	4	3	1	8 (0.02)	42	14	38	94 (0.53)	102 (0.17)
Cardiospermum galapageium	32		84	116 (0.27)		1		1 (0.01)	117 (0.19)
Castela galapageia		81	1	82 (0.19)	56	45	21	122 (0.69)	204 (0.34)
Chamaesyce sp.							806	806 (4.54)	806 (1.34)
Clerodendrum molle	5	5	3	13 (0.03)	2		5	7 (0.04)	20 (0.03)
Commicarpus tuberosus	310	159	662	1131 (2.68)	3	14	112	129 (0.73)	1260 (2.1)
Cordia leucophlyctis	541	190	1026	1757 (4.16)	619	138	660	1417 (7.98)	3174 (5.28)
Cordia lutea	701	235	1623	2559 (6.05)	27	4	66	97 (0.55)	2656 (4.42)
Croton scouleri	99	29	214	342 (0.81)	94	129	1005	1228 (6.91)	1570 (2.61)
Cryptocarpus piryformis	8695	5483	1856	16034 (37.94)					16034 (26.67)
Evolvulus convolvuloides	8	7		15 (0.04)	127	135	171	433 (2.44)	448 (0.75)
Evolvulus simplex							8	8 (0.05)	8 (0.01)
Galactia striata	44	47	1047	1138 (2.69)	20	2		22 (0.12)	1160 (1.93)
grass	18	18	715	751 (1.78)	330	281	2221	2832 (15.94)	3583 (5.96)
Heliotropium angiospermum						6	4850	4856 (27.34)	4856 (8.08)

Appendix 2.5 (continuation). Available food resources for Darwin's finches on Santa Cruz Island, Galápagos, Ecuador. The total represent number of food item per species found at different sites. Percentages are given in parenthesis.

	Academy Bay			Total	El Garrapatero			Total	Grand Total
Plant species	2005	2006	2007		2005	2006	2007		
human food	24	14	57	95 (0.22)					95 (0.16)
insect	4			4 (0.01)	20			20 (0.11)	24 (0.04)
Lantana peduncularis	42	40	259	341 (0.81)	3	12	693	708 (3.99)	1049 (1.74)
Opuntia echios	63	92	75	230 (0.54)	1	17	6	24 (0.14)	254 (0.42)
Parkinsonia aculeata					14	6	3	23 (0.13)	23 (0.04)
Passiflora foetida	72	77	34	183 (0.43)	4	7	4	15 (0.08)	198 (0.33)
Physalis pubescens	8	10	9	27 (0.06)	40	55	67	162 (0.91)	189 (0.31)
Portulaca oleracea	102	46	1217	1365 (3.23)	49	52	38	139 (0.78)	1504 (2.5)
Prosopis juliflora	8	75	6	89 (0.21)					89 (0.15)
Rhynchosia minima	24	8	134	166 (0.39)		6	263	269 (1.51)	435 (0.72)
Scutia spicata	533	3782	270	4585 (10.85)	112	244	27	383 (2.16)	4968 (8.26)
Sida ciliaris	135	45	155	335 (0.79)		4		4 (0.02)	339 (0.56)
Sida spinosa	7	2		9 (0.02)	48	220	35	303 (1.71)	312 (0.52)
Sidia rhombifolia							38	38 (0.21)	38 (0.06)
Tephrosia decumbens	23			23 (0.05)	67	48	25	140 (0.79)	163 (0.27)
Tournefortia psilostachya	4889	828	2141	7858 (18.59)	180	71	115	366 (2.06)	8224 (13.68)
Tournefortia pubescens	408	97	1102	1607 (3.8)	1	65	1	67 (0.38)	1674 (2.78)
Trianthema portulacastrum	130	51	107	288 (0.68)					288 (0.48)
Tribulus cistoides							61	61 (0.34)	61 (0.1)
Tribulus triangularis							101	101 (0.57)	101 (0.17)
Vallesia glabra	3			3 (0.01)	60	59	352	471 (2.65)	474 (0.79)
Waltheria ovata					316	271	268	855 (4.81)	855 (1.42)
Grand Total	17190	11746	13331	42267	2625	2433	12706	17764	60126

Linking statement between Chapter 2 and Chapter 3

In Chapter 1, I found that the ground finches represent imperfect generalists, showing only partial overlap in diets that vary in space and time, perhaps promoting their coexistence. Among these species, *Geospiza fortis* was the morphologically and ecologically most variable, including having the greatest overall niche width. *G. fortis* also shows strong bimodality on Santa Cruz Island. This raises the question of whether this species is a true generalist or might instead be composed of a collection of individual specialists? Such a determination is important because theoretical and experimental studies have shown that individual specialization can promote the evolution of niche expansion, and thereby promote and maintain morphological variation and disruptive selection. In Chapter 2, I therefore ask 1) what are the levels of individual specialization within *G. fortis?*, and 2) are these levels of individual specialization related to the morphological and genetic variation? This determination will allow me to consider the implications of individual specialization for the initial stages of adaptive divergence within an adaptive radiation.

CHAPTER 3

Individual specialization in Darwin's finches, with implications for their adaptive radiation

3.1 Abstract

Empirical and theoretical studies suggest that individual specialization can be an important diversifying force. However, few studies of natural populations – and none for birds – have explicitly considered the impact of individual specialization on adaptive divergence. We attempted such a consideration for Darwin’s finches from the Galapágos. We focused on the medium ground finch (*Geospiza fortis*) from El Garrapatero on Santa Cruz Island, a population showing large morphological and genetical variation resulting from ecologically-based adaptive divergence. We studied the interaction between individual specialization and adaptive/genetic divergence within this population. We found high levels of individual specialization and an association between individual diet differences and head size differences – the latter being an important determinant of feeding performance. Expected, but non-significant, trends were also seen for relationships between diet differences and either beak size or genetic differences. Overall, these findings are consistent with the hypothesis that individual specialization might contribute to the initial stages of adaptive divergence in Darwin’s finches. To our knowledge, this is the first study that combines morphological and genetic data to study the interplay between individual specialization and adaptive divergence in natural populations.

***Key words*:** Darwin’s finches, Galápagos, adaptive radiation, diversification, intra-specific competition, niche variation, ecological speciation.

3.2 Introduction

Generalist species can be composed of either generalist individuals, who use broad and similar ranges of resources, or specialist individuals, who use narrow and different ranges of resources (Van Valen 1965; Roughgarden 1972). The latter case, known as “individual specialization”, now appears to be a widespread phenomenon, evident across multiple taxa (Bolnick *et al.* 2003) and it has attracted increasing interest in the context of evolutionary diversification (Smith & Skúlason 1996; Bolnick *et al.* 2003; Bolnick *et al.* 2007). Indeed, individual specialization forms the core of several theoretical models of adaptive diversification (Wilson & Turelli 1986; Rueffler *et al*. 2006; Abrams *et al.* 2009; Day & Young 2009); and empirical studies suggest that it can promote the evolution of niche expansion (Svanbäck & Persson 2004; Bolnick *et al.* 2007; Svanbäck & Bolnick 2007), enhance adaptive divergence (Bolnick 2001; Martin & Pfennig 2009), and generate and maintain morphological and genetic variation (Bolnick & Paull 2009; Agashe & Bolnick 2010). Individual specialization thus could be an important evolutionary diversifying force. Few studies (Bolnick 2004; Martin & Pfennig 2009); however, have explicitly considered its role in promoting adaptive divergence in natural populations. In particular, individual specialization and its potential for divergence has been very rarely studied in bird populations, even though a number of examples have been confirmed in this taxon (e.g., Grant *et al.* 1976b; Price 1987; Werner & Sherry 1987; Scott *et al.* 2003).

Our goal in the present paper is to quantify individual resource use in a bird population that appears to be in the midst of an adaptive split driven by diet. We first use a series of niche specialization indices to quantify the degree of individual specialization. We then ask whether diet differences among individuals are associated with morphological (Bolnick & Paull 2009) or neutral genetic differences among individuals. In this regard, we predict that individuals with increasing diet differences will have increasing differences in foraging-related morphological traits. If the morphological traits are also associated with reproductive isolation, as is the case in our study group, we might expect an association between individual diet differences and individual neutral genetic differences. To our knowledge, ours is the first study to test for this latter association.

3.2.1 Darwin's finches

Darwin's finches of Galapágos are considered a classic example of adaptive radiation (Lack 1947; Grant 1999; Schluter 2000; Grant & Grant 2008b). In particular, different species show beak morphologies that are seemingly well suited for the exploitation of different food resources, such as seeds, fruits, insects, or nectar (Lack 1947; Bowman 1961; Abbott *et al.* 1977; Schluter & Grant 1984; Grant 1999; Schluter 2000; Grant & Grant 2008b). In one part of this radiation, the granivorous ground finches, beak size and shape are best suited for feeding on seeds with different size and hardness (Lack 1947; Bowman 1961; Abbott *et al.* 1977b; Schluter & Grant 1984; Grant 1999; Schluter 2000; Grant &

Grant 2008b). The small ground finch (*Geospiza fuliginosa)* has a small beak and feeds mostly on small and soft seeds. The medium ground finch (*Geospiza fortis*) has an intermediate beak and feeds mostly on intermediate seeds. The large ground finch (*Geospiza magnirostris*) has a large beak and feeds mostly on large and hard seeds.

Inter-specific niche specialization thus plays an important role in maintaining the adaptive radiation of Darwin's finches. We suggest that this inter-specific niche specialization might have originated from intra-specific (individual) specialization during the initial stages of population divergence. *G. fortis* on Santa Cruz Island are an excellent population to test this hypothesis because they often show highly variable, and sometimes bimodal, distributions of beak morphology (Hendry *et al.* 2006), bite force (Herrel *et al.* 2005a), and song characteristics (Podos *et al.* 2004; Huber & Podos 2006; Podos 2007; Herrel *et al.* 2009). The corresponding large and small beak size morphs also mate assortatively (Huber *et al.* 2007), experience disruptive selection (Hendry *et al.* 2009), and show modest differentiation at neutral genetic markers (Huber *et al.* 2007; De León *et al.* 2010).

Previous studies of *G. fortis* have suggested that it is cosmopolitan, possessing the widest niche breath of the ground finches species (Abbott *et al.* 1977; Smith *et al.* 1978; Schluter 1982; Boag & Grant 1984; Grant 1999). However, this ecological generality of *G. fortis* could be the result of high between-individual variation in resource use (i.e., individual specialization). Individual specialization has only been considered for Darwin's finches in two instances. First, Werner and Sherry (1987) found high individual specialization in the generalist Cocos finch (*Pinaroloxias inornata*). They suggested that this specialization might be related to behavior rather than morphology given the low morphological variation within this species (Werner & Sherry 1987; Grant 1999). Second, Price (1987) and Grant *et al*. (1976) found a strong positive association between diets and beak morphology among *G. fortis* on the small island of Daphne Major. They suggested that individual specialization might explain the high morphological variability in this population (Grant *et al.* 1976; Price 1987).

Our study differs from this earlier work by examining in more detail individual specialization in a bimodal population of *G. fortis*. If individual specialization promotes adaptive diversification, we would expect strong individual specialization in this population related to beak morphology and, perhaps, neutral genetic variation. We test for these associations by calculating and comparing individual diets (feeding observations), morphology (head dimension, beak dimension), and genetic variation (microsatellite markers).

3.3 Methods

3.3.1 Sampling, morphology, and genetics

We studied the diet of G. *fortis* at El Garrapatero on Santa Cruz Island, Galápagos, Ecuador. This site is located at the eastern edge of the island, and the dominant vegetation is *Bursera graveolens*, *Cordia lutea,* and the cactus *Opuntia echios.* Sampling took place between January and April over five consecutive years (2003 to 2007).

G. fortis were captured in mist nets and banded with unique combinations of three colored leg bands and a metal leg band with a unique code. These bands enabled us to avoid re-measuring the same birds, and to individually identify free-ranging birds. Beak dimensions were measured following Grant *et al*. (1985), and head dimensions were measured following Herrel *et al*. (2005b). The specific measurements include beak length (anterior edge of nares to anterior tip of upper mandible), beak depth (at the nares), beak width (at the base of the lower mandible), head length (from the tip of the upper mandible to the back of the head), head depth (at the deepest part of the head posterior to the orbits), and head width (the widest part posterior to the orbits).

Blood samples were taken from each bird by pricking the ulnar vein with a needle, and blotting the blood on filter paper treated with EDTA, as described in Chapter 4. DNA was amplified by PCR and screened for allelic variation at 10 di-nucleotide microsatellite loci: Gf03, Gf04, Gf05, Gf07, Gf08, Gf09, Gf10, Gf11, Gf12, Gf13 and Gf16 (Petren 1998). More details on these genetic methods are provided in Chapter 4.

3.3.2 Diet data

Feeding observations were conducted during morning and afternoon walks at each site. Each time a banded bird was seen, it was followed and observed through binoculars until a foraging event occurred. This was possible because Darwin’s finches are very tame and can be easily observed through binoculars at short distances (2-5 m) while engaged in normal feeding behavior (Lack 1947; Grant

1999). For each feeding observation, we recorded the food item (e.g., plant species or arthropod) and, if applicable, the specific plant part (e.g. seeds, fruits, leaves). Plant identification was achieved by reference to Wiggins and Porter (1971) and by comparison to seed collections at the Charles Darwin Research Station. When the specific food item could not be confirmed, we used more inclusive diet categories including “grass” (several Gramineae species with small and soft seeds), “ground” (unidentified small seeds), and “arthropods” (e.g., butterfly larvae, spiders, and grasshoppers). After a single observation of a given bird, we began searching for other banded birds. This “point-observation” procedure was chosen, rather than following individual birds for longer periods of time (Abbott *et al.* 1977; Smith *et al.* 1978), in an effort in increase independence among the observations for a given bird. These observations represent a subset (only banded individual birds) of the total observations presented in Chapter 2.

3.3.3 Data analysis

PCA was used to develop an overall measure of beak size (PC1 from length, depth, and width) and head size (PC1 from length, depth, and width). In addition, we classified individual *G. fortis* into small or large beak classes (here “morphs”) following the methods of Huber *et al*. (2007a). Here we used PC1 for beak dimensions in a cluster analysis to determine the best cut-off between large and small beak size classes. Only adult birds with at least six feeding observations were included in these analyses.

Indices of niche use were calculated using the software IndSpec1 (Bolnick *et al.* 2002). Total niche width (TNW) for each morph was calculated (Roughgarden 1979). TNW was then divided into two components: within-individual niche variation (WIC), reflecting the within-individual variation in resource use; and the between-individual niche variation (BIC), reflecting the between-individual variation in resource use. The ratio WIC/TNW then describes the degree of individual specialization, with low values indicating high individual niche specialization relative to the total niche width of the group. We next described the variation in resource use among individuals by calculating the proportional similarity index (*PSi*) (Schoener 1968; Bolnick *et al.* 2002). This index estimates specialization for each individual, and Mann Whitney U tests can be used to examine differences between groups (Araújo *et al.* 2007), here beak morphs. This analysis was performed using R (R Development Core Team 2007). Finally, we estimated the predominance of individual specialization within *G. fortis* by using a second likelihood index (*Wi*), which estimates the probability that the diet of individuals is drawn from the population's diet (Petraitis 1979; Bolnick *et al.* 2002). All the above indices range from 0 (indicating high specialization) to 1 (indicating complete generalization). Statistical significance of these indices was obtained through 10,000 Monte Carlo permutations implemented in IndSpec 1.0 (Bolnick *et al.* 2002). These analyses were based both on plant species ignoring specific plant items (e.g. seed, fruit, leave, etc) within each plant species, and on the combination of plant species and item.

To test for associations between individual specialization and morphological and genetic variation, we first built a matrix of pairwise diet similarity among individuals based on the proportional similarity index (*PSij*) (Schoener 1968; Bolnick *et al.* 2002). We then constructed two matrices of pairwise morphological distances among the same individuals based on the Euclidean distances for beak and head morphology separately. Finally, we built a matrix of genetic distance among individuals based on pairwise Wright's F-statistics (FST) distance according to (Weir & Cockerham 1984). We then tested for correlations between the above matrices using a simple Mantel test (Araújo *et al.* 2007; Bolnick & Paull 2009). Statistical significance was evaluated through 10,000 permutations. These analysis were performed in R (www.r-project.org), except for the matrix of genetic distance which was calculated using GenePop (Raymond & Rousset 1995).

3.4 Results

3.4.1 Individual specialization in G. fortis

We collected 810 feeding observations across 152 individually banded birds (Table 3.1). For each bird, we also had detailed data on beak and head morphology and genotype data at 10 microsatellite DNA markers. Some of the morphological data have been previously reported in earlier work (Herrel *et al.* 2005a; Hendry *et al.* 2006), and the microsatellite data are a subset of those reported in Chapter 4.

Table 3.2 provides a summary of the different niche indices. All of the WIC/TNW ratios were lower (0.318 to 0.600) than expected by chance, suggesting that the total niche width of the population is significantly influenced by diet variation among individuals.

Table 3.1 Summary of feeding observations of individually-banded *G. fortis* across years at El Garrapatero on Santa Cruz Island.

Year	Total observations	Individual birds
2003	88	16
2004	156	33
2005	245	38
2006	304	73
2007	17	2
Total	810	152

Table 3.2 Individual niche properties and estimates of individual specialization in *G. fortis*. These analyses were based on plant species and food items across morphs, and on food items for small and large morphs. The estimators represent total niche width (TNW), the within-individual niche component of niche width (WIC), the between individual component of niche width (BIC), and the degree of individual specialization (WIC/TNW). Also shown is average individual specialization (*IS*) based proportional similarity index (*PSi*) (Bolnick *et al*. 2002) and the likelihood index (*Wi*) of specialization (Petraitis 1979; Bolnick *et al*. 2002). These estimators range from 0 (indicating complete specialization) to 1 (indicating complete generalization) and statistical significance was obtained through 10,000 Monte Carlo permutations implemented in IndSpec 1.0 (Bolnick *et al*. 2002). Values in bold were significantly lower (P < 0.05) than the expected null model shown parenthesis.

Estimator	Species	Items	Small	Large
WIC	1.019	0.536	0.997	1.068
BIC	0.813	1.148	0.768	0.713
TNW	1.832	1.685	1.766	1.781
WIC/TNW	**0.556** (0.60**)**	**0.318 (**0.40)	**0.565** (0.61)	**0.600** (0.63)
IS (*PSi*)	**0.463** (0.48)	**0.403** (0.43)	**0.457** (0.49)	**0.524** (0.53)
Wi	0.404	0.368	0.416	0.438

Individual specialization based on the proportional similarity index (*PSi*) was also significantly lower than expected by chance (mean observed *PSi* = 0.39, mean expected *PSi* = 0.48), suggesting that individuals commonly exploit private resources in a manner not proportional to the total populations' diet. The small and large beak size morphs did not differ in *PSi* (Fig. 3.1; U = 452.5, p = 0.83), suggesting that individual specialization is not limited to one of the morphs but rather is characteristic of the species as a whole. The likelihood index (*Wi*) also revealed high levels of individual specialization (Table 3.2). When examining frequency distributions of *PSi* and *Wi*, a bias is seen toward low values, indicating strong individual specialization for most birds and generalism for a few (Fig. 3.2). In summary, these results suggest high levels of individual specialization in *G. fortis* Santa Cruz Island.

3.4.2 Associations with morphological and genetic divergence

Results were consistent when performed on either plant species or plant species/items, and so we here discuss and show only the latter. Across all birds, *PSi* was negatively correlated with morphological distance for head size but (marginally) not correlated with beak size (Fig. 3.3). That is, birds that differed more in head dimensions had more different diets. These results suggest that individual specialization in resource use is associated with some morphological variation in *G. fortis*. *PSi* was not significantly correlated with genetic differences between individual birds (Fig. 3.4). The trend was negative, suggesting that a large data set might have revealed that birds that are more genetically different have more different diets. In all cases, however, the data were quite variable and the relationships weak even if significant – as is the case for similar analyses of other taxa (Araújo *et al.* 2009; Bolnick & Paull 2009; Agashe & Bolnick 2010).

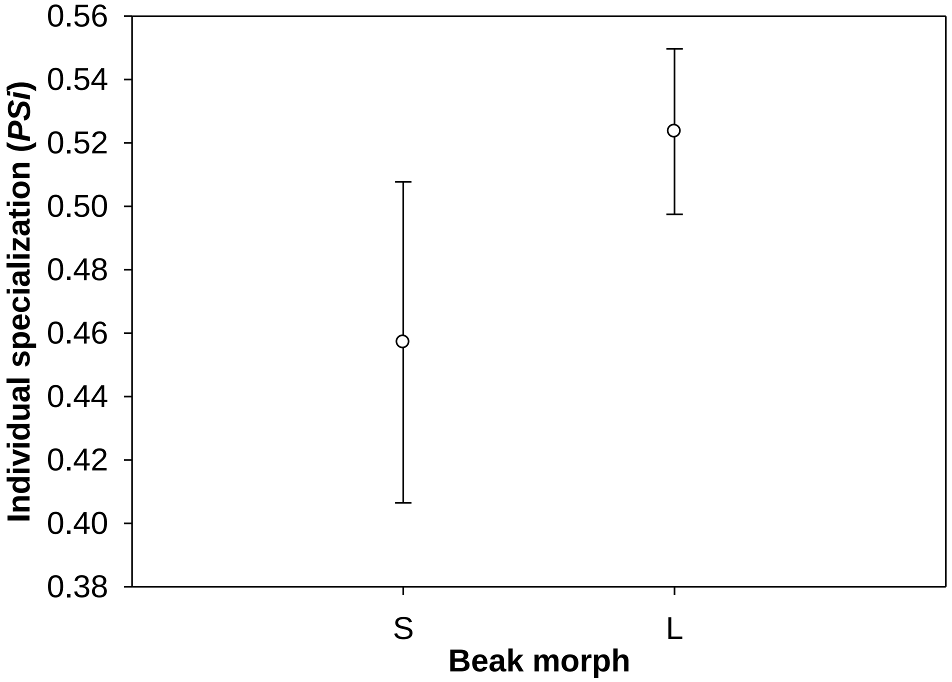

Figure 3.1 Mean individual specialization (*PSi*) in resource use in the small (S) and large (L) beak morphs of *G. fortis* at El Garrapatero on Santa Cruz Island. Individual specialization was not significantly different between the morphs (U = 452.5, p=0.83). Bars represent standard errors.

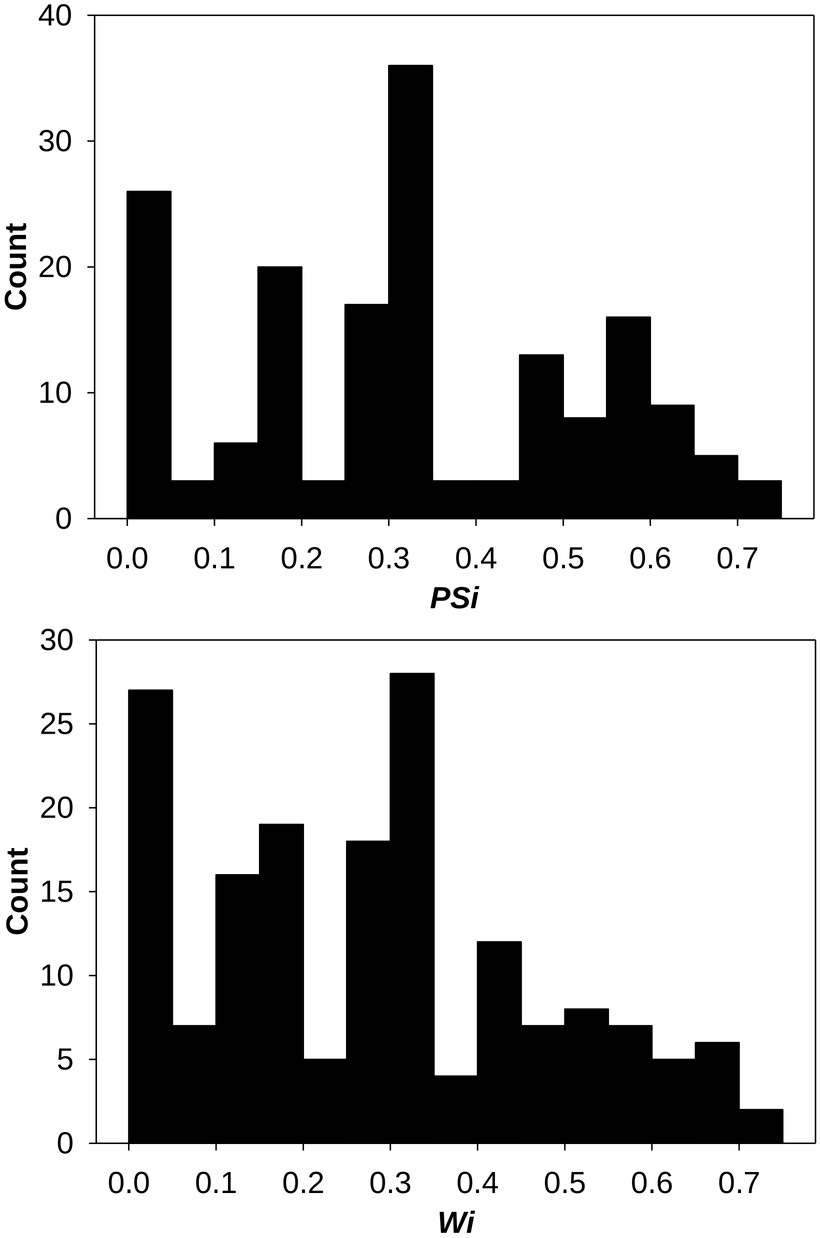

Figure 3.2 Frequency distribution of overall individual diet specialization in *G. fortis* on Santa Cruz Island. Upper panel shows individual specialization based proportional similarity index (*PSi*) (Schoener 1968); (Bolnick *et al.* 2002) and lower panel shows individual specialization using a second likelihood index (*Wi*) (Petraitis 1979; Bolnick *et al.* 2002).

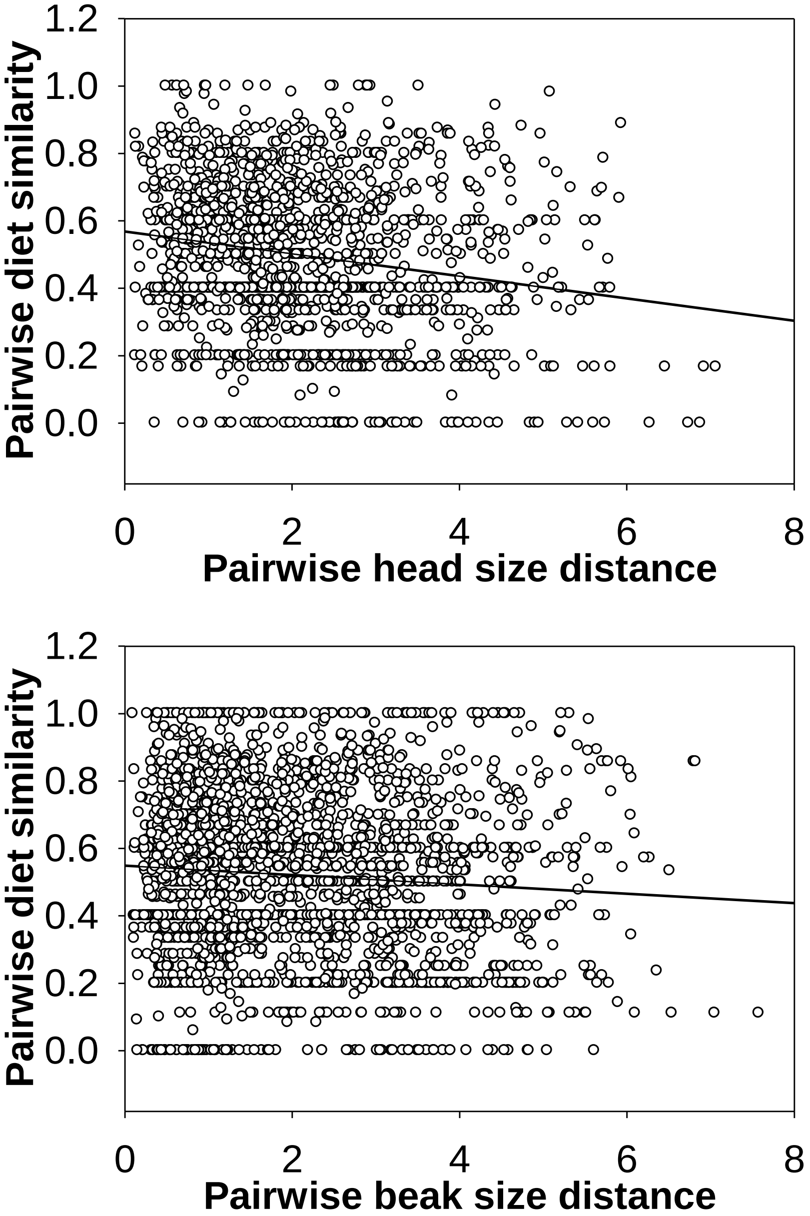

Figure 3.3 Mantel test on the correlation between matrices of proportional diet similarity (*PSij*) and Euclidean morphological distance (PC1 of either beak or head dimensions) among individuals. The upper panel shows the correlation based on head dimension (r= -0.1993, p=0.001). The lower panel shows the correlation based on beak dimension (r= -0.0793, p=0.094). Significance was estimated based on 1000 permutations implemented in IndSpec 1.0.

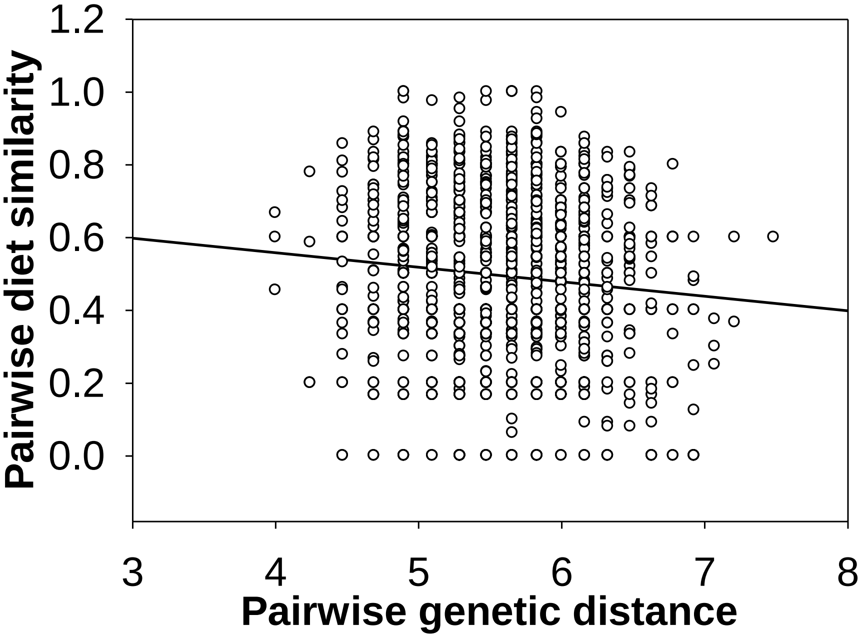

Figure 3.4 Mantel test on the correlation between matrices of proportional diet similarity (*PSi*) and genetic distance (pairwise Fst differences) in individuals of *G. fortis* on Santa Cruz Island. Although the trend was consistent with our predictions, the correlation was not significant (r = -0.0534, p=0.182). Significance was obtained after 1000 permutations.

3.5 Discussion

3.5.1 Individual specialization

Although different Darwin's finch species are clearly adapted to use different food resources (Lack 1947; Bowman 1961; Grant 1999), their diets are quite variable within a species and overlap to a certain degree between species (Chapter 1). Among the ground finches, *G. fortis* has the most variable diet, which also overlaps considerably with its smaller (*G. fuliginosa*) and larger (*G. magnirostris*) congeners. Each of these species, and the ground finch group as a whole, therefore might be considered generalists – at least within the broad class of seed/fruit foods. But a generalist group of this sort might be composed of a diversity of individual specialists. Hinting at this possibility, individual specialization has been documented in several bird species (see examples in Bolnick *et al*. 2003; Scott *et al*. 2003), including some populations of Darwin's finches (Grant *et al.* 1976; Price 1987; Werner & Sherry 1987). We have expanded on this work by formally calculating indices of individual specialization and comparing them to null expectations. Another novelty of our work is that the study population appears to be diverging along the same morphological axis (beak size) that characterizes differences among its granivorous congeners.

Overall, we found high levels of individual specialization in *G. fortis* on Santa Cruz Island (Table 3.1, Fig. 3.1). Average individual specialization was quite high and the distribution was biased toward individuals with high specialization. These properties were true for both the small and large beak size morphs. These results indicate that individual specialization is indeed a property of heterogeneous populations (Bolnick *et al.* 2007), perhaps resulting from intense intraspecific competition and disruptive selection (Bolnick 2001; Svanbäck & Bolnick 2005, 2007; Martin & Pfennig 2009; Agashe & Bolnick 2010). These results also conform to previous studies suggesting that many apparently generalist species are composed of a variety of specialist individuals (Van Valen 1965; Roughgarden 1972; Werner & Sherry 1987; Bolnick *et al.* 2003).

In Darwin's finches, individual specialization might be especially common, given their great variation in feeding traits. Our data demonstrate this possibility for *G. fortis*, but it might also be true of other species, such as the highly variable small ground finch (*G. fuliginosa*) (Kleindorfer *et al.* 2006). High individual specialization in Darwin's finches could be promoted by the high diversity of the underlying ecological resources (flora and fauna) and the substantial geographical heterogeneity (size and altitude) within and among different islands in the Archipelago. In other bird species, individual specialization might also be common; however, more studies are needed to get a better understanding of its prevalence and its ecological and evolutionary implications.

3.5.2 Diversification of individual specialists

Individuals that differed more in morphology (head size) differed more in diet. A similar, but marginally non-significant, pattern was suggested for beak size. The stronger result for head size than beak size fits with suggestions that head size (as opposed to beak size) is the most important determinant of bite force, the key feeding performance indicator that determines what seeds can be consumed by an individual finch (Herrel *et al.* 2005a, b). That is, diet divergence selects for bite force divergence, which selects for larger head muscles (causing larger head size), which then selects for larger beaks to resist the greater strains incurred when applying high bite force to hard seeds (Herrel *et al.* 2009).

Our specific study population (El Garrapatero *G. fortis*) was of particular interest because it has diverged to some extent into large and small beak size morphs (Hendry *et al.* 2006). These morphs are under disruptive selection (Hendry et al. 2009), mate assortatively (Huber *et al.* 2007), and are differentiated at neutral markers (Huber *et al.* 2007, Chapter 4). Our documentation of high individual specialization within this population appears to be consistent with empirical and theoretical suggestions that individual specialization can be an important diversifying force (Wilson & Turelli 1986; Skulason & Smith 1995; Bolnick *et al.* 2003; Rueffler *et al*. 2006; Day & Young 2009). Specifically, specialization by individuals on alternative resources can generate frequency dependent competition that generates strong disruptive selection (Skulason & Smith 1995; Bolnick 2001; Svanbäck & Bolnick 2007; McCormack & Smith 2008; Martin & Pfennig 2009). This interaction can in turn generate adaptive divergence and ecological speciation (Schluter 1994; Bolnick 2004; Polechova & Barton 2005; Rundle & Nosil 2005). Of course, confirmation of this hypothesis for our study population would require a demonstration that individual specialization in El Garrapatero *G. fortis* is higher than that in bird populations that are not in the process of diversifying – and such data are not yet available.

The diversifying force of individual specialization could also influence genetic variation in natural populations (Bolnick *et al.* 2007; Agashe & Bolnick 2010). In particular, greater diet differences should be associated with greater genetic differences at neutral markers – assuming diet differences are the driver of reproductive isolation. This appears to be the case for different *G. fortis* populations on Santa Cruz Island, where stronger associations between diet and beak size, and greater bimodality in beak size, are associated with greater differences in neutral genetic markers (Chapters 3 and 4). It is also the case for within- versus between-species comparisons: different ground finch species differ more in diet, morphology, and genetic markers than do the two *G. fortis* morphs (Chapters 1 and 4). At the individual level within El Garrapatero *G. fortis*, we did not find a significant association between diet and genetic differences (Fig. 3.4) – although the trend was consistent with the prediction. As ours is the first study to compare diet differences to genetic differences within species, we cannot state whether or not our results are typical. It is true, however, that associations between diet and *morphology* are usually quite weak in other taxa (Araújo *et al.* 2009; Bolnick & Paull 2009). Thus, perhaps diet differences are associated with genetic differences but the trend is so weak that it will be hard to detect. Indeed, recent simulation studies have shown that neutral genetic markers can be poor indicators of the degree to which divergent selection reduces gene flow (Thibert-Plante & Hendry 2010). Additional studies of the sort conducted here would therefore benefit from the use of a wider range of genetic markers and even larger sample sizes.

3.5.3 General implications

Species with high levels of individual specialization might support higher population sizes through reductions in intra-specific competition (Bolnick 2001; Bolnick 2004; Svanbäck & Bolnick 2007). In our case, *G. fortis* is not only the most variable species but also the most abundant and widespread in Galapágos (Grant *et al.* 1976; Grant 1999; Hendry *et al.* 2006). On the other hand, species with high levels of individual specialization might also have a greater tendency to diverge into new species. For instance, *G. fortis* shows great morphological and genetic divergence across Santa Cruz Island (Huber *et al.* 2007; De León *et al.* 2010). Thus, individual specialization might lead to greater genetic variation and diversification (*sensu* Bolnick *et al*. 2007), which can in turn produce a greater eco-evolutionary stability in natural populations. Individual specialization in *G. fortis* could also be seen as an 'eco-genetic trait' arising from a combination of morphological, ecological, and genetic factors. These assertions are, of course, merely hypotheses that warrant further investigation.

Our results fit the paradigm of ecological speciation in Darwin's finches by showing that diet differences could reinforce adaptive divergence after secondary contact (Lack 1947; Bowman 1961; Grant 1999; Schluter 2000; Grant & Grant 2008). However, we here complement previous studies by suggesting that individual specialization might promote and maintain the initial stages of adaptive divergence even sympatry. For instance, the morphological divergence within *G. fortis* parallels the same axis of divergence observed among the ground finch species (Hendry *et al.* 2006; Foster *et al.* 2008), and because beak size differences are highly heritable both within and between species (Boag & Grant 1978; Boag 1983; Keller *et al.* 2001; Abzhanov *et al.* 2004), we expect similar parallelism in the ecological factors promoting the initial stages of divergence. Thus, divergence in resource use at individual level might play an important role in promoting the initial stages of adaptive divergence in Darwin's finches.

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Linking statement between chapter 3 to Chapter 4

Results from the previous chapters (Chapter 2, 3) showed that the interplay between ecological heterogeneity, morphological variation and diet specialization might have promoted the adaptive divergence in *G. fortis* on Santa Cruz Island. This is in line with one of the predictions of the ecological theory of adaptive radiation that adaptive divergence drives the evolution of reproductive isolation as byproduct. If this is the case in Darwin's finches, we would expect that different species and beak-size morphs show limited gene flow even in sympatry. In the next chapter I will use DNA microsatellite markers to quantify gene flow among different species of ground finches and between large- and small-beaked *G. fortis* within and among three sites on Santa Cruz Island. I expect that gene flow will be low between the recognized species and higher, but still only modest between the two beak size morphs.

CHAPTER 4

Divergence with gene flow as facilitated by ecological differences: within-island variation in Darwin’s finches

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4.1 Abstract

Divergence and speciation can sometimes proceed in the face of, and even be enhanced by, ongoing gene flow. We here study divergence with gene flow in Darwin's finches, focusing on the role of ecological/adaptive differences in maintaining/promoting divergence and reproductive isolation. To this end, we survey allelic variation at 10 microsatellite loci for 989 medium ground finches (*Geospiza fortis*) on Santa Cruz Island, Galápagos. We find only small genetic differences among *G. fortis* from different sites. We instead find noteworthy genetic differences associated with beak. Moreover, *G. fortis* at the site with the greatest divergence in beak size also showed the greatest divergence at neutral markers; i.e., the lowest gene flow. Finally, morphological and genetic differentiation between the *G. fortis* beak size morphs was intermediate to that between *G. fortis* and its smaller (*Geospiza fuliginosa*) and larger (*Geospiza magnirostris*) congeners. We conclude that ecological differences associated with beak size (i.e., foraging) influence patterns of gene flow within *G. fortis* on a single island, providing additional support for ecological speciation in the face of gene flow. Patterns of genetic similarity within and between species also suggest that inter-specific hybridization might contribute to the formation of beak size morphs within *G. fortis*.

***Key words*:** sympatric speciation, ecological speciation, disruptive selection, reproductive isolation, gene flow, Darwin's finches.

4.2. Introduction

Strict geographic isolation surely aids speciation in many cases (Mayr 1963; Felsentein 1981; Coyne & Orr 2004), but there are several contexts where divergence can proceed despite (or even be enhanced by) a lack of geographic isolation. “Context 1” occurs when some initial divergence has occurred in allopatry, and this divergence is then strengthened during a period of secondary contact. Mechanisms that can be important here include ecological character displacement to reduce competition (Schluter 2000) and reproductive character displacement to reduce maladaptive interbreeding (Brown & Wilson. 1956; Grant 1972; Servedio & Noor 2003). “Context 2” is the fission of one initial species into two or more species without strict geographical isolation: i.e., parapatric or purely sympatric speciation. One mechanism likely to be important here is strong disruptive selection (owing to competition or discrete resources) that acts on traits also linked to assortative mating (Fry 2003; Gavrilets 2004; Bolnick & Fitzpatrick 2007). “Context 3” is hybridization between groups following their secondary contact, which can sometimes cause the formation of a new hybrid species (Seehausen 2004; Mallet 2007). Recent theoretical and empirical work on these contexts has spurred a resurgence of interest in longstanding (Smith 1966; Endler 1973; Felsenstein 1988) hypothesis of “divergence with gene flow” (Rice & Hostert 1993; Dieckmann & Doebeli 1999; Piertney *et al.* 2001; Bolnick & Fitzpatrick 2007; Doebeli *et al.* 2007; Berner *et al*. 2009). Our work focuses on divergence with gene flow in Darwin’s finches of the Galápagos Islands, where all three of the above contexts have been invoked.

Context 1: The classic view of speciation in Darwin's finches envisions a three-phase process (Lack 1947; Grant 1999; Schluter 2000; Grant 2001; Petren *et al.* 2005; Grant & Grant 2008). In the first phase, a single founding species from the mainland colonizes an island. In the second phase, migrants from that first island colonize additional islands that have different ecological resources, such as different food types. These ecological differences cause divergent selection on foraging traits, particularly beak size and shape, which then undergo adaptive divergence between the islands. In the third phase, a new round of migration between the islands brings partially-divergent forms back into secondary contact, where competition further enhances divergence (Lack 1947; Mayr 1963; Grant 1999; Schluter 2000; Grant & Grant 2006). In this three-phase model, the incipient species continue to diverge following secondary contact because allopatric divergence has lead to assortative mating and selection against hybrids that is then manifest in sympatry (Grant & Grant 1993; Grant & Grant 1996a, b; Grant 1999; Grant & Grant 1997a).

Context 2: Two potential cases of purely sympatric speciation have been discussed in Darwin's finches. In one, Grant & Grant (1979) described a population of *Geospiza conirostris* (large cactus ground finch) on Genovesa Island that was composed of two male types singing different songs and having different beak sizes and foraging habits. These initial distinctions subsequently broke down (Grant & Grant 1989), and no further attention has been directed toward this population. In the other case, Ford *et al.* (1973) described a population of *Geospiza fortis* (medium ground finch) at Academy Bay on Santa Cruz Island that was bimodal for beak size. The authors suggested that bimodality was the result of disruptive selection and assortative mating – but this was not tested – and bimodality has since weakened (Hendry *et al.* 2006). A case has also been made for possible parapatric speciation in *Geospiza fuliginosa* (small ground finch) between elevation zones on a single island (Kleindorfer *et al.* 2006).

Context 3: Darwin's finches frequently hybridize (Grant & Grant 1994; Grant & Grant 1997a; Grant & Grant 1998; Grant 1999; Satto *et al*. 1999; Zink 2002; Grant *et al*. 2005; Grant & Grant 2008), which might have several consequences for their diversification. First, hybridization between two species on the small island of Daphne Major has led to their morphological and genetic convergence (Grant & Grant 2002; Grant *et al*. 2004). This convergence was partly the result of changing ecological conditions that increased the fitness of hybrids (Grant & Grant 1996b). Second, hybridization could help to generate new phenotypes that might be able to adapt to new resources (Grant & Grant 1994) - although this has not been confirmed for Darwin's finches.

Common to all of the above contexts is the potential importance of ecological differences that cause divergent or disruptive selection, and thereby promote adaptive divergence. This divergence then becomes coupled to reproductive isolation, a process now called "ecological speciation." Ecological speciation has considerable support from theory and from many natural systems (Schluter 2000; Rundle & Nosil 2005; Hendry *et al*. 2007). In Darwin's finches, ecological speciation has been invoked through comparisons of established species (Grant 1999; Grant & Grant 2008), and our work extends these inferences to divergence within species.

4.2.1 Our study

Our work has concentrated on a population of *G. fortis* at El Garrapatero on Santa Cruz Island that is bimodal for beak size (Fig. 1.4, 1.5; Hendry *et al.* 2006). The two morphs (1) have beaks adapted for different food types (Herrel *et al*. 2005; Foster *et al.* 2008), (2) produce distinctive vocal mating signals (Podos *et al.* 2004; Huber & Podos 2006; Herrel *et al*. 2009) and respond differently to those signals (J. Podos unpubl. data), (3) show higher survival than birds with intermediate-sized beaks (Hendry *et al.* 2009), (4) pair assortatively by beak size (Huber *et al.* 2007), and (5) show some evidence of genetic divergence (Huber *et al.* 2007). In short, this population shows potential signs of ecological differentiation in the face of some gene flow.

The origin of these beak size morphs is unknown, with the different possibilities paralleling the general contexts introduced above. For context 1, the two morphs may have originated owing to adaptive divergence on different islands and then came into secondary contact on Santa Cruz (Grant & Grant 2008). As an extension of this context, the two morphs may have originated owing to adaptive divergence between sites on the same island, and then come into secondary contact across the island. For context 2, variation in resources at a given site, or competition for those resources, might have led to a purely sympatric origin of the morphs (Ford *et al.* 1973). For context 3, hybridization between *G. fortis* and *Geospiza magnirostris* (large ground finch) might have originated the large *G. fortis* morph (Grant & Grant 2008). In the present study, we will not conclusively discriminate among these possibilities. We will instead focus on how ecological/adaptive differences might influence gene flow between the morphs.

Several observations would be particularly informative. First, substantial genetic differences among *G. fortis* populations at different sites on Santa Cruz would suggest (although not confirm) that spatial isolation on a single island could contribute to speciation. Second, genetic clustering across the island by beak size rather than by location would suggest that gene flow is primarily reduced by ecology/adaptation (diet and beak size) rather than by geography (location). Third, evidence that gene flow is lower between the morphs at the sites where beak size divergence is greater would further suggest a link between ecology/adaptation and reproductive isolation. Fourth, a continuity in genetic divergence between the *G. fortis* morphs to divergence between *G. fortis* and its smaller (*G. fuliginosa*) and larger (*G. magnirostris*) congeners, would suggest a possible continuity of process from intra-specific divergence to speciation. This last pattern would also be consistent with a possible role for hybridization.

We test for the above signatures of ecological speciation by using microsatellite loci to analyze population structure on Santa Cruz Island, specifically in relation to (1) the small and large *G. fortis* beak size morphs (Fig. 1.5), (2) the different sampling sites (Fig. 1.2), and (3) the different granivorous ground finch species (*G. fuliginosa*, *G. fortis*, *G. magnirostris*). At one of the collection sites (El Garrapatero), both small and large beak size morphs are present and the beak size distribution is bimodal (Hendry *et al*. 2006; Huber *et al.* 2007; Fig. 4.1). At a second site (Academy Bay), both small and large morphs are present, but the historically-strong bimodality in beak size demonstrated by Ford *et al.* (1973) has since weakened (Hendry *et al.* 2006; Fig. 4.1). At a third site (Borrero Bay), the large morph is rare (Grant *et al*. 1976; Hendry *et al.* 2006; Fig. 4.1).

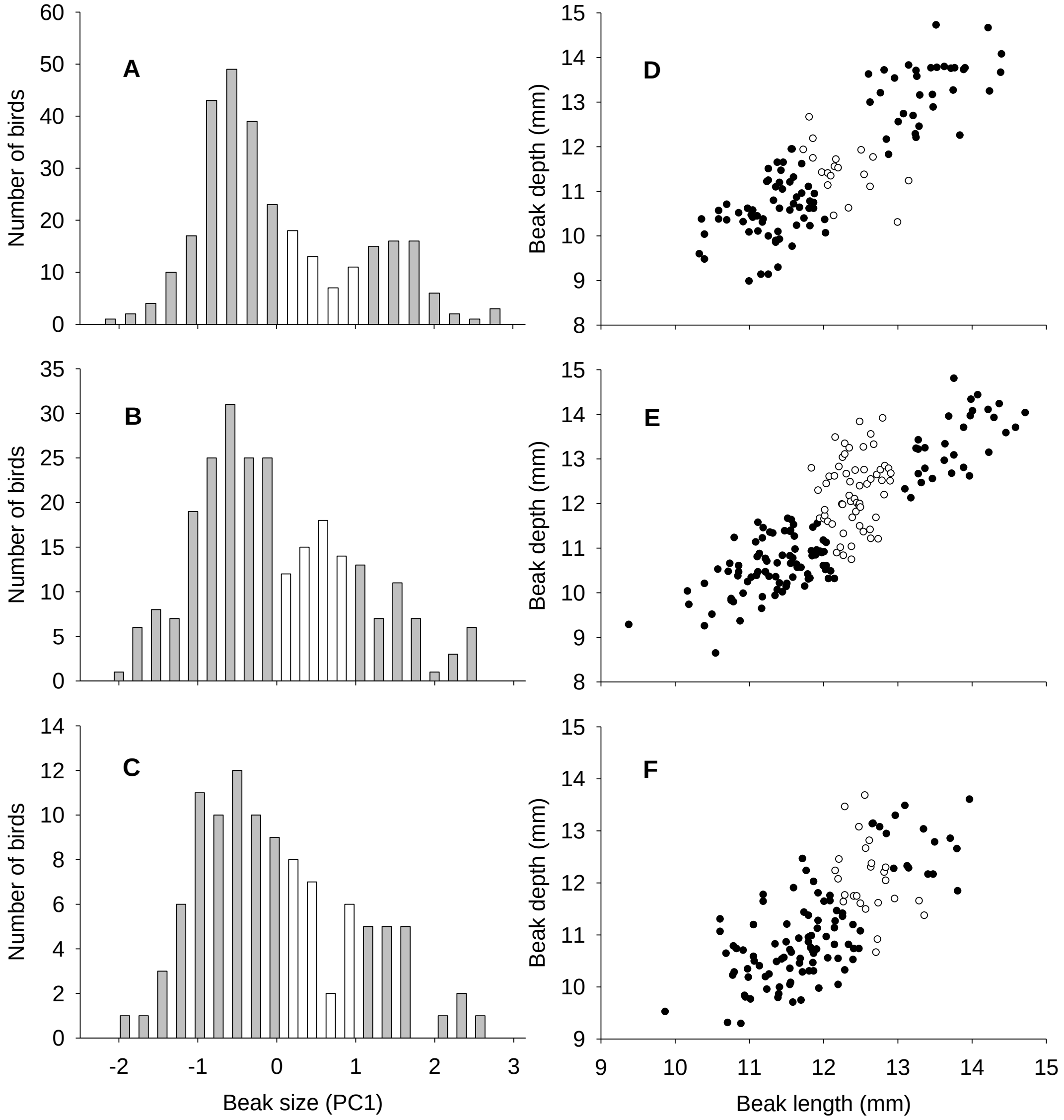

Figure 4.1 Distribution of beak sizes in *G. fortis* at three different sites on Santa Cruz Island in 2004. At El Garrapatero, small and large morphs are both common (Panel D) and intermediates are relatively few (A and D). At Academy Bay, small and large morphs are both common (E) and intermediates are less rare (B and E). At Borrero Bay, the large morph is rare (C and F). These data are from Hendry *et al.* (2006). The white sections on the histograms (A-C) and the open circles on panels (D-F) represent the "intermediate beak size class" omitted from the genetic analyses (see methods).

4.3. Material and Methods

4.3.1 Sampling

Sampling took place in January through March of 2003-2005. Finches were captured in mist nets and then measured (following Grant 1999) for beak length (anterior edge of nares to tip of upper mandible), beak depth (at the nares), and beak width (base of lower mandible). The ulnar vein was pricked with a needle, and the blood was blotted onto filter paper treated with EDTA. Captured birds were banded to avoid repeated sampling, and were then released at their site of capture. Sample sizes for *G. fortis* were 518 birds at Academy Bay, 419 birds at El Garrapatero, and 113 birds at Borrero Bay. All of these sites are low-elevation arid zone habitats (Wiggins & Porter 1971), where *G. fortis* is most abundant. Logistic and monetary constraints prevented sampling on additional islands.

We also took blood samples from the other granivorous ground finches found on Santa Cruz: 10 *Geospiza fuliginosa* (small ground finch) from El Garrapatero, 6 *Geospiza magnirostris* (large ground finch) from El Garrapatero, and 14 *G. magnirostris* from Academy Bay. Larger sample sizes were not possible owing to limitations on our permits. We did not analyze the closely related *Geospiza scandens* (cactus finch) because divergence for this species is on a different divergent ecological (cactus seeds, pollen, and nectar) and morphological (beak length relative to depth) axis than for the other three *Geospiza* (Lack 1947; Bowman 1961; Grant 1999; Foster *et al.* 2008).

Individual *G. fortis* with beak size data were assigned either to the small or large beak size categories. Specifically, we used PC1 scores (based on beak length, depth, and width) in a two-step cluster analysis with all *G. fortis* together to identify the beak size dividing point that best separated the two clusters (PC1= 0.312). Birds close to the dividing point could not be confidently assigned to either category, and so we defined three beak-size "classes." The large beak size class had 150 birds with beaks more than 0.5 standard deviations *larger* than the dividing point defined by the cluster analysis. The small beak size class had 342 birds with beaks more than 0.5 standard deviations *smaller* than the dividing point. The intermediate beak size class had 174 birds with beak sizes within 0.5 standard deviations of the dividing point. We focus our presentation on comparisons of the small and large beak size classes – because we can be certain that these birds represent the small and large beak size morphs. Regardless, our conclusions do not change in analyses that (1) consider the intermediate size class as an additional group (Appendix 4.1), or (2) divide the birds into small and large beak size categories based strictly on the above dividing point – i.e., without excluding any *G. fortis* (Appendix 4.2).

4.3.2 Genetic data

Total DNA was extracted from blood samples with a modified standard proteinase K phenol-chloroform protocol (Sambrook *et al*. 1989). DNA was amplified by PCR and screened for allelic variation at 11 di-nucleotide microsatellite loci: Gf03, Gf04, Gf05, Gf07, Gf08, Gf09, Gf10, Gf11, Gf12, Gf13, and Gf16 (Petren 1998). Multiplex PCR reactions in a final volume of 10 µl were carried out under the thermocycling conditions specified by Petren (1998).

Four fluorescent-labeled primers (FAM, HEX, PET, and TET) were used to label the 5' tail of the different loci. A multi-capillary ABI 3100-base station was used to score the microsatellite fragments.

Raw genotypes were imported into GENALEX V6 (Peakall & Smouse 2006), where they were converted into formats suitable for various software packages. We then used GENEPOP (Raymond & Rousset 1995) V.3.4 and FSTAT (Goudet 1995) V.2.9.3.2 to calculate basic population genetic parameters: allelic diversity, observed (Ho) and expected (He) heterozygosities, and fixation indices (F_{IS}). We also used GENEPOP to test for Hardy-Weinberg deficits and for linkage disequilibrium.

4.3.3 Population structure

Analyses of *G. fortis* population structure began with statistical comparisons between *a priori* defined groups based on various combinations of beak size class (large or small) and collection site (El Garrapatero, Academy Bay, or Borrero Bay). We first partitioned the total genetic variation with Analysis of Molecular Variance (AMOVA) based on 10,000 permutations in ARLEQUIN V.3.1 (Excoffier *et al.* 2005). We then used hierarchical F-statistics in HIERFSTAT (Goudet 2005). Both analyses were performed for (1) beak size classes nested within sites and (2) sites nested within beak size classes.

We next tested for genetic differences between (1) all combinations of beak size class and site, (2) beak size classes only (i.e., sites pooled within a beak size class), and (3) sites only (i.e., beak size classes pooled within a site). These analyses involved (1) Fisher's exact tests for genic differentiation in GENEPOP (Raymond & Rousset 1995), (2) Wright's F-statistics (F_{ST}) according to Weir & Cockerham (1984) and with confidence intervals from FSTAT (Goudet 1995), and (3) Slatkin's (1995) R-statistics (R_{ST}).

Finally, we used multilocus genotypes to infer population structure. Two of these analyses were conducted without reference to *a priori* defined groups. First, we used the Bayesian clustering method in STRUCTURE (Pritchard *et al*. 2000) V.2.1 to infer the likelihood of K = 1-5 clusters that minimize Hardy-Weinberg and linkage disequilibrium. Given the modest genetic differences (see Results), these analyses were run under an admixture model with correlated allele frequencies using a 50,000 burn-in period and 500,000 Monte Carlo Markov Chain iterations. Second, we performed similar analyses in BAPS (Corander *et al.* 2004) V.4.14, here using an admixture model and runs consisting of 10,000 iterations. Third, we used Factorial Correspondence Analysis in GENETIX (Belkhir *et al.* 2004) V.4.0 to visualize variation between the *a priori* defined (as above) small and large beak size classes at each site.

Variation among the three *Geospiza* species was examined by repeating the above analyses (as appropriate) using the three species as separate groups. For this analysis, all *G. fortis* individuals, including the intermediate size class, were pooled.

4.3.4 Gene flow

We also estimated contemporary gene flow to check for congruence with the above analyses of historical population structure. For this analysis, we used the Partial Bayesian assignment method (Cornuet *et al*. 1999; Paetkau *et al*. 2004) implemented in GeneClass (Piry *et al.* 2004) with 10000 simulations. This method (1) estimates contemporary gene flow as the number of first generation "migrants" (and is therefore conceptually quite different from the historical inferences of population structure reported above), and (2) is still useful when some potential source populations (here other sites on Santa Cruz or other islands) have not been sampled (Cornuet et al. 1999). We felt that GENECLASS was more appropriate than STRUCTURE for assignment tests because the latter assigns proportions of genomes to candidate populations and assumes all the candidate populations have been included in the analysis. Previous studies have shown that GENECLASS performs well in detecting contemporary migrants (Cornuet *et al*. 1999; Eldridge et al. 2001; Berry *et al.* 2004; Paetkau *et al*. 2004). We did not use MIGRATE because simulation studies have found it performs poorly when estimating gene flow (Abdo *et al.* 2004; Slatkin 2005; Chapuis 2009).

4.4. Results

4.4.1 Variation within G. fortis

All of the loci showed moderate to high levels of variation (Table 4.1). One locus (Gf10) turns out to be Z-linked (Petren *et al*. 2005) and was therefore omitted from subsequent analyses. Of the ten remaining loci, six (Gf03, Gf04, Gf09, Gf11, Gf12, Gf16) showed significant heterozygote deficits when all *G. fortis* were pooled together (four after sequential Bonferroni; Table 4.1). Five pairings of loci in this pooled sample showed significant linkage disequibrium (two pairs after sequential Bonferroni, results not shown). These deviations from Hardy-Weinberg and linkage equilibria likely reflect population structure within *G. fortis* – because these loci apparently lack null alleles (Petren 1998; Keller 2001; Petren *et al.* 2005) and are not physically linked to each other (Petren 1998). In the following two paragraphs, we first describe differences between *a priori* defined beak size classes (small vs. large) and collection sites (Academy Bay, El Garrapatero, Borrero Bay) based on standard population genetic tests. We then examine the groupings revealed by analyzing multilocus genotypes, whether defined *a priori* or not.

Table 4.1 Allelic diversity in the combined sample of all *G. fortis* from Santa Cruz Island, Galapágos. Columns indicate the total number of individuals genotyped (N), the number of alleles identified (Na), observed heterozygosity (Ho), expected heterozygosity (He), F_{IS} estimates following Weir & Cockerham (1984), and the significance of Hardy-Weinberg deficits. Bold entries are those that remain significant after sequential Bonferroni correction.

Locus	N	Na	Ho	He	F_{IS}	HW
Gf03	986	17	0.827	0.841	0.017	**<0.001**
Gf04	989	8	0.479	0.469	-0.020	0.012
Gf05	983	13	0.663	0.664	0.002	0.276
Gf07	861	20	0.849	0.873	0.028	0.137
Gf08	902	28	0.906	0.927	0.023	0.056
Gf09	903	21	0.630	0.636	0.011	**<0.001**
Gf10	988	14	0.242	0.473	0.489	**<0.001**
Gf11	860	35	0.890	0.936	0.049	**<0.001**
Gf12	781	23	0.874	0.900	0.029	**<0.001**
Gf13	847	16	0.850	0.862	0.014	0.401
Gf16	857	13	0.799	0.797	-0.002	0.014
Average	897	18	0.728	0.762	0.058	**<0.001**

Some subtle genetic differences were evident among the sites (Tables 4.2-4.4), but we here concentrate on the much greater differences between the beak size classes. First, the percentage of molecular variation attributable to beak size classes was greater than that attributable to differences between collection sites, and the former was highly significant when size classes were nested within collection sites (Table 4.2). Second, hierarchical F-statistics revealed that differentiation between beak size classes (classes nested within total: $F_{classes/total}$ =0.011, p=0.001 and classes nested within sites: $F_{classes/sites}$ = 0.0203, p=0.035) was greater than that between collection sites (sites nested within total: $F_{sites/total}$ =0.021, p=0.737 and sites nested within classes: $F_{sites/classes}$ = 0.0122, p=0.001). Third, the two beak size classes were quite distinct when the birds were pooled across all sites: exact test $p < 0.0001$, F_{ST} = 0.011 (CI = 0.006 - 0.034), and R_{ST} = 0.017. Fourth, when birds were not pooled across sites, all pair-wise comparisons between the small and large beak size classes (within or between sites) were significant – except for those involving the rare large Borrero Bay birds (Table 4.3). Amplifying this last point, differentiation between beak size classes at a given site was greatest for El Garrapatero, lower for Academy Bay, and absent for Borrero Bay (Table 4.3).

The above findings were mirrored in analyses of multilocus genotypes. Bayesian analyses in STRUCTURE recovered two clusters (Fig. 4.2) that roughly corresponded to the small and large beak size classes. For example, 63% of birds in the large beak size class were assigned with the highest probability to one cluster, whereas 60% of birds in the small beak size class were assigned with the highest probability to the other cluster. When each collection site was considered separately, STRUCTURE found only a single cluster at each site, which is not surprising given the very limited power of this method when genetic differentiation is modest (Pritchard *et al.* 2000; Evanno *et al.* 2005; Waples & Gaggiotti 2007). Similar to STRUCTURE, BAPS largely recovered the two beak size classes when using the entire data set, but not when each site was analyzed separately (results not shown). Finally, Factorial Correspondence Analysis revealed that the differences between morphs were greatest at El Garrapatero, lower at Academy Bay, and absent at Borrero Bay (Fig. 4.3).

Table 4.2 Analysis of Molecular Variance (AMOVA) for Santa Cruz *G. fortis*. Sampled birds represent two beak size classes (small and large) from the three collection sites (Academy Bay, Borrero Bay, and El Garrapatero). These analyses were based on ten polymorphic microsatellite loci (i.e., excluding Gf10). Levels of significance were extracted after 10,000 permutations, as implemented by Arlequin V3.1.

Source of variation	Sum of squares	Variance components	Percentage of variation	P-value
Sites nested within beak size classes				
Between size classes	23.249	0.04373	1.11	0.096
Among sites within size classes	19.734	0.00706	0.18	<0.001
Within sites	3786.277	3.87145	98.70	<0.001
Beak size classes nested within sites				
Among sites	12.238	-0.01902	-0.49	0.793
Between size classes within sites	30.745	0.04602	1.18	<0.001
Within size classes	10244.091	3.89103	99.31	<0.001

Table 4.3 Genetic differentiation in *G. fortis* between beak size classes (S = small, L = large) from different collection sites (AB = Academy Bay, BB = Borrero Bay, EG = El Garrapatero). N represents sample sizes. P values for genic differentiation are above the diagonal. Bold entries are those that remained significant after sequential Bonferroni correction. F_{ST} values are below the upper diagonal, with asterisks (*) indicate 95% confidence intervals that do not overlap with zero. R_{ST} values are below the lower diagonal.

	N	ABS	ABL	BBS	BBL	EGS	EGL
Academy Bay S (ABS)	118		**<0.001**	0.029	0.004	**0.001**	**<0.001**
Academy Bay L (ABL)	60	0.012*		**<0.001**	0.023	**<0.001**	0.101
Borrero Bay S (BBS)	53	0.001	0.015*		0.093	**0.002**	**<0.001**
Borrero Bay L (BBL)	24	0.004*	0.007*	0.001		**<0.001**	0.044
El Garrapatero S (EGS)	171	0.002*	0.019*	<0.001	0.005*		**<0.001**
El Garrapatero L (EGL)	66	0.012*	0.0001	0.013*	0.005	0.016*	
Academy Bay S (ABS)	118						
Academy Bay L (ABL)	60	0.017					
Borrero Bay S (BBS)	53	0.001	0.021				
Borrero Bay L (BBL)	24	0.009	0.008	0.007			
El Garrapatero S (EGS)	171	0.003	0.023	<0.001	0.017		
El Garrapatero L (EGL)	66	0.025	<0.001	0.029	<0.001	0.030	

Table 4.4 Genetic differentiation between *G. fortis* (all three beak size classes pooled) from the three collection sites on Santa Cruz Island. P values for genic differentiation are above the diagonal. Bold entries are those that remained significant after sequential Bonferroni correction. F_{ST} values are below the upper diagonal, with asterisks (*) indicate 95% confidence intervals that do not overlap with zero. R_{ST} values are below the lower diagonal.

	Academy Bay	Borrero Bay	El Garrapatero
Academy Bay		**0.010**	**<0.001**
Borrero Bay	0.002*		**<0.001**
El Garrapatero	0.003*	0.002*	
Academy Bay			
Borrero Bay	0.001		
El Garrapatero	0.003	0.003	

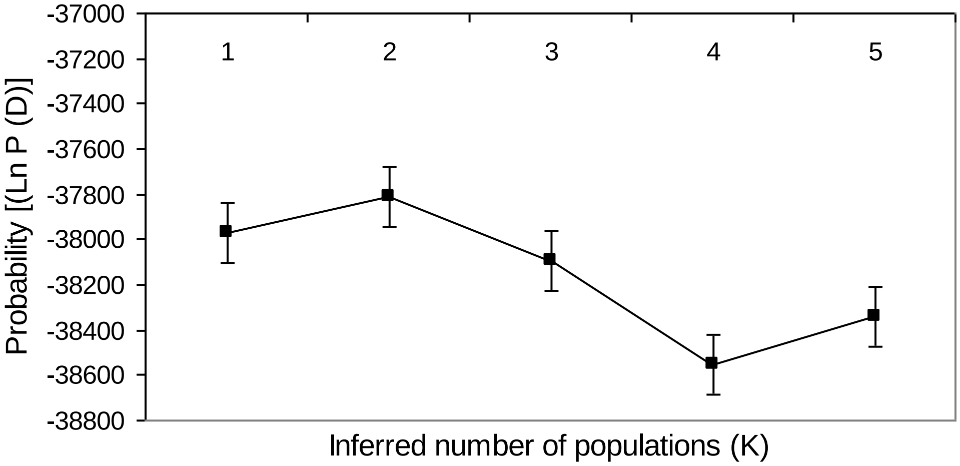

Figure 4.2 Two clusters are most likely when combining all *G. fortis* in STRUCTURE V2.1. This conclusion holds when using the ad hoc criterion of Evanno *et al.* (2005). Shown is the consensus of five simulations following the parameters described in methods. Error bars show the variation in the probability of assignment.

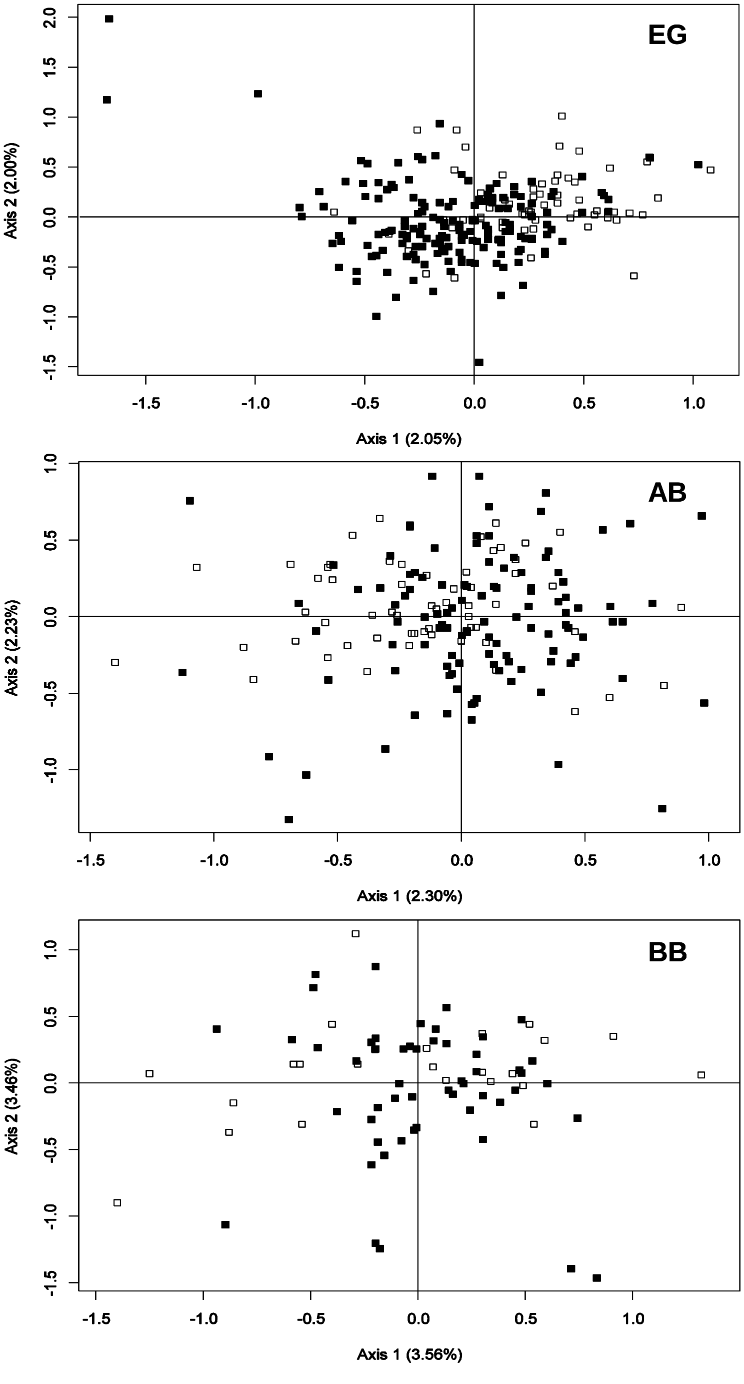

Figure 4.3 Population structure in *G. fortis* as visualized through Factorial Correspondence Analysis of multilocus genotypes (GENETIX V3.1). The black and white squares are small and large beak size classes, respectively. At El Garrapatero (EG), the two morphs occupy different, although partly overlapping distributions. At Academy Bay (AB) and Borrero Bay (BB), the two morphs occupy similar distributions, although some slight separation is evident, particularly for Academy Bay.

4.4.2 Variation among species

Genetic differentiation between the *G. fortis* beak size classes represents a small-scale version of the differences between *G. fortis* and its smaller (*G. fuliginosa*) and larger (*G. magnirostris*) granivorous congeners. First, differentiation (F_{ST}) between the small and large beak size classes (pooled across sites) was roughly one-half to one-third of that between *G. fortis* (pooled across morphs and sites) and either *G. fuliginosa* or *G. magnirostris* (Table 4.5). Second, small *G. fortis* were genetically more similar to *G. fuliginosa* than to *G. magnirostris,* whereas large *G. fortis* were genetically more similar to *G. magnirostris* than to *G. fuliginosa* (Table 4.5). These results were supported in analyses of multilocus genotypes. When STRUCTURE (Fig. 4.4) and BAPS (results not shown) were asked to assign birds to only two clusters, these programs found (1) a strong separation between *G. fuliginosa* and *G. magnirostris*, and (2) that small *G. fortis* tended to be placed in the *G. fuliginosa* cluster and large *G. fortis* tended to be placed in the *G. magnirostris* cluster.

4.4.3 Gene flow

Estimates of contemporary gene flow from GeneClass were consistent with key results from the above analyses of population structure: (1) gene flow was lower between beak size morphs than within them, both within and between sites; and (2) gene flow was lower between the beak size classes at El Garrapatero than at other sites (Tables 4.6 and 4.7). Similar results were obtained both when including the intermediate size class and when using a single dividing point between the large and small size class (Appendix 4.3, 4.4).

Table 4.5 Genetic differentiation within and between the three granivorous ground finch species. *G. fortis* is represented by the small and large beak size classes and by all size classes (including intermediates) pooled. P values for genic differentiation are above the diagonal. Bold entries are those that remained significant after sequential Bonferroni correction. F_{ST} values are below the upper diagonal, with asterisks (*) indicate 95% confidence intervals that do not overlap with zero. R_{ST} values are below the lower diagonal.

	G. fortis (small)	*G. fortis* (large)	*G. fuliginosa*	*G. magnitrostris*	*G. fortis* (all)
G. fortis (small)		**<0.001**	**0. 01**	**<0.001**	-
G. fortis (large)	0.011*		**<0.001**	**<0.001**	-
G. fuliginosa	0.022*	0.041*		**<0.001**	**<0.001**
G. magnitrostris	0.031*	0.021*	0.082*		**<0.001**
G. fortis (all)	-	-	0.029*	0.026*	
G. fortis (small)					
G. fortis (large)	0.017				
G. fuliginosa	0.001	0.036			
G. magnitrostris	0.014	0.001	0.054		
G. fortis (all)	-	-	0.011	0.001	

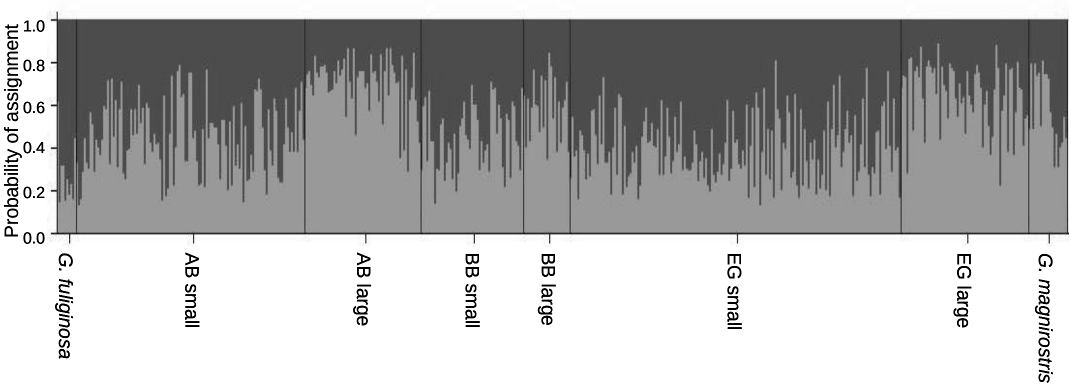

Figure 4.4 Bayesian clustering analysis of population structure performed in STRUCTURE V2.1. Black bars represent the separation between different groups: *G. fuliginosa*, *G. fortis* (AB small, AB large, BB small, BB large, EG small, and EG large), and *G. magnirostris*.

Table 4.6 Bidirectional rates of contemporary gene flow in *G. fortis* between beak size classes (S = small, L = large) from different collection sites (AB = Academy Bay, BB = Borrero Bay, EG = El Garrapatero). N and P represent sample sizes and statistical significance of the probability of assignment respectively. These results were obtained using the Paetkau *et al. (*2004) assignment method as implemented in the software GeneClass 2.0.

	N	P	ABS	ABL	BBS	BBL	EGS	EGL
Academy Bay S (ABS)	118	0.014		7	8	1	12	6
Academy Bay L (ABL)	60	0.015	4		1	2	4	14
Borrero Bay S (BBS)	53	0.006	7	1		6	8	3
Borrero Bay L (BBL)	24	0.011	6	3	4		2	4
El Garrapatero S (EGS)	171	0.021	12	3	9	1		5
El Garrapatero L (EGL)	66	0.021	3	12	2	3	2	

Table 4.7 Bidirectional rates of contemporary gene flow in *G. fortis* (all three beak size classes pooled) from the three collection sites on Santa Cruz Island. P represents statistical significance of the probability of assignment. These results were obtained using the Paetkau *et al. (*2004) assignment method as implemented in the software GeneClass 2.0.

	P	Academy Bay	Borrero Bay	El Garrapatero
Academy Bay	0.001		21	26
Borrero Bay	0.001	14		16
El Garrapatero	0.001	33	23	

4.5 Discussion

All of our data and analyses were congruent in revealing significant population structure and limitations to gene flow within *G. fortis* on Santa Cruz Island. This result shows the potential for partial reproductive isolation to be maintained (or perhaps to even originate) on a single island. This conclusion seems to run counter to persistent skepticism about bird diversification on single islands (Coyne & Price 2000; Grant 2001; Phillimore *et al.* 2008). Perhaps divergence can be more easily maintained here owing to the reasonable size (986 km^2), elevation (869 masl), and ecological diversity of Santa Cruz Island (Wiggins &

Porter 1971; Grant 1999; Parent & Crespi 2006). That is, larger and higher islands often have a greater diversity of niches into which adaptive radiation may proceed (Ricklefs & Lovette 1999; Losos & Schluter 2000; Ryan *et al.* 2007). In addition, larger islands allow for greater isolation-by-distance, which may facilitate divergence in response to spatially-structured selection (Doebeli & Dieckmann 2003; Gavrilets & Vose 2005; Gavrilets *et al.* 2007). We now detail how the population structure of *G. fortis* on Santa Cruz is associated with space (collection sites) and with ecological traits (beak size). We then turn to a further consideration of the origins/maintenance of this variation.

4.5.1 Patterns of differentiation and gene flow

A significant fraction of the observed population structure and limitations to gene flow in *G. fortis* on Santa Cruz Island could be attributed to differences among collection sites (Table 4.3). This result seems to support sporadic suggestions that spatial separation, particularly when coupled with ecological differences, might contribute to the divergence of birds on a single island (see also Blondel *et al.* 1999; Postma & van Noordwijk 2005; Kleindorfer *et al*. 2006; Ryan *et al*. 2007; Christensen & Kleindorfer 2007). In our study, however, the population structure attributable to space was very small – although it might have been greater if we had examined *G. fortis* at more distant and ecologically-divergent sites, such as different altitudinal zones (e.g., for *G. fuliginosa* see Kleindorfer *et al*. 2006). At present, however, we must conclude that although spatial separation can certainly aid diversification in parapatry (Doebeli & Dieckmann 2003; Gavrilets & Vose

2005; Gavrilets *et al.* 2007), we have no evidence that this process has been particularly important in Darwin's finches.

The most striking pattern in our data was that most of the population structure and gene flow restriction was associated with beak size. That is, birds in the small and large beak size classes showed moderate, and highly significant, genetic differentiation both within and between sites (Table 4.3). Indeed, birds with similar beak sizes were more genetically similar across sites than were birds of different beak sizes within sites (Table 4.3). These patterns likely reflect limited overall gene flow between the morphs, rather than linkage to genes for beak size (e.g., BMP4, Abzhanov *et al.* 2004), because similar patterns were evident at multiple unlinked neutral loci. The observed clustering by beak size, rather than by site, could have two basic explanations. One is that the beak size morphs originated at one or a few sites and then spread out to occupy more sites with limited gene flow between the morphs at each site. Another is that the two morphs split independently at multiple sites and then interbred across sites within each morph. Either way, patterns of gene flow within *G. fortis* on Santa Cruz Island are mainly associated with ecology (different foraging adaptations) rather than geography.

Evidence for the importance of ecology is strengthened when divergence and gene flow between the small and large *G. fortis* morphs is compared across the three collection sites. Genetic differentiation is greatest, and gene flow lowest, at El Garrapatero (Table 4.3, Fig. 4.3), where the population is currently bimodal for beak size (Fig. 4.1; Hendry *et al.* 2006). Genetic differentiation is weaker at

Academy Bay (Table 4.3; Fig. 4.3), where bimodality was strong in the past (Ford *et al.* 1973) but has since weakened (Hendry *et al.* 2006). Genetic differentiation is largely absent at Borrero Bay (Table 4.3; Fig. 4.3), where the large morph is rare (Hendry *et al*. 2006). This spatial coupling of genetic and phenotypic differentiation might reflect either of two opposite, but complementary, causal effects (Räsänen & Hendry 2008). On the one hand, increasing gene flow between the morphs might constrain their ability to differentiate, with this effect being greatest at Borrero Bay. On the other hand, increasing adaptive divergence between the morphs might reduce gene flow (i.e., ecological speciation), with this effect being greatest at El Garrapatero.

Additional insights are made possible by comparing divergence and gene flow within *G. fortis* to that between *G. fortis* and its smaller (*G. fuliginosa*) and larger (*G. magnirostris*) congeners. First, divergence between the *G. fortis* beak size morphs was approximately half of that between *G. fortis* and each of the two other species. Second, the small *G. fortis* morph was genetically most similar to *G. fuliginosa* and the large *G. fortis* morph was genetically most similar to *G. magnirostris*. Here, again, greater ecological/adaptive differences (between relative to within species) is associated with lower gene flow. In addition, the fact that divergence within *G. fortis* is a small scale version of divergence among the three species suggests that processes maintaining reproductive isolation between the two morphs, such as disruptive selection (Hendry *et al*. 2009) and assortative mating (Huber *et al.* 2007), might reflect those that contribute to speciation in the group. Our analysis of within-species variation thus supports previous arguments based on between-species variation (Lack 1947; Grant 1999; Grant & Grant 2008) about the importance of ecological speciation in Darwin's finches.

4.5.2 Potential scenarios for diversification

As noted in the introduction, several scenarios have been proposed for the origin of *G. fortis* beak size morphs, each matching a different expected context for divergence with gene flow. We cannot here determine which scenario is correct, but we can provide some further insight into the various possibilities.

The first context was initial divergence in different locations followed by further divergence after secondary contact. For Darwin's finches, this initial divergence is typically postulated to have occurred among islands (Lack 1947; Grant 1999; Schluter 2000; Grant 2001; Petren *et al.* 2005; Grant & Grant 2008), whereas our data are relevant to the possibility of initial divergence between sites on the same island. We found relatively little support for this possibility given that genetic divergence was very small between sites on the same island (Table 4.4). This result is not definitive, however, because substantial divergence in selected traits/genes might occur even when divergence in neutral markers is absent (Nosil *et al.* 2009). Moreover, we did not examine all possible divergent environments on Santa Cruz, with higher elevations being a possible site of greater divergence (Kleindorfer *et al*. 2006).

The second context was fully sympatric speciation. We have no data to directly address this possibility but it seems worthwhile to at least entertain further. The reason is that the "magic trait" conditions thought to favor fully sympatric speciation (Fry 2003; Gavrilets 2004; Bolnick & Fitzpatrick 2007) are present in *Geospiza*. First, disruptive selection on beak size occurs between sympatric species (Schluter & Grant 1984; Schluter *et al*. 1985; Grant & Grant 2006) and between the sympatric *G. fortis* morphs (Hendry *et al*. 2009). Second, differences in beak size, and the resulting differences in song (Podos 2001; Huber & Podos 2006; Herrel *et al*. 2009), generate assortative mating between sympatric species (Ratcliffe & Grant 1983; Grant & Grant 1996a, Grant & Grant 1997b) and between the sympatric *G. fortis* morphs (Huber *et al*. 2007). Third, beak size is highly heritable (Keller *et al.* 2001), males learn their songs from their fathers (Grant & Grant 1989), and females prefer to mate with males that sing songs similar to their father (Grant & Grant 1998). In short, beak size in Darwin's finches seems a particularly likely candidate for a magic trait.

The third context was inter-specific hybridization leading to the origin of one of the morphs. The most likely scenario here would be that the large morph was originally formed through interbreeding between *G. magnirostris* and small-beaked *G. fortis*. This possibility is worth considering given that (1) the small *G. fortis* morph is widespread whereas the large *G. fortis* morph is not (Grant *et al.* 1976; Hendry *et al.* 2006), (2) the large *G. fortis* morph is morphologically and genetically intermediate between the small *G. fortis* morph and *G. magnirostris*, and (3) we recorded one instance of a large morph *G. fortis* female mating with a *G. magnirostris* male (Huber *et al.* 2007). Our study system might therefore represent a chance to further consider the possibility of ecologically-based hybrid speciation.

4.5.3 Summary

The beak size morphs of *G. fortis* on Santa Cruz Island represent a case of divergence with gene flow being maintained (or driven) by ecological differences. The two morphs have different foraging morphology that is clearly adapted for different food types, and this divergence has lead to reproductive barriers that include disruptive selection and assortative mating. These associations are largely maintained across Santa Cruz Island as a whole, overwhelming minor spatial restrictions on gene flow. Space does interact with beak size, however, in that sites where birds show greater morphological divergence are also sites were they show greater genetic divergence (lower gene flow). Moreover, patterns of genetic variation are associated with beak size not only within species, but also between species. All of these observations point to ecological/adaptive differences as the main driver of the reproductive isolation in this system.

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4.8 Appendix

Appendix 4.1 Genetic differentiation between the three beak size classes (L = large, I = intermediate, and S = small) from the different collection sites. P values for genic differentiation are above the diagonal. Bold entries are those that remained significant after sequential Bonferroni correction. F_{ST} values are below the upper diagonal, with asterisks (*) indicate 95% confidence intervals that do not overlap with zero. R_{ST} values are below the lower diagonal.

	ABS	ABI	ABL	BBS	BBI	BBL	EGS	EGI	EGL
Academy Bay S (ABS)		0.868	**<0.001**	0.003	0.026	0.004	**<0.001**	**<0.001**	**<0.001**
Academy Bay I (ABI)	<0.001		0.028	0.550	0.2735	0.145	0.291	0.009	**<0.001**
Academy Bay L (ABL)	0.012*	0.008*		**<0.001**	<**0.001**	0.342	<**0.001**	**<0.001**	0.101
Borrero Bay S (BBS)	0.001	<0.001	0.015*		0.396	0.062	**0.002**	**<0.001**	**<0.001**
Borrero Bay I (BBI)	0.001	<0.001	0.016*	<0.001		0.049	0.009	**<0.001**	**<0.001**
Borrero Bay L (BBL)	0.004*	<0.001	0.007*	0.001	0.001		**0.001**	0.999	0.044
El Garrapatero S (EGS)	0.002*	0.003*	0.019*	<0.001	0.002	0.005*		**<0.001**	**<0.001**
El Garrapatero I (EGI)	0.009*	0.005*	0.015*	0.005*	0.010*	0.003	0.004*		**<0.001**
El Garrapatero L (EGL)	0.012*	0.007*	<0.001	0.013*	0.015*	0.005	0.016*	0.008*	
Academy Bay S (ABS)									
Academy Bay I (ABI)	<0.001								
Academy Bay L (ABL)	0.017	0.006							
Borrero Bay S (BBS)	0.001	<0.001	0.021						
Borrero Bay I (BBI)	0.005	0.005	0.015	<0.001					
Borrero Bay L (BBL)	0.009	0.002	0.008	0.007	0.002				
El Garrapatero S (EGS)	0.003	0.007	0.023	<0.001	0.001	0.017			
El Garrapatero I (EGI)	0.006	0.002	0.005	0.001	0.002	0.002	<0.001		
El Garrapatero L (EGL)	0.025	0.010	<0.001	0.029	0.020	<0.001	0.030	0.011	

Appendix 4.2 Genetic differentiation in *G. fortis* between large (above the dividing point in cluster analysis) and small (below the dividing point) birds, and among the different collection sites. P values for genic differentiation are above the diagonal. Bold entries are those that remained significant after sequential Bonferroni correction. F_{ST} values are below the upper diagonal, with asterisks (*) indicate 95% confidence intervals that do not overlap with zero. R_{ST} values are below the lower diagonal.

	ABS	ABL	BBS	BBL	EGS	EGL
Academy Bay S (ABS)		**<0.001**	0.061	0.093	**<0.001**	**<0.001**
Academy Bay L (ABL)	0.006*		**<0.001**	0.131	**<0.001**	0.061
Borrero Bay S (BBS)	0.001	0.007*		0.096	**0.001**	**<0.001**
Borrero Bay L (BBL)	0.003*	0.003*	0.001		**0.001**	0.051
El Garrapatero S (EGS)	0.003*	0.011*	0.002	0.003		**<0.001**
El Garrapatero L (EGL)	0.009*	0.003*	0.009*	0.007*	0.011*	
Academy Bay S (ABS)						
Academy Bay L (ABL)	0.021					
Borrero Bay S (BBS)	0.001	0.022				
Borrero Bay L (BBL)	0.012	<0.001	0.008			
El Garrapatero S (EGS)	0.020	0.024	0.002	0.012		
El Garrapatero L (EGL)	0.020	<0.001	0.022	<0.001	0.018	

Appendix 4.3 Bidirectional rates of contemporary gene flow in *G. fortis* between beak size classes (S = small, I = intermediate, L = large) from different collection sites (AB = Academy Bay, BB = Borrero Bay, EG = El Garrapatero). P represents statistical significance of the probability of assignment. These results were obtained using the Paetkau *et al. (*2004) assignment method as implemented in the software GeneClass 2.0.

	P	ABS	ABI	ABL	BBS	BBI	BBL	EGS	EGI	EGL
Academy Bay S (ABS)	0.012		7	4	6	2	0	11	2	3
Academy Bay I (ABI)	0.021	12		6	6		2	7	3	5
Academy Bay L (ABL)	0.001	2	3		0	2	1	2	3	10
Borrero Bay S (BBS)	0.004	8	5	0		3	3	7	1	1
Borrero Bay I (BBI)	0.013	3	1	3	4		2	5	0	2
Borrero Bay L (BBL)	0.011	3	4	4	5	1		0	2	1
El Garrapatero S (EGS)	0.002	6	7	4	6	3			8	1
El Garrapatero I (EGI)	0.005	4	0	3	4	0	0	8		4
El Garrapatero L (EGL)	0.015	1	4	9	1	1	2	1	3	

Appendix 4.4 Bidirectional rates of contemporary gene flow in *G. fortis* between large (above the dividing point in cluster analysis) and small (below the dividing point) birds, and among the different collection sites. P represents statistical significance of the probability of assignment. These results were obtained using the Paetkau *et al. (*2004) assignment method as implemented in the software GeneClass 2.0.

	P	ABS	ABL	BBS	BBL	EGS	EGL
Academy Bay S (ABS)	0.021		13	7	3	11	2
Academy Bay L (ABL)	0.012	7		6	3	9	8
Borrero Bay S (BBS)	0.003	5	6		11	8	1
Borrero Bay L (BBL)	0.034	5	8	8		6	2
El Garrapatero S (EGS)	0.002	12	7	12	4		8
El Garrapatero L (EGL)	0.013	7	14	3	3	9	

Linking statement between Chapter 4 and Chapter 5

Previous chapters revealed that the medium ground finch (*G. fortis*) seems to be in an early stage of adaptive diversification at El Garrapatero. That is, the two beak-size morphs show strong divergence in morphology, ecology, and genetic markers. At another site (Academy Bay), however, morphological (Hendry *et al*. 2006) and genetic (Chapter 4) divergence in *G. fortis* is less strongly associated with beak size. This lack of divergence at Academy Bay appears to be related to human activities at this site (Hendry *et al*. 2006). This is interesting because many studies have shown that humans can impact the evolution of natural populations in contemporary time, but not many have specifically considered effects on adaptive radiation. In the next chapter, I therefore examined how human activities might weaken the strength of associations between morphology (beak and head dimensions), ecology (diet and food resource), and performance (bite force). If so, this would strength the hypothesis that the specific human influences are related to their alteration of food resources for Darwin's finches in ways that convert the rugged adaptive landscapes that promote diversification (Chapter 1) into smooth landscapes that hamper it.

CHAPTER 5

Exploring possible human effects on the evolution of Darwin’s finches

***** A version of this chapter appears as: Exploring possible human effects on the evolution of Darwin’s finches. Submitted to Evolution

5.1 Abstract

Humans present an increasingly common influence on the evolution of natural populations. Potential arenas of influence include the evolution of traits within populations and the process of divergence among populations. We consider this latter possibility for the medium ground finch (*Geospiza fortis*) of Santa Cruz Island, Galápagos, Ecuador. Our study compared the *G. fortis* population at a relatively undisturbed site, El Garrapatero, to that at a severely disturbed site, Academy Bay. The former population currently shows beak size bimodality that is tied to reproductive isolation, whereas the latter population was historically bimodal but has since lost this property – in conjunction with a dramatic increase in human population density. In the present study, we evaluate potential ecological-adaptive drivers of these differences by quantifying relationships between morphology (beak and head dimensions), functional performance (bite force), and environmental characteristics (diet). Our main finding is that associations among beak size, bite force, and diet are all weaker at Academy Bay than at El Garrapatero. These results are consistent with the hypothesis that the rugged adaptive landscapes promoting and maintaining diversification in nature can be smoothed by human activities, thus hindering ongoing adaptive radiation.

Key words: Galápagos, adaptive radiation, diversification, human impacts, contemporary evolution, ecological speciation

5.2 Introduction

The environmental conditions experienced by natural populations are increasingly altered by human activities (Pimm *et al.* 1995; Vitousek *et al.* 1997). Potential consequences extend from extirpations and extinctions (Hughes *et al.* 1997; Pimm & Raven 2000; Mace *et al.* 2003) to a variety of other ecological and evolutionary effects (Smith and Bernatchez 2008). The best-studied evolutionary effects involve changes in the mean values of presumed-adaptive traits (Reznick & Ghalambor 2001; Stockwell *et al.* 2003; Hendry *et al.* 2008), as well as related changes in individual fitness, population productivity, and probability of persistence (Bürger & Lynch 1995; Gomulkiewicz & Holt 1995; Kinnison & Hairston Jr. 2007; Pelletier *et al.* 2007; Gordon *et al.* 2009). In the present paper, we focus on a different type of eco-evolutionary effect about which relatively little is known: the consequences of human activities on the process of evolutionary diversification.

Human activities might influence evolutionary diversification in several ways (Seehausen *et al.* 1997; Hendry *et al.* 2006; Taylor *et al.* 2006; Seehausen *et al.* 2008; Slabbekoorn & Ripmeester 2008; Smith *et al.* 2008), and these can be considered either as inflationary (increasing diversity) or deflationary (decreasing diversity). On the inflationary side, humans might enhance diversification by fragmenting formerly continuous species ranges, thereby increasing the potential for different populations to proceed on independent evolutionary trajectories. One example might be the increased divergence between *Timema* walking-stick host races following a human-caused isolation of two host plant types (Nosil 2009). In addition, humans can provide new environments or resources that are distinct from existing environments or resources. If these new environments can be colonized by local individuals, population divergence can proceed between the ancestral and derived types. A classic example is the evolution of new insect host races on introduced plants (Bush 1969; Carroll *et al.* 1997). On the deflationary side, humans might hamper diversification by increasing contact between populations or species that cannot then maintain their integrity and instead fuse into a single species. Examples include the frequent hybridization of native and introduced species (Rhymer & Simberloff 1996; Streelman *et al.* 2004). In addition, humans might alter resource distributions so that they are no longer so multi-modal, essentially smoothing adaptive landscapes and reducing the divergent-disruptive selection that maintains separate populations or species. A possible example involves human-caused changes in the food resources of Darwin's finches (Hendry *et al.* 2006) – a possibility we investigate in the present paper.

The fusion of two species into one has been called speciation reversal (Taylor *et al.* 2006; Seehausen *et al.* 2008). We suggest that speciation reversal resulting from human induced changes in the adaptive landscape would be most likely under several conditions. The first is when sympatric forms show adaptive divergence and reproductive isolation that is due mainly to ecological differences (rather than, for example, intrinsic genetic incompatibilities). The second is when ample genetic variation is present in the adaptive traits, making them responsive to altered selection. And the third is when adaptive divergence occurs along a resource axis (e.g., diet) that is impacted by humans. Each of these three conditions is met in some species of Darwin's finch, allowing us to use this group for examining how humans might impact adaptive divergence by altering adaptive landscapes.

5.2.1 Darwin's finches: Beaks, bites, diets, and human impacts

Darwin's finches include 14 recognized species that radiated from a common ancestor after colonization of the Galapágos Archipelago approximately 3 million years ago (Lack 1947; Grant 1999; Grant & Grant 2008b). The different species show dramatically different beak morphologies (size and shape) that are well suited for exploiting different food resources, such as seeds of different size and hardness, fruits, insects, and nectar (Lack 1947; Bowman 1961; Abbott *et al.* 1977b; Schluter & Grant 1984; Grant 1999; Schluter 2000; Foster *et al.* 2008; Grant & Grant 2008b). This adaptive diversification is thought to have driven the reproductive isolation currently evident among these species (Schluter 2000; Grant and Grant 2008). The most important reproductive barriers are ecological selection against hybrids (Grant & Grant 1993; Grant & Grant 2008b) and assortative mating based on beak size and song (Ratcliffe & Grant 1983; Podos 2001; Grant & Grant 2008b).

Our work focuses on a part of this radiation, the ground finches (*Geospiza* spp.), that is in the early stages of diversification. In particular, these species separate only at the tips of the Darwin's finch phylogeny and they are not yet reciprocally monophyletic (Petren *et al*. 1999; Sato et al. 1999; Zink 2002).

Second, the different species do not show intrinsic genetic incompatibilities that would be typical of a long period of isolation (Grant & Grant 1992; 1996a; 1997a; 1998; Grant *et al.* 2005). On Santa Cruz Island, up to four *Geospiza* species can occur in sympatry, and their foraging traits apparently correspond to alternative fitness peaks on a diet-based adaptive landscape (Lack 1947; Bowman 1961; Abbott *et al.* 1977; Schluter & Grant 1984; Grant 1999; Schluter 2000; Grant & Grant 2008b). *Geospiza scandens* has a long beak and is a cactus specialist, whereas the other three species have deeper and blunter beaks and are more commonly granivorous. These latter species include the small ground finch (*Geospiza fuliginosa*), which has a small beak and commonly eats small, soft seeds, the medium ground finch (*Geospiza fortis*), which has an intermediate beak and commonly eats intermediate seeds, and the large ground finch (*Geospiza magnirostris*), which has a large beak and commonly eats large, hard seeds (Abbott *et al.* 1977; Smith *et al.* 1978). These beak size differences are highly heritable both within and between species (Boag & Grant 1978; Boag 1983; Keller *et al.* 2001; Abzhanov *et al.* 2004), and the three granivorous species do not differ in beak shape after controlling for allometry (Foster *et al.* 2008; Campàs *et al.* 2010).

G. fortis is the primary focus of our work because some populations of this species show signs of an incipient split into two forms. At El Garrapatero, in particular, two beak size morphs (small and large) are currently present in a bimodal distribution (Hendry *et al.* 2006). Although the origin of these morphs is not known, they are currently sympatric and differ strongly in bite force (Herrel *et* *al.* 2005a), song characteristics (Podos *et al.* 2004; Huber & Podos 2006; Podos 2007; Herrel *et al.* 2009), responses to song characteristics (Podos 2010), and mate preferences (Huber *et al.* 2007). The two morphs also experience disruptive viability selection on beak size (Hendry *et al.* 2009), and they are differentiated at neutral genetic markers (Huber *et al.* 2007; De León *et al.* 2010). Importantly, all of these differences are intermediate to those between *G. fortis* and its smaller (*G. fuliginosa*) and larger (*G. magnirostris*) congeners. The factors promoting and constraining evolutionary divergence within the *Geospiza* therefore might be profitably informed by comparing *G. fortis* populations that differ in their degree of bimodality.

In this comparative context, a suitable contrast to the strongly bimodal population at El Garrapatero is the *G. fortis* population at Academy Bay. This population was strongly bimodal in beak size prior to the 1970s but no longer has this property (Ford *et al.* 1973; Hendry *et al.* 2006). The conclusion was reached based on quantitative analysis of raw data collected using similar methods from 1964, 1968, 1973, 1988, and 1999 to the present (Hendry *et al.* 2006). In essence, conclusive statistical support for a bimodal distribution was evident in 1964 and 1968 (and alluded to in earlier work by Lack 1947 and others) but not in later samples. Hendry *et al*. (2006) hypothesized that the decrease in bimodality at Academy Bay was the consequence of the dramatic intensification of human activities at this site. This conclusion was reached because although many environmental conditions change through time, these do not show consistent differences between time periods (e.g., rainfall: Hendry *et al*. 2009), in contrast to human population size at the directly adjacent town of Puerto Ayora. This has increased from approximately 500 in the 1960s to more than 19,000 in 2006 (Watkins & Cruz 2007). Because the Galapágos are a park, increases in human population density are extremely localized, and so are unlikely to influence finch populations more than a few kilometers distant – including El Garrapatero.

The specific hypothesis is that humans have altered the availability of food types at Academy Bay in ways that weaken the selective forces otherwise maintaining bimodality (Hendry *et al*. 2006). In the present paper, we test several predictions that derive from this hypothesis. First, we predict that beak size should be less closely associated with diet at Academy Bay than at El Garrapatero. The idea here is that the food types introduced by humans at Academy Bay (but not at El Garrapatero) can be used by birds of all beak sizes, thus reducing the need for diet specialization related to beak size. Second, and for the same reason, we predict that bite force should be less closely associated with diet at Academy Bay than at El Garrapatero. Third, we predict the same for beak size in relation to bite force. One reason here could be a breakdown of the correlational selection that normally maintains this association: i.e., birds no longer need to have a close match between their bite force and beak size. Another reason could be that birds with large beaks now have less "training" in cracking hard seeds, which might normally be necessary to develop the muscles for high bite force (i.e., plasticity). Fourth, and for the same reasons, we predict that bite force at a given beak size should be lower at Academy Bay than at El Garrapatero.

We test these predictions using a large, multi-year data set on available foods and individual diets, bite forces, and beak dimensions at Academy Bay and El Garrapatero. This analysis is correlative and so causation is difficult to conclusively infer. Moreover, Academy Bay is the only site where a known bimodal finch population has come into contact with a rapidly expanding human population. And El Garrapatero is the only documented bimodal population that remains separate from human populations. For this reason, replication of the "disturbed" and "undisturbed" contexts is not possible. We therefore restrict our main inferences to our specific study populations, rather than the general situation where humans come into contact with bimodal populations. Our results however; might still exemplify some of the ways of how humans could impact adaptive divergence.

5.3 Methods

5.3.1 Ecological context

We studied *G. fortis* at two sites on Santa Cruz Island, Galapágos, Ecuador. One site, Academy Bay, is located on the south shore and is contiguous with the town of Puerto Ayora. Some of the direct human influences at this site include a high occurrence of exotic plant species and human foods, both of which finches consume (see below). Like human foods, the exotic plants are particularly abundant in open areas along the roadsides and around the human settlement in Puerto Ayora. These include *Lantana camara*, *Momordica charantia*, *Pasiflora foetida*, *Capsicum annuum*, *Chloris virgata*, *Eragrotis ciliaris*, and *Penisetun* *purpurium*. The other site, El Garrapatero, is located on the eastern shore of the island approximately 11 km north-east of Puerto Ayora. El Garrapatero is not adjacent to human settlements (Fig. 5.1) and experiences minimal direct human influences. Indirect influences do exist in the form of browsing by feral goats and donkeys. Importantly, however, non-native plant species and human foods are rare at El Garrapatero in comparison to Academy Bay. The contrast in human influences between these two sites provides the opportunity to explore how humans might influence evolutionary diversification in Darwin’s finches (Hendry *et al.* 2006).

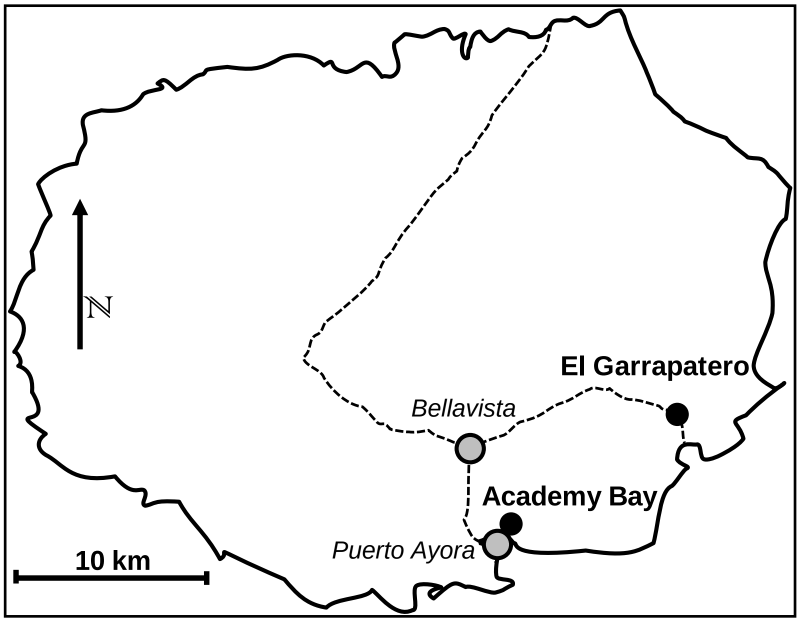

Figure 5.1 Study sites (black circles) and human settlements (gray circles) on Santa Cruz Island, Galapágos, Ecuador. The dotted lines represent roads.

5.3.2 Available resources and diets

We surveyed the food resources available at each site by updating the methods of Abbott *et al*. (1977). At El Garrapatero, we used GPS coordinates to randomly designate 50 different plots, each 1 m^2. The same numbers of plots were randomly designated at Academy Bay within accessible areas (in some areas the vegetation is too dense for sampling). At each plot, we tallied the abundance of different food items (seeds, flowers, and fruit) on each plant species in the standing vegetation. Plant species were identified by reference to published catalogues (Wiggins & Porter 1971; McMullen 1999) and by consulting herbarium specimens at the Charles Darwin Research Station. For a 10 cm^2 sub-plot within each plot, we then tallied the abundance of seeds of each plant species on the surface of the ground. At the same time, we also tallied other potential food items, including arthropods and human foods (e.g., rice, bread, potato chips). For each sub-plot, we also collected superficial soil samples (~45 g) that were examined under a stereoscope for additional seeds. Sub-plot seed counts, determined as the sum of the counts on the ground surface and in the soil samples, were extrapolated to whole plot, and added to counts of the same items in the standing vegetation. This yielded a total count of each food type for each plot. At both Academy Bay and El Garrapatero, we sampled the same plots, but different sub-plots, in each of three years (2004, 2005, and 2007).

For each plant species, we measured the length, width, depth, and hardness of ten intact seeds haphazardly collected from the ground. Hardness was estimated by cracking individual seeds with a Kistler force transducer attached to a Kistler charge amplifier (Kistler Inc., Winterthur, Switzerland). Following Abbott *et al*. (1977), we then estimated a weighted index of seed size-hardness for each plant species. This index (H*i*) was obtained by combining mean seed depth (*D*) in millimeters with mean seed hardness (*H*) in Newtons ($Hi=\sqrt{HD}$; Abbott *et al*. 1977), and it is intended to capture the likely efficiency with which birds of a given beak size can handle different seeds types. That is, it should be more difficult to crack seeds that are harder and that are larger (Grant *et al.* 1976; Abbott *et al.* 1977; Boag & Grant 1984). We also categorized plant species into three seed size/hardness categories: small-easy (*Hi* < 3.0), intermediate (4.0 > *Hi* < 11.0), and large-hard (*Hi* > 11.0).

G. fortis were captured in mist nets and measured for beak dimensions following Grant *et al*. (1985), and for head dimensions and bite force following Herrel *et al*. (2005a, 2005b, 2009). Specific measurements included beak length (anterior edge of nares to anterior tip of upper mandible), beak depth (at the nares), beak width (at the base of the lower mandible), head length (from the tip of the upper mandible to the back of the head), head depth (at the deepest part of the head posterior to the orbits), and head width (the widest part posterior to the orbits). For beak traits, three measurements were taken and the median was used for analysis. For head dimensions, only a single measurement was required. We also classified *G. fortis* individuals into "small" and "large" beak size categories (referred to here as "morphs"). This was done by using PC1 scores based on beak dimensions (beak length, depth, and width) in a cluster analysis (e.g., Huber *et al*. 2007) conducted separately for each site. Bite force was measured separately at the tip and the side of the beak using a Kistler force transducer attached to a Kistler charge amplifier (Kistler Inc., Winterthur, Switzerland) (see Herrel *et al*. 2005a for details). All of these measurements are highly repeatable (Herrel *et al.* 2005a). At first capture, each bird was banded with a unique combination of three colored leg bands and a metal leg band. This allowed us to avoid unknowingly measuring the same bird multiple times, and it enabled individual identification of free-ranging birds. Finally, we crudely estimated relative abundance of birds at the two sites based on capture rates per net-hour over our entire study period.

Feeding observations took place between January and April over five consecutive years (2003 to 2007; Table 5.1). Data were collected during morning and afternoon walks at the study sites. Each time a banded bird was seen; it was followed and observed through binoculars until a foraging event was observed. This was possible because Darwin’s finches are very tame and easily observed through binoculars at close range without being disturbed. For each feeding observation, we recorded the food item (e.g., plant species or “arthropod”) and, if applicable, the specific plant part (e.g., seeds, fruits, leaves, flowers). When this precision was not possible, we used more inclusive diet categories, including “grass” (several Gramineae species with small-soft seeds), “ground” (unidentified small seeds), and “human food” (Appendix 5.1). After a single observation of a given bird, we began searching for other banded birds. This “point observation” method, as opposed to following individual birds for extended periods of time (e.g., Abbott *et al*. 1977; Smith *et al*. 1978; Price 1987), was intended to maximize the independence of different foraging observations of individual birds.

From the resulting count data, we estimated the proportion of different food types in the diets of *G. fortis* individuals. For this analysis, we only included data for birds with at least six independent feeding observations. If these counts were converted to biomass or energy, then the relative importance of large seeds would increase and that of small seeds would decrease. This conversion was not attempted here because the necessary data were not available and because seeds cannot currently be exported from Galapagos. Nevertheless, seed size is strong correlated with energy content (Schluter 1982). For this reason, and because we are interested in relative differences between sites, counts should be sufficient for the inferences attempted here.

Individual-based diet data were only possible for banded birds and banding was only allowed in locations where tourists are not common. Thus, we could only assess diets at Academy Bay at sites adjacent to the main town. However, we also wished for some additional species-level data on finch diets within the town itself. In 2005 and 2006, we therefore performed similar feeding observations during walks in different places (along the main streets and restaurants) in Puerto Ayora.

Table 5.1 Summary of feeding observations on individual *G. fortis* in each year at each site.

	Academy Bay			El Garrapatero		
Year	Total observations	Individual birds	Different food types	Total observations	Individual birds	Different food types
2003	52	22	10	82	16	8
2004	226	45	18	148	33	13
2005	37	19	7	241	38	11
2006	9	3	5	304	73	9
2007	1	1	1	15	2	4
Total	325	90	41	790	162	45

5.3.3 Data analysis

We first used Pearson Product Moment correlations to test for associations among ecologically-important morphological traits (beak size [PC1], head size [PC1]), performance traits (bite force [tip and side]) and diet (average *Hi* for the seed types eaten, weighted by the proportion of each type eaten). Similar results were obtained for beak size and head size, and so we here only report the former. We then compared these associations between Academy Bay and El Garrapatero by testing for significant differences between correlation coefficients (Cohen *et al.* 1983). Additionally, we used two-way ANOVA to test for (fixed) effects of site and beak morph on bite force and *Hi*. We also performed a similar analysis but with ANCOVA using beak size (PC1) as a continuous variable instead of discrete beak morph categories as in ANOVA. Data for all years of observation were pooled in the analysis because of the large number of feeding observations required per bird. When necessary, the data were log-transformed to improve normality.

Given that diet is inherently multivariate and probably not best encapsulated by a single average index of seed size-hardness, we also employed permutational multivariate analysis of variance with diet (frequencies of feeding on different food types) as the response variable. The first analysis used beak morph (small or large) as a grouping variable and was based on Bray-Curtis distance matrices of proportionally scaled diets using the Adonis function (Anderson 2001) in the software R (R Development Core Team 2007). Statistical significance was obtained through 1000 permutations of the raw data. We next conducted canonical redundancy analysis (RDA) to examine the relationship between diet (response variable) and a matrix of phenotypic traits (explanatory variables). These traits included beak dimensions, head dimensions, and bite force. This analysis identifies optimal linear combinations (Thompson 1984; Ter Braak 1986; Lepš & Šmilauer 2003) of phenotypic traits that best explain the variation in diet. Statistical significance was obtained through 1000 Monte Carlo permutations.

To graphically illustrate the association between diet and morphology at each site, we performed Non-Metrical Multidimensional Scaling (NMDS) based on Bray-Curtis distances. We then used the first two NMDS axes to create a 3D surface plot, adding a third axis based on the most influential linear combination of morphological traits (beak and head dimensions) and performance measures (tip and side bite force). The resulting plots helped visualize the degree to which particular morphological traits are associated with diet differences at each site. The software package R (R Development Core Team 2007) was used for all analyses, unless otherwise noted.

Differences in the morphological distribution of *G. fortis* between the two sites could influence the above analyses. For instance, the average beak size of birds in each morph category was larger at El Garrapatero than at Academy Bay (small morph: $t=2.5$, $p=0.01$; large morph: $t=4.8$, $p<0.001$). In order to make the two sites directly comparable with respect to beak size, we therefore “trimmed” the data by excluding extreme individuals at both sides of the distribution to make them more similar between the two sites. After this procedure, beak size differences were no longer evident between the sites for the small (t=0.76, p=0.45) or large (t=11.53, p=0.13.) morphs. We then repeated the above analyses using this new trimmed data set.

5.4 Results

5.4.1 Available resources and diets

We recorded 44 different available food items, including 24 different plant species (Appendix 2.5). Most seeds were quite small-soft but some large-hard seeds were also present (Fig. 5.2). If these counts were converted to biomass or energy, then the relative importance of large seeds would increase and that of small seeds would decrease. This conversion was not attempted here because the necessary data were not available and because seeds cannot currently be exported from Galapagos. Nevertheless, seed size is strongly correlated with energy content (Schluter 1982). For this reason, and because we are interested in relative differences between sites, counts should be sufficient for the inferences attempted here.

El Garrapatero and Academy Bay differed in available food resources in several ways. First, seeds of all types combined were more than twice as abundant at Academy Bay as at El Garrapatero (Table 5.2). Second, the overall frequency distribution of the seed size-hardness index differed between the sites (Fig. 5.2). In particular, relatively more small-soft seeds (*Hi* <3.0) were present at Academy Bay (65.9%) than at El Garrapatero (57.7%). Third, human foods (e.g., rice, bread, and potato chips) were found only at Academy Bay (0.22%) (Appendix 2.5).

We obtained a total of 1115 point observations of 252 *G. fortis* individuals (Table 5.1) feeding on 28 different plant species (Appendix 5.1). One major difference between the two sites was that the proportion of feeding observations on “grass” (small grass seeds) and “arthropods” (consuming or searching for arthropods along branches) was at least three times higher at El Garrapatero than at Academy Bay. Another difference was that feeding observations of banded birds on human food items occurred only at Academy Bay (5.85%). In addition, for unbanded birds in the town on Puerto Ayora, we recorded 194 observations of finches feeding on human foods, including bread, cooked beans, rice, ice cream cones, and chips. During these observations, no finch was observed feeding on natural food items. Overall, birds frequently exploited human food items at Academy Bay but not at El Garrapatero.

Data on capture rates in mist nets suggests that the abundance of birds is more than three-fold higher at Academy Bay (birds=575, net-hours=350, birds/net-hour=1.64) than at El Garrapatero (birds=806, net-hours=2224, birds/net-hour=0.36). The direction of this difference was consistent within each study year (results not shown).

Table 5.2 Summary of available seed types across all years according to an index (Abbott *et al*. 1977) of seed size-hardness (*Hi*): small-soft (<3.0N), intermediate (4.0N >*Hi*>11.0N) and large-hard (>11N). Differences between sites in the frequencies of seeds in these different categories were statistically significant as given by a Chi-square test ($\chi^2 = 519.71$, $p<0.01$). Percentages are given in parenthesis.

Seed type	Academy Bay	El Garrapatero	Total
Small-soft	27834 (65.9)	10243 (57.7)	38077 (63.4)
Intermediate	7722 (18.3)	4665 (26.3)	12387 (20.6)
Large-hard	6711 (15.9)	2856 (16.1)	9567 (15.9)
Total	42267 (70.4)	17764 (29.6)	60031

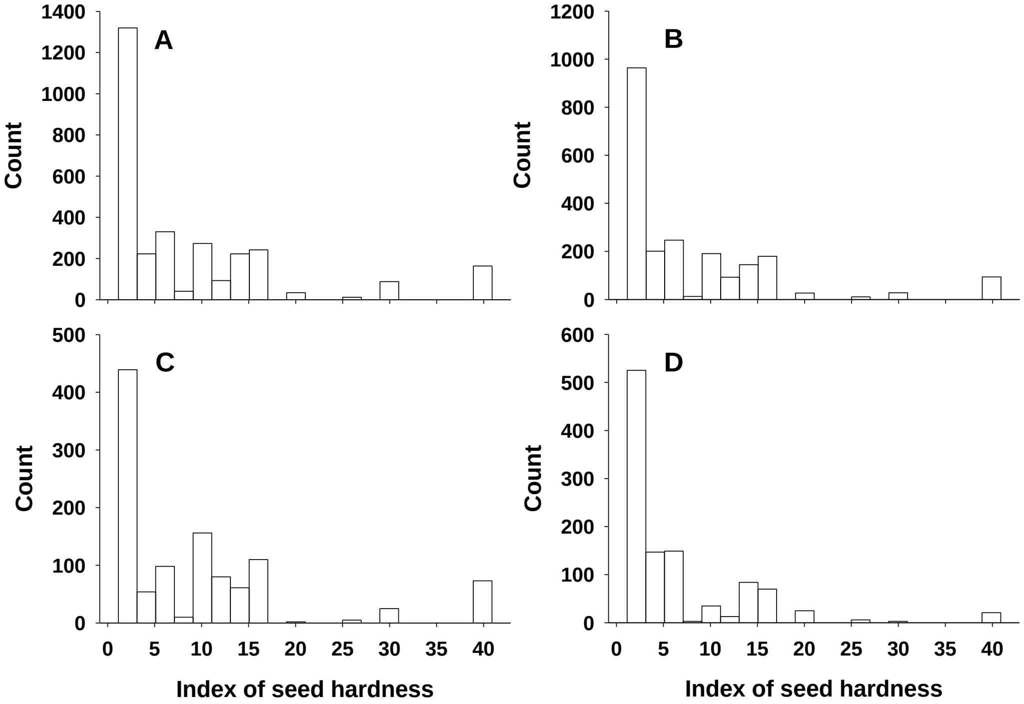

Figure 5.2 Abundances (counts) of available seeds of different size-hardness (*Hi*) at Academy Bay (Panels A and C) and El Garrapatero (Panels B and D). Seed types that are shared across both sites are shown in Panels A and B, and seeds of all types are shown in Panels C and D. The distribution differs statistically between the two sites for the former (D=0.129, p<0.001) and the latter (D=0.282, p<0.001).

5.4.2 Beak size and diet

In a simple correlation across all individuals at both sites and in all years, beak size (PC1), and the index of seed size-hardness (*Hi*) were positively correlated (r=0.227, p=0.002; Fig. 5.3). This result mirrors the findings from another *G. fortis* population (Price 1987). A comparison of the two sites did not reveal significant differences between these correlations (Fig. 5.3). An analysis based on the beak morph categories, rather than the above continuous beak size variation, was more suggestive of a difference between the two sites. In particular, the small morph appeared to feed on smaller-softer seeds than did the large morph at El Garrapatero but not at Academy Bay (Fig. 5.4). This difference (i.e., interaction between morph and site) was not, however, statistically significant (Table 5.3). Differences in *Hi* were, however, evident based on ANCOVA in the form of a statistically significant interaction between beak size and site (F=4.137, p=0.043). In addition, a multivariate analysis of diets revealed a strong interaction between beak morph and site. In particular, the two morphs have similar diets at Academy Bay but different diets at El Garrapatero (Fig. 5.5; Table 5.4).

When repeating the above analyses using the “trimmed data set” that equalized beak sizes in the two “morph” categories (see Methods), the following results were obtained. First, for beak size (PC1) and diet (*Hi*), the correlation was non-significant for both sites combined (r=0.08, p=0.26) and within each site (Academy Bay: r=0.09, p=0.29); El Garrapatero: r=0.10 p=0.22). This contrasts with previous results and we attribute the difference to the reduced range of variation in beak size. Results were similar, however, in that comparisons of correlations and the two-way ANOVA failed to reveal a significant interaction between morph and site (F=1.66, p=0.68). Also as before, ANCOVA (F=3.331, p=0.04) and multivariate analysis of variance (F=2.068, p=0.01) did find a statistically significant interaction between beak size and site. Overall, then, we conclude that the strength of associations between beak size and diet are weaker at Academy Bay than at El Garrapatero, but that this association is only statistically confirmed in more sophisticated analyses.

5.4.3 Bite force and diet

In a simple correlation across all individuals at both sites and in all years, bite force was positively correlated with seed size-hardness (Fig. 5.6). This association appears to be stronger than the one between beak size and seed size-hardness (Fig. 5.3), which matches expectations. Specifically, bite force is a composite performance trait (*sensu* Arnold 1983; Irschick *et al.* 2008), influenced by variation in both beak size and muscle mass, and is therefore a better predictor of feeding performance than is beak size alone (Bowman 1961; Herrel *et al.* 2005a, b; Soons *et al.* 2010). In a comparison of the two sites, the correlations between bite force and seed size-hardness appeared weaker at Academy Bay than at El Garrapatero although this was not statistically significant (Fig. 5.6). In ANCOVA, however, seed size-hardness increased more rapidly with bite force at El Garrapatero than at Academy Bay (F=84.21, p<0.001).

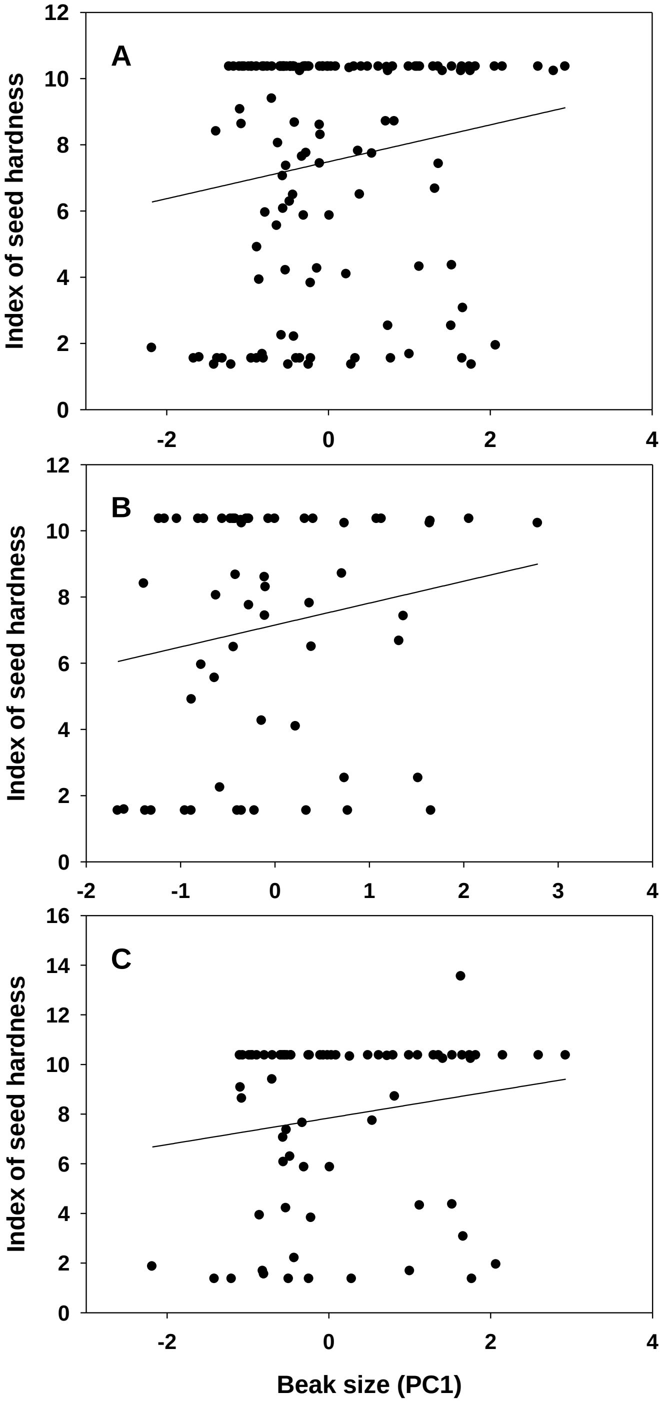

Figure 5.3 Correlations between beak size and diet. Panel A shows the correlation across birds at both sites. Panel B shows the correlation for Academy Bay (r=0.17, p<0.001). Panel C shows the correlation for El Garrapatero (r=0.17, p<0.001). The two correlations are not significantly different between sites (Z=0, p=0.5).

Table 5.3 Two-way Analysis of Variance (ANOVA) of the mean seed size-hardness (*Hi*) consumed and the bite force of individual *G. fortis* according to their beak size morph and site (El Garrapatero and Academy Bay).

	Df	F	Pr(>F)
Hi			
Morph	1,155	1.47	0.23
Site	1, 155	16.68	< 0.001
Morph*site	1, 155	0.022	0.88
Tip bite force			
Morph	1,72	43.65	< 0.001
Site	1,72	14.343	< 0.001
Morph*site	1,72	2.81	0.09
Side bite force			
Morph	1,72	42.59	< 0.001
Site	1,72	12.68	< 0.001
Morph*site	1,72	2.73	0.10

Table 5.4 Permutational multivariate analysis of variance using matrices of distances between individuals in the frequencies of different food types consumed. These matrices were calculated using Bray-Curtis distances. Each model first shows results for an interaction between beak size (morph) and location (site), and then shows results for a comparison of the two morphs within each site: Academy Bay (AB) and El Garrapatero (EG).

	Df	F	R^2	Pr(>F)
Analysis based on Plant items				
a) Morph*site	1	2.460	0.009	0.013
b) AB morph	1	1.220	0.0138	0.270
c) EG morph	1	2.972	0.018	0.006

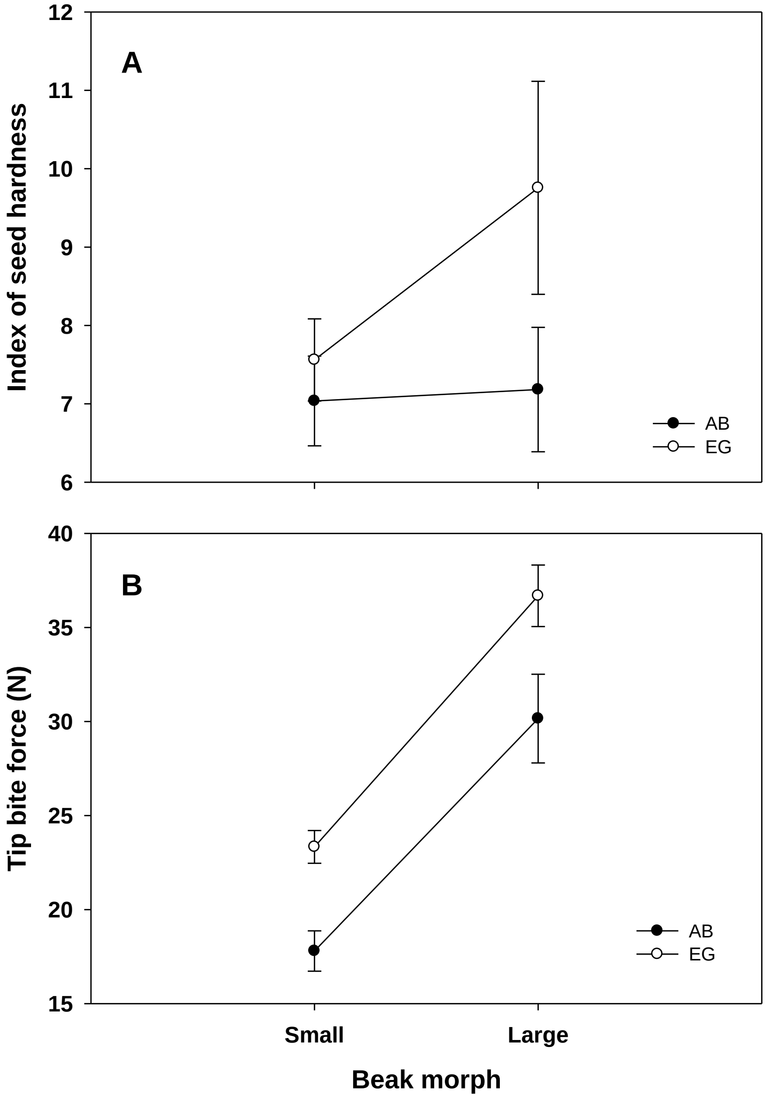

Figure 5.4 Effects of beak morph on diet and bite force. Panel A shows the average size-hardness (*Hi*) of seeds consumed by *G. fortis* in each beak size category (small and large) at Academy Bay (AB) and El Garrapatero (EG). No significant differences are evident (Table 5.3) in a two-factor ANOVA (site and beak morph) but multivariate analyses confirm that the two morphs differ in diet at El Garrapatero but not at Academy Bay (Table 5.4; Fig.5.5). Panel B shows that bite force as measured at the tip of the beak is significantly different between beak morphs and sites, being higher in the large morph and at El Garrapatero (EG). Error bars indicate standard error.

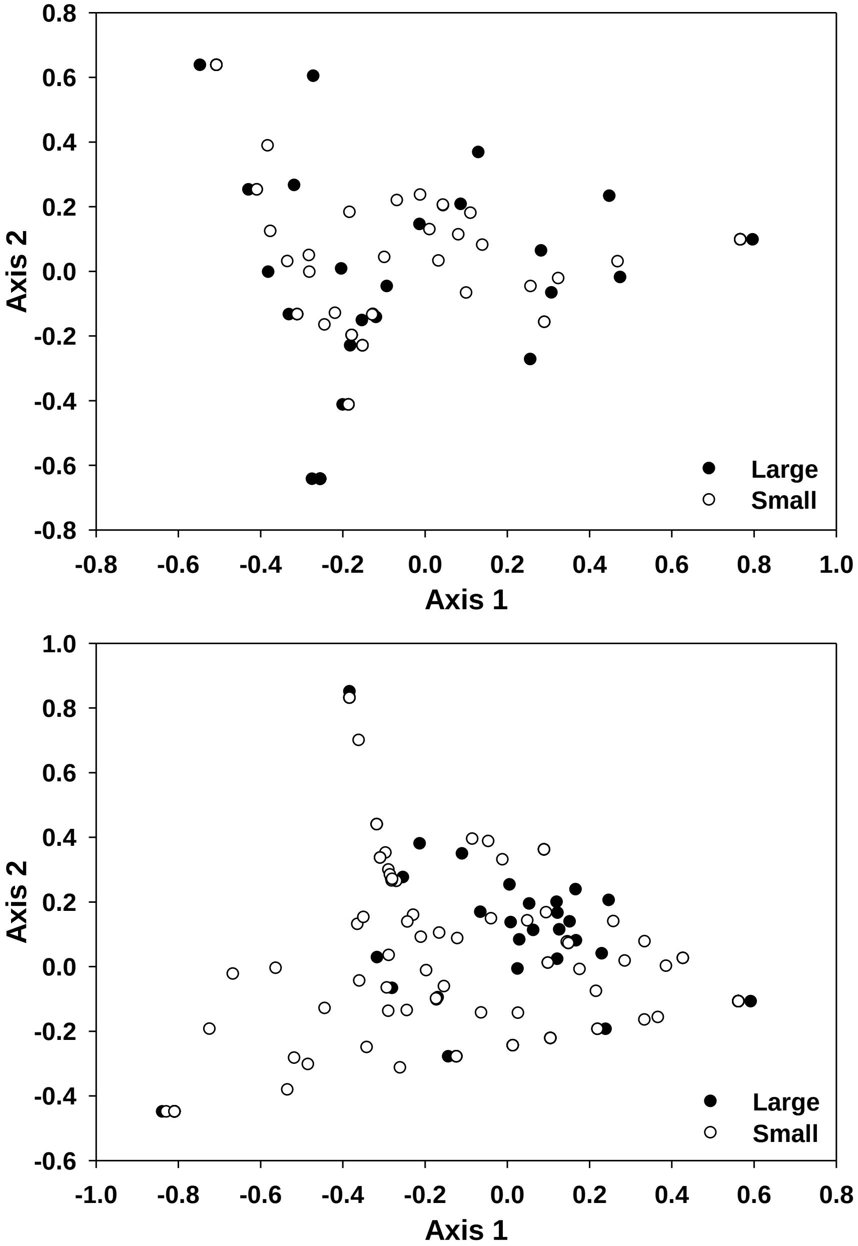

Figure 5.5 Non-metrical Multidimensional Scaling (NMDS) plot of the diets of individual small (open circles) and large (filled circles) beak size morphs of *G. fortis* at Academy Bay (Panel A) and El Garrapatero (Panel B). The data represent diets as derived from specific plant items and the differences between morphs are significant at El Garrapatero but not at Academy Bay (Table 5 4). Some of the point coordinates were slightly modified to avoid overlap and so make them more visible.

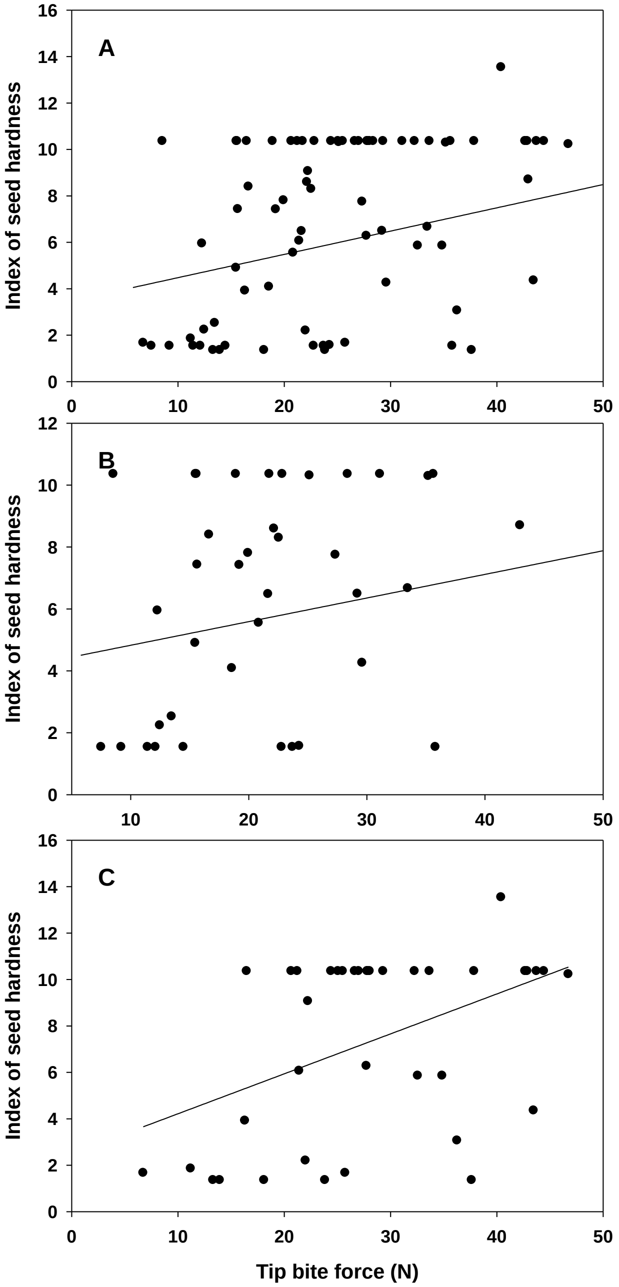

Figure 5.6 Bite force of individual birds correlates with the average size-hardness (*Hi*) of the seeds they eat. Panel A shows the significant relationship for pooled data from the two sites (r=0.41, p<0.001). Panel B shows the significant relationship for Academy Bay (r=0.32, p<0.001). Panel C shows the significant relationship for El Garrapatero (r=0.45, p<0.001). Similar correlations were obtained using side bite force instead of tip bite force (results not shown). No significant differences in these relationships were detectable between the two sites (tip: Z=0.63, p=0.26; side: Z=0.28, p=0.39).

For the trimmed data, bite force and diet correlations were positive across both sites (r=0.22, p=0.01) and at El Garrapatero (r=0.27 p=0.02). The correlation was not significant at Academy Bay (r=0.21, p=0.09), and no statistical difference could be detected for El Garrapatero versus Academy Bay (Z=0.33, p=0.37). However, ANCOVA once again showed that seed size-hardness increased more rapidly with bite force at El Garrapatero than at Academy Bay (F=72.26, p=0.001). Overall, then, we conclude that seed size-hardness increases more rapidly with bite force at El Garrapatero than at Academy Bay.

5.4.3 Beak size and bite force

In a simple correlation across all individuals at both sites and in all years, beak size was positively correlated with bite force (Fig. 5.7). Considering the sites separately, these correlations were stronger at El Garrapatero than at Academy Bay (Fig. 5.7). An analysis based on the morph categories, rather than the above continuous beak size variation, yielded several conclusions: (1) small-beaked birds had lower bite force than did large-beaked birds at both sites, (2) each beak size morph had lower bite force at Academy Bay than at El Garrapatero, and (3) the relationship between beak morph and bite force differed between the two sites (Fig. 5.4). Finally, multivariate analyses (RDA) showed that the strength of associations between morphology/performance and diet were weaker at Academy Bay than at El Garrapatero (Fig. 5.8; Table 5.5).

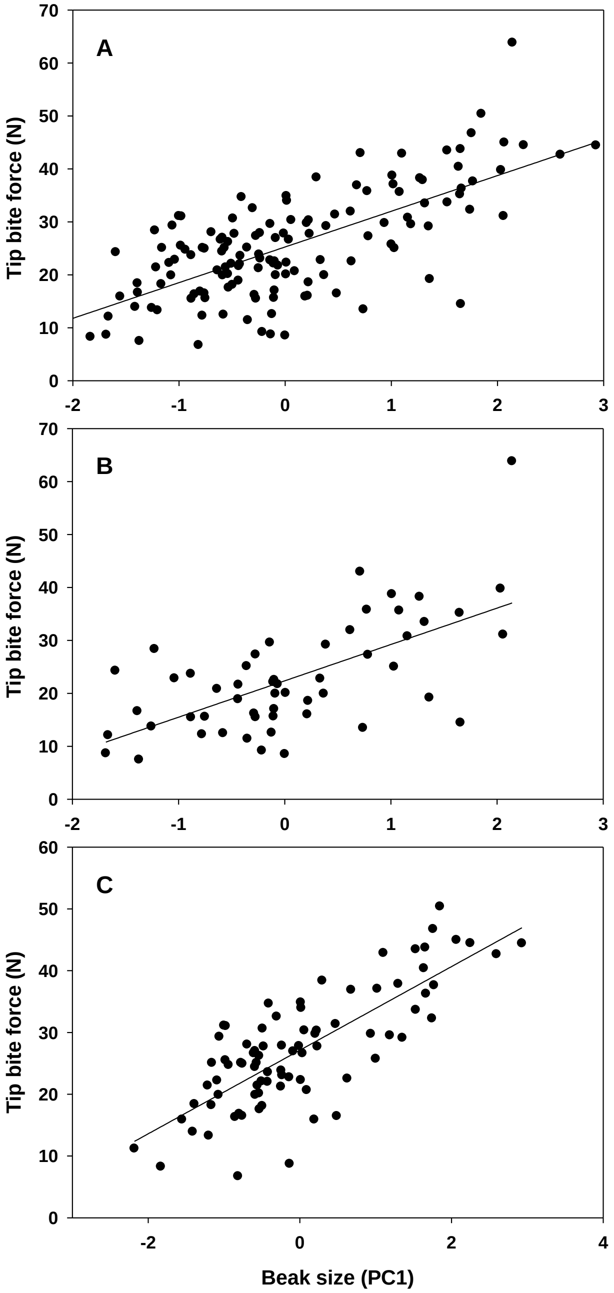

Figure 5.7 Beak size of individual birds correlates with their bite force. Panel A shows the significant relationship for pooled data from the two sites (r=0.69, p<0.001). Panel B shows the significant relationship for Academy Bay (r=0.64, p=<0.001). Panel C shows the significant relationship for El Garrapatero (r=0.78, p=<0.001). The relationship is stronger at El Garrapatero than at Academy Bay for both tip bite force (Z=1.6, p=0.05) and side bite force (not shown; Z=2.24, p=0.01).

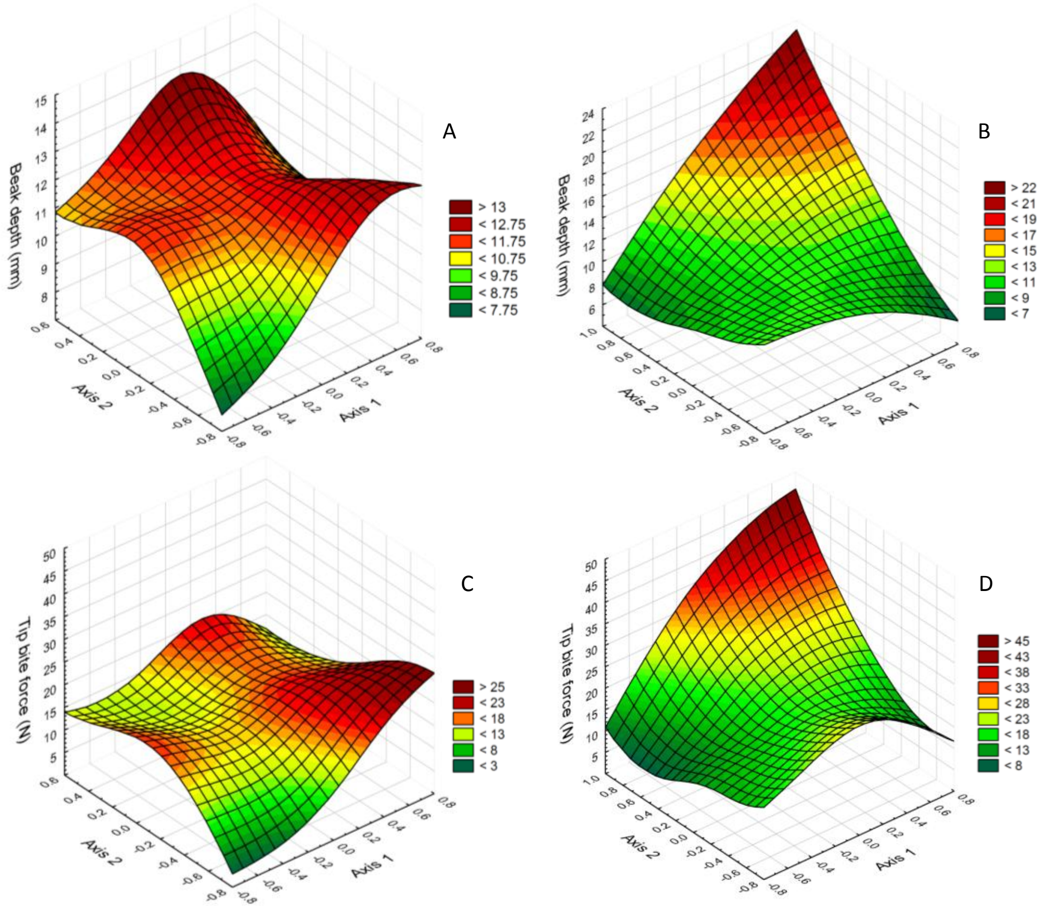

Figure 5.8 Non-metrical Multidimensional Scaling (NMDS) 3-D surface plots of mean beak depth (A,B) and tip bite force (C,D) Academy Bay (A, C) and El Garrapatero (B, D) against the first two diet axes. The plots show that birds exploiting different food items have different traits at each site, but the association is stronger at El Garrapatero than at Academy Bay. Similar results were obtained for all morphological traits (results not shown).

Table 5.5 Permutation tests for Canonical Redundancy Analysis (RDA) of the contribution of morphology/performance to the difference in diet between the two *G. fortis* beak-size morphs at each site.

Test	Df	Variance	F	Permutations	Pr(>F)
a) Academy Bay	8	3.166	0.399	99.00	0.29
Residual	16	15.834			
b) El Garrapatero	8	2.8	0.368	199.00	0.005
Residual	17	16.2			

In analyses of trimmed data, the beak size and bite force correlation was positive across both sites (r=0.65, p<0.001) and within each site (Academy Bay: r=0.64, p<0.001; El Garrapatero: r=0.73 p<0.001) although, the correlation was not significantly different (Z=0.88, p=0.19). Finally, multivariate analyses (RDA) also showed that the strength of associations were weaker at Academy Bay than at El Garrapatero (F=1.788, p=0.01). Overall, then, we conclude that the strength of the associations between morphology and performance are weaker at the human disturbed site than at the undisturbed site.

5.5 Discussion

5.5 .1 Context

Previous studies of Darwin's finches have revealed that evolutionary diversification is powered by tight associations between environment (available foods and diets), performance (bite force), and morphology (beaks, heads, and bodies) (Lack 1947; Bowman 1961; Abbott *et al.* 1977; Schluter & Grant 1984; Grant 1999; Herrel *et al.* 2005a; Grant & Grant 2008b). For example, if a group of finches begins feeding on larger/harder seeds, they should be selected for stronger bite forces, which are achieved through larger jaw muscles, which in turn select for larger beaks to resist the resulting stresses (Bowman 1961; Herrel *et al.* 2010a; Soons *et al.* 2010). Spin-off effects are larger heads and bodies (Herrel *et al.* 2005b). This correlated variation should be present among species and populations and also within populations – the latter because broad resource distributions and competition should generate selection for individual specialization (Bolnick *et al.* 2007). This specialization might then provide the seeds of diversification into multiple species (Chapter 3).

In Darwin's finches, we thus expect strong associations among diet, morphology, and performance. We also expect that morphology and performance distributions will match the distribution of available food types. Supporting the first expectation, strong associations have been reported between diet, morphology, and bite force both within and among Darwin's finch species and populations (Lack 1947; Bowman 1961; Price 1987; Herrel *et al.* 2005a, b). Supporting the second expectation, the Darwin's finch species found at a given site have beak characteristics that are well suited for the locally available food types (Schluter & Grant 1984). Given that these resource-based promoters of diversification are ecological, they might be sensitive to environmental change wrought by humans.

5.5.2 Primary conclusions

In the population where human influences on the environment are relatively minor (El Garrapatero), we found that environment-morphology-performance associations were very strong. In particular, the birds in this population manifest strong correlations between diet, beak size, and bite force. In addition, these characteristics show a bimodal distribution (Hendry *et al.* 2006) that is associated with low between-morph gene flow (De León *et al.* 2010). This partial split within *G. fortis* appears to be maintained by assortative mating that limits the production of intermediate forms (Huber *et al.* 2007), and viability selection against any intermediates that are formed (Hendry *et al.* 2009). The specific source of viability selection is unknown but might relate to a relative scarcity of intermediate seeds (Fig. 5.2) or to strong competition for those seeds.

In the population where human influences on the environment are strong (Academy Bay), we found that environment-morphology-performance associations were generally weaker – and that bite force is low for a given beak size. In short, this population appears to have undergone a breakdown of the expected associations that drive diversification. This is consistent with the idea that the adaptive landscape for this population has become flat with respect to beak size. The result should be a reduction in disruptive selection (intermediate birds might no longer be at a disadvantage) and this might ultimately reduce assortative mating (intermediate birds might come to be produced at a higher rate). Although selection and mating have not yet been studied for Academy Bay *G. fortis*, the population does show the anticipated evolutionary outcome of these changes: bimodality is weak or absent (Hendry *et al.* 2009) and genetic differentiation is lower than at El Garrapatero (De León *et al.* 2010). In short, the historical divergence noted for Academy Bay *G. fortis* (Ford *et al.* 1973) has been reversed, perhaps because environmental conditions have changed with the dramatic increase in the local human population.

This reversal of diversification might reflect a combination of genetic and plastic effects. Genetic effects likely predominate for beak morphology given its very high hereditability (Boag 1983; Keller *et al.* 2001; Abzhanov *et al.* 2004). The same might be true for head size and bite force given their strong correlation with beak size (Herrel *et al.* 2005a, b). However, plastic effects might also be more likely for head size and bite force. For instance, increased feeding on hard seeds might lead to the development of larger jaw abductor muscles, which would then lead to higher bite force and a larger head (Bowman 1961; van der Meij & Bout 2004; Herrel *et al.* 2005b). We attempted to understand the potential for such training effects by comparing the bite force of fully grown young birds (1-3 year old) with that of older birds (> 3 years old), expecting that older birds would have more training. First, two-way ANOVA (to test for the effects of age on bite force, controlling for site and mophology) showed that younger birds have lower bite force than older birds (F=6.91, p=0.001). Second, at a similar age and morphology, correlation analyses revealed that birds at Academy Bay (juveniles: r=0.07, p=0.85; adults: r=0.60, p<0.001) have lower bite force than at El Garrapatero (juveniles: r=0.59, p=0.41; adults: r=0.78, p<0.001). Thus, although training does influence bite force, standardizing for one aspect of training (age) did not change the difference between sites. Of course, other effects of plasticity might remain.

Our hypothesis that the loss of bimodality is the result of human disturbance requires that Academy Bay finches consume foods that have been introduced into the environment by humans. Our data revealed that this behavior occurs at Academy Bay but never at El Garrapatero (Appendix 5.1). Moreover, our intensive surveys of banded birds underestimate the true frequency with which Academy Bay finches consume human foods – because we were not allowed to band birds in tourist areas. Surveys of unbanded birds in Puerto

Ayorra, however, revealed *G. fortis* frequently consuming human foods but not natural foods (see Results). In addition, large numbers of *G. fortis* routinely feed on rice at human-maintained "feeders" within 100 m of our study site (Hendry *et al.* 2006). Importantly, both large and small *G. fortis* can consume these food types because they do not require a particular beak size or bite force – in contrast to the situation for many natural foods (Grant 1999).

5.5.3 Alternative Hypotheses

Our main hypothesis is that human influences on the resource distribution have caused the collapse of strong bimodality at Academy Bay. However, because our study is correlative and unreplicated (both limitations by necessity), alternative hypotheses should be considered.

Some alternatives are possible to the inference that humans are the cause of the decrease in bimodality. One alternative is changes in *immigration*. However, this would require that intermediate-sized immigrants have recently become proportionally more common at Academy Bay but not at El Garrapatero. We have no reason to suspect that this is the case – and it would not explain the reduced environment-morphology-performance correlations documented here. A second possibility is increased h*ybridization* between beak morphs at Academy Bay but not El Garrapatero. This is certainly possible, but mate choice in Darwin's finches is closely linked to beak size and the resulting songs (Ratciliffe & Grant 1983; Grant 1999; Podos 2001; Podos *et al.* 2004), and so changes in beak size would likely precede changes in assortative mating. And, again, this would not explain the reduced environment-morphology-performance correlations. A third possibility is that some environmental factor unrelated to local human activities has changed at Academy Bay but not El Garrapatero. However, most such environmental variables would vary more randomly through time and likely do so similarly at both sites (e.g., rainfall). The conclusions that humans are the primary driver, and that this is in some way related to feeding ecology, seems fairly robust.

Other alternatives relate to the specific human-mediated driver of bimodality collapse. We have generally argued that humans have altered the underlying resource distribution available to finches. But it is also possible that human activities have caused a general *relaxation of competition*, which has smoothed the adaptive landscape without altering the shape of the underlying resource distribution. For instance, humans may have increase the mortality of finches for other reasons (e.g., parasites or cats) or may have increase food resources to the point that some other factor becomes limiting. However, our catch-per-unit effort data suggest that finch abundance is more than three-fold higher at Academy Bay than at El Garrapatero, perhaps maintaining competition and thereby making the underlying resource distribution important. We cannot conclusively distinguish between these two alternatives with our correlative data but both alternatives still invoke human-induced effective changes in the adaptive landscape for finches.

The postulated human impacts could extend to other situations in Galapágos. On Santa Cruz, for instance, introduced agricultural plants are widely cultivated around Bellavista (Fig. 5.1), and some of these (and other) plants have become invasive (Mauchamp 1997; Tye 2001). Moreover, human settlements on the islands of San Cristobal and Isabela are also increasing dramatically in population density (Watkins & Cruz 2007). Perhaps *G. fortis* in these other areas will be similarly impacted – although the extent to which they were historically bimodal is not known. It is also possible that such effects could influence separation *among* the recognized finch species, given their lack of intrinsic genetic incompatibilities and the corresponding importance of reproductive barriers based on ecological selection against hybrids (Grant & Grant 1989; Grant & Grant 1993). If that selection is removed owing to environmental change, the species could converge. As a telling natural example, severe El Nino conditions eliminated selection against hybrids between *G. fortis* and *G. scandens* on Daphne Major Island, with the result being ongoing adaptive merging of the two species (Grant & Grant 2002; Grant *et al.* 2004). Perhaps the increased availability of human foods and introduced plants could have similar effects.

5.5.4 Summary

The ongoing and increasing human influences in Galapágos appear to negatively influence at least some part of the adaptive radiation of Darwin's finches. We have specifically argued that the introduction of new food types at Academy Bay may be eroding the disruptive diet-based selection that previously maintained distinctive *G. fortis* beak-size morphs in sympatry. In contrast, the relative scarcity of human influences at a nearby site (El Garrapatero) has allowed the two beak morphs to remain bimodal, preserving their ecological, performance, and genetic differences and correlations.

Our findings suggest at least three broader implications for the conservation of biological diversity, considered here to be encapsulated by adaptively divergent and at least partially reproductively isolated groups. First, we have illustrated yet another way in which evolutionary processes can be influenced by human disturbances (Smith & Bernatchez 2008). Second, our results highlight the importance of conserving the processes that generate and maintain biodiversity, rather than just the product of those processes (Moritz 2002). Third, we have raised the specter of new conservation concerns in Galapágos, a hotspot for both biodiversity (Myers *et al.* 2000) and evolution (*sensu* Davis *et al.* 2008; Grivet *et al.* 2008) that has recently been added to the list of World Heritage Sites in Danger (UNESCO 2007). A region so important in our original understanding of evolutionary processes could now prove important for understanding how humans alter those processes and thereby modify future evolutionary trajectories.

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Appendix 5.1 Common specific feeding categories (food items) consumed by *G. fortis* on Santa Cruz Island. The data represent the frequency of feeding observations of individual banded birds. Percentages are given in parenthesis.

		Academy Bay					Total	El Garrapatero					Total	Grand Total
Plant species	Food item	2003	2004	2005	2006	2007		2003	2004	2005	2006	2007		
Abutilon depauperatum	flower		2				2 (0.62)							2 (0.18)
Acacia nilotica	flower								3				3 (0.38)	3 (0.27)
arthropod	insect		1	13	4	1	19 (5.85)	19	15	47	98	180	359 (45.44)	378 (33.9)
Boerhaavia caribaea	fruit		1				1 (0.31)	4	2		1		7 (0.89)	8 (0.72)
	seed										1		1 (0.13)	1 (0.09)
Bursera graveolens	bud							5	1		3		9 (1.14)	9 (0.81)
	flower							2					2 (0.25)	2 (0.18)
	fruit		2				2 (0.62)	5	2	2			9 (1.14)	11 (0.99)
	green seed		2				2 (0.62)		2	4			6 (0.76)	8 (0.72)
	seed		5	1			6 (1.85)	4	1	1			6 (0.76)	12 (1.08)
Castela galapageia	seed							4					4 (0.51)	4 (0.36)
	fruit								5	1			6 (0.76)	6 (0.54)
	seed								1				1 (0.13)	1 (0.09)
Commicarpus tuberosus	flower	2	10				12 (3.69)		4	6			10 (1.27)	22 (1.97)
	fruit										1		1 (0.13)	1 (0.09)

A**ppendix 5.1** (continuation). Common specific feeding categories (food items) consumed by *G. fortis* on Santa Cruz Island. The data represent the frequency of feeding observations of individual banded birds. Percentages are given in parenthesis.

		Academy Bay					Total	El Garrapatero					Total	Grand Total
Plant species	Food item	2003	2004	2005	2006	2007		2003	2004	2005	2006	2007		
Cordia leucophlyctis	seed			1			1 (0.31)			3			3 (0.38)	4 (0.36)
	fruit											1	1 (0.13)	1 (0.09)
Cordia lutea	flower										1		1 (0.13)	1 (0.09)
	seed				1		1 (0.31)							1 (0.09)
Croton scouleri	green seed						0 (0)					1	1 (0.13)	1 (0.09)
Cryptocarpus pyriformis	fruit	1					1 (0.31)							1 (0.09)
	leave	2	2				4 (1.23)							4 (0.36)
grass	seed							22	2	1			25 (3.16)	25 (2.24)
Evolvulus convolvuloides	seed									1			1 (0.13)	1 (0.09)
ground	na	14	58	2	1		75 (23.08)	16	50	54	58	2	180 (22.78)	255 (22.87)
Heliotropium angiospermum	seed		1				1 (0.31)							1 (0.09)
human food	na		6	10	3		19 (5.85)							19 (1.7)
Lantana peduncularis	seed		8				8 (2.46)							8 (0.72)
Lycium minimum	leave										2		2 (0.25)	2 (0.18)
Opuntia echios	na	2	1				3 (0.92)	2	1				3 (0.38)	6 (0.54)
	flower								3				3 (0.38)	3 (0.27)
	seed		2				2 (0.62)		1	1			2 (0.25)	4 (0.36)

Appendix 5.1 (continuation). Common specific feeding categories (food items) consumed by *G. fortis* on Santa Cruz Island. The data represent the frequency of feeding observations of individual banded birds. Percentages are given in parenthesis.

		Academy Bay					Total	El Garrapatero					Total	Grand Total
Plant species	Food item	2003	2004	2005	2006	2007		2003	2004	2005	2006	2007		
Portulaca oleracea	bud/seed	4	24	6			34 (10.46)							34 (3.05)
Rhynchosia minima	seed							3	1				4 (0.51)	4 (0.36)
Scutia spicata	na	15	23	2	1		41 (12.62)		8	13	13		34 (4.3)	75 (6.73)
	fruit			1	1		2 (0.62)			4	14		18 (2.28)	20 (1.79)
	green fruit			2			2 (0.62)			16	12		28 (3.54)	30 (2.69)
	green seed			2			2 (0.62)			11			11 (1.39)	13 (1.17)
	seed		31	6	1		38 (11.69)		13	16	14		43 (5.44)	81 (7.26)
Sida ciliaris	flower		2				2 (0.62)							2 (0.18)
Tournefortia psilostachya	seed	8	7				15 (4.62)		1				1 (0.13)	16 (1.43)
	bud		10				10 (3.08)							10 (0.9)
Tournefortia pubescens	flower	1					1 (0.31)			1			1 (0.13)	2 (0.18)
	seed		1				1 (0.31)							1 (0.09)
	fruit					1	1 (0.31)							1 (0.09)
Trianthema portulacastrum	bud/seed	2	13				15 (4.62)							15 (1.35)
Vallesia glabra	fruit		1				1 (0.31)							1 (0.09)
	seed		1				1 (0.31)				3		3 (0.38)	4 (0.36)
Waltheria ovata	seed								1				1 (0.13)	1 (0.09)
Grand Total		51	214	46	12	2	325	86	117	182	221	184	790	1115

Linking statement between Chapter 5 and Chapter 6

In this final chapter, I summarize the significance of my findings for our understanding of the process of adaptive diversification.

CHAPTER 6

General discussion and implications

6.1 Introduction

The study of adaptive radiation is essential to understanding observed patterns of biological diversity (Lack 1947; Simpson 1953; Grant 1999; Schluter 2000). My thesis has investigated the ecological basis of one of the classical examples of the adaptive radiation, Darwin's finches of the Galápagos. I used a combination of ecological observations, genetic tools, and statistical analyses to study patterns of sympatric coexistence, niche utilization, adaptive divergence, and gene flow in a part of this radiation. I also quantified the possible impact of human disturbances on this adaptive radiation. In this final chapter, I summarize my main findings, their general implications, and the contribution of my research to our understanding of the processes underlying adaptive diversification.

6.2 Main findings

My first data chapter suggests that Darwin's ground finches correspond to a model of the adaptive radiation of "imperfect generalists" (*sensu* Barrett *et al*. 2005). In this model, coexistence is promoted even in the case of overlapping diets because species retain some food types for which they are best adapted. The unique contribution of my work to this idea is the discussion of how spatial and temporal variation in niche overlap, which is dramatic in Darwin's finches, can aid the coexistence of imperfect generalists. This generalizes previous suggestions that coexistence of sympatric species can be facilitated by certain (perhaps rare) periods during which resources are low and species' diets diverge to those foods for which they are best adapted (e.g., Smith *et al.* 1978).

My second data chapter supported the idea that an apparent generalist species might instead be composed of a variety of individual specialists. Specifically, I found high levels of individual specialization within the bimodal population of *G. fortis* at El Garrapatero. Individual specialization in this population seems to be related to morphological variation (beak and head dimensions) but not to genetic variation. These findings are consistent with the idea that individual specialization might be important in promoting the initial stages of adaptive divergence (Bolnick *et al.* 2003).

My third data chapter confirmed that reproductive isolation in Darwin's finches is driven by differences in beak size. This has previously been suggested to be the case based on differences between species (Lack 1947; Grant 1999) and my contribution was to show that the same applies to two partially-divergent morphs within a single species (*G. fortis*) on a single island. This demonstration increases confidence in the idea that beak size evolution is the specific cause of the reproductive isolation observed in this radiation. Overall, these results suggest the controversial (Smith 1966; Endler 1973; Felsenstein 1981) possibility of genetic divergence in the face of gene flow in sympatric populations on a single island.

My final data chapter suggests that human activities might be negatively impacting adaptive divergence within *G. fortis*. Specifically I found that the correlation between ecology (diet), morphology (beak size) and performance (bite force) is weaker at a disturbed than at an undisturbed site. These findings expanded previous work by Hendry *et al.* (2006) which suggested that human activities might explain the loss of bimodality within *G. fortis* at Academy Bay. My contribution was to identify the specific mechanisms responsible for weakening this adaptive divergence. This example contributes to a growing body of evidence suggesting that humans may be becoming a ubiquitous evolutionary force in natural populations.

Overall, my study provides new insights into the mechanisms involved in both promoting and constraining the initial stages of divergence within natural populations and permitting the coexistence of young divergent species within an adaptive radiation. In the following sections, I return to several themes that have recurred through my work and discuss how they may be integrated with previous ideas about adaptive radiation. Here I attempt to go beyond my data and try to integrate a variety of information from finches and other natural systems to present some ideas that might prove interesting to consider in future work.

6.3 The importance of spatial and temporal variation

Ecological variation is thought to be one of the main underpinnings of adaptive radiation (Schluter 2000). In Darwin's finches, spatial and temporal ecological heterogeneity can have profound implications for this process (Lack 1947; Grant 1999). My results suggest that Santa Cruz Island is more ecologically heterogeneous than previously thought, presenting not only elevational variation (Wiggins & Porter 1971; Grant 1999; Kleindorfer *et al.* 2006), but also considerable variation within a given elevation, as evidenced by large differences in seed types across locations (Chapter 2). Santa Cruz Island also shows high temporal variation, resulting from extreme oscillations between wet and dry periods determined by the El Niño effect (Smith *et al.* 1978; Gibbs & Grant 1987; Grant & Grant 2002). This variation has important implications for the distribution of available resources, as evidenced by changes in both the abundance and diversity of seed types available for finches (Chapter 2).

The combination of spatial variation with the ability of ground finches to adapt to different food resources (Chapter 2) could generate a complex adaptive landscape with multiple adaptive peaks exploited by different coexisting species (Grant *et al.* 1976; Schluter & Grant 1984; Grant 1999). This ecological-spatial heterogeneity could also contribute to diversification by: (1) allowing/promoting the coexistence of incipient species after secondary contact (Lack 1947; Grant 1999), (2) promoting within-island divergence (Chapter 4), and (3) by sustaining a large number of stable resident finch populations/species (Chapter 2).

Temporal ecological heterogeneity might influence movements of species between adaptive peaks, which can either promote further divergence or convergence of the interacting species (Boag & Grant 1981; Grant & Grant 2002; Grant & Grant 2008). For instance, when seasonal changes in environmental conditions produce a scarcity/surplus of available resources this can drive species to diverge/converge in the use of such resources (Smith *et al.* 1978; Grant & Grant 2002).

Overall, the interplay between spatial and ecological heterogeneity seems to be essential in driving and maintaining the isolation barriers that allow coexistence of species in the initial stages of adaptive radiation.

6.4 Isolation barriers within an adaptive radiation

Sympatric coexistence of closely related species depends not only on ecological differences (as discussed above) but also on reproductive barriers. These barriers might reflect intrinsic genetic incompatibilities (Smith 1966) or they might depend on the environmental context (Rundle *et al.* 2000). Either of these might be driven by divergent selection (i.e., ecological speciation) but the former are unlikely to evolve on time frames of less than a few million years (Coyne & Orr 2004; Price 2008). Thus, reproductive barriers in Darwin's finches are likely environment-dependent and, indeed, intrinsic genetic incompatibilities are absent (Grant & Grant 1992; Grant *et al.* 1996; Zink 2002; Grant *et al.* 2004).

The presence of environmentally-dependent barriers implies that the boundaries between morphs (and species) may be dynamic. For instance, in some cases, reproductive barriers might break down and the speciation process will cease or reverse (Taylor *et al.* 2006; Seehausen *et al.* 2008). In Darwin's ground finches, different species interbreed at a modest frequency and the hybrids have high fitness under benign environmental conditions (Grant & Grant 1996; Grant *et al.* 2004; Grant *et al.* 2005). In at least one instance, this has led to the ongoing fusion of two species, *G. fortis* and *G. scandens*, on Daphne Major (Grant *et al.* 2004b). This is not surprising because these species also show high levels of overlap in their use of feeding resources. I found a similar effect between the beak size morphs of *G. fortis* at Academy Bay (Chapter 5). Despite considerable overlap and interbreeding, the species are still regarded as reasonably independent evolutionary units (Grant & Grant 2008). This raises the question: what maintains the long term separation of seemingly different species coexisting on a single island?

I here briefly considered this possibility by creating an index of pair-wise species differences based on the ecological, morphological, functional, and genetic variables measured in this study (Fig. 6.1). This analysis revealed what might be the natural path leading to reproductive isolation in Darwin's finches. Specifically, the observed pairwise differences suggest a chain of multiple synergetic factors linking divergence in ecology (diet) to morphology (beaks) to function (bite force), to signals (songs) and mating (preferences), to genetics, and finally to reproductive isolation (Fig. 6.2).

At the initial stages of divergence, the interaction among these factors might lead to different outcomes such as convergence, further divergence or maintenance of a highly variable population at equilibrium. This can result in periods of fission (Huber *et al.* 2007; De León *et al.* 2010) and fusion (Grant *et al.* 2004; Grant & Grant 2008) through time among interacting populations/species. If divergence proceeds further, the contribution of each one of these factors to reproductive isolation can vary (Coyne & Orr 2004). This suggests that in the long run, reproductive isolation between species might be consistent with a model of multiple isolation barriers in which each barrier contributes to some proportion of the total reproductive isolation (Fig. 6.1, 6.2). The coupling of multiple isolation barriers seems to be facilitated when selection acts on traits that are more vulnerable to speciation such as so-called "magic traits" (Gavrilets 2004).

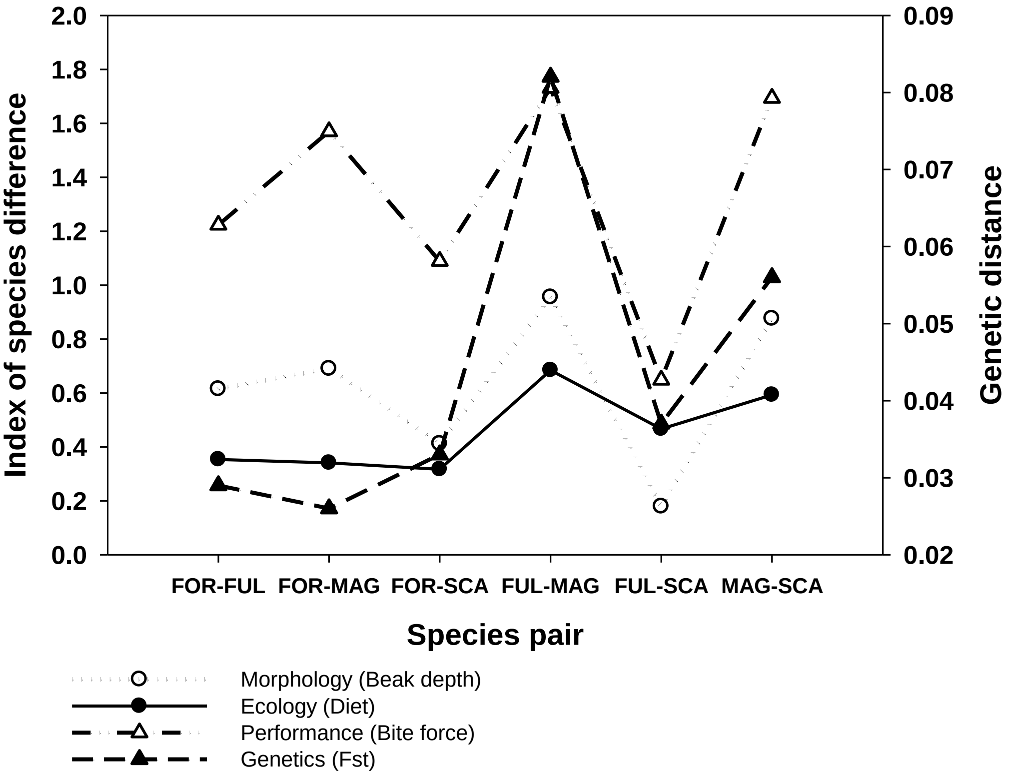

Figure 6.1 Contribution of multiple isolation barriers promoting reproductive isolation and adaptation in Darwin's ground finches. The data represent an index of species differences based on pairwise species distance in morphology (log beak depth), ecology (Pianka's niche overlap), performance (bite force) and genetics (Fst).

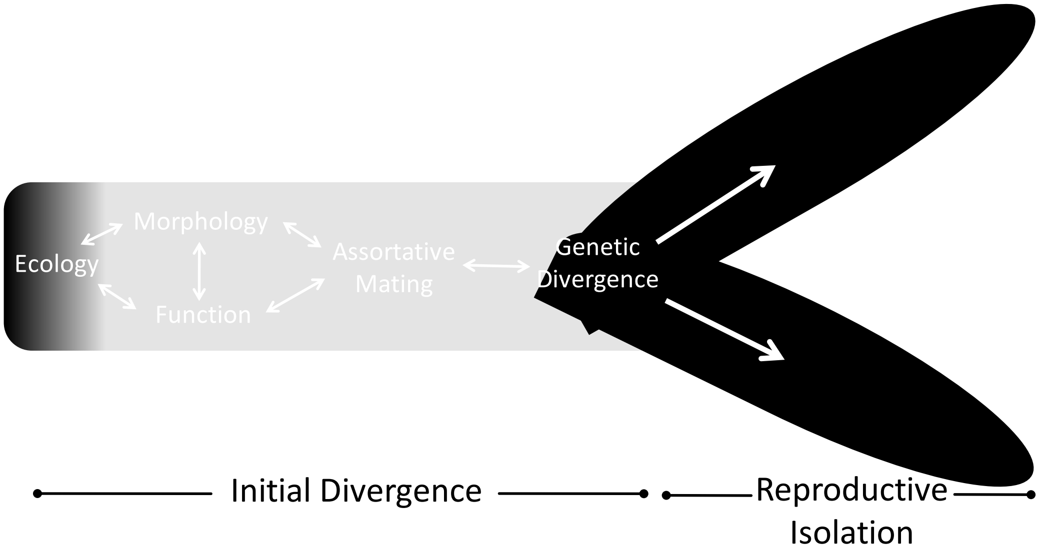

Figure 6.2 Synergetic factors leading to reproductive isolation within an adaptive radiation. In this model, initial divergence (left side) starts with minor ecological differences that can link divergence in morphology, to function, to assortative mating, to genetics, and finally to reproductive isolation (right side). This functional link from ecology to reproductive isolation can be facilitated when selection is acting on magic traits.

6.5 Implications for “magic trait” speciation and de-speciation in Darwin’s finches

”Magic traits” (*sensu* Gavrilets 2004) are traits that are under divergent natural selection and also influence mate choice, such that adaptive divergence necessarily causes reproductive isolation. Magic traits are important to consider because they should dramatically ease evolutionary diversification in sexual organisms. Potential examples of magic trait speciation include colour in Hamlet fishes (Puebla *et al.* 2007), poison-dart frogs (Maan & Cummings 2008; Noonan & Comeault 2009), and butterflies (Jiggins *et al.* 2001), as well as body size (Boughman 2001; McKinnon *et al.* 2004; Vines & Schluter 2006; Maccoll 2009) and parasite immunity (Eizaguirre *et al.* 2009a; Eizaguirre *et al.* 2009b) in threespine stickleback. The beak of Darwin’s finches has been suggested as another magic trait because it influences both disruptive-divergent selection (based on diet and assortative mating both within (Huber *et al.* 2007; Hendry *et al.* 2009) and among species (Ratcliffe & Grant 1983; Grant 1999). Magic traits thus seem to have facilitated the initial stages of divergence in the medium ground finch (*G. fortis*). Specifically, the direct functional link between the ability of a bird to crack seeds (Herrel *et al.* 2005a, b) and its vocal repertoire (Podos 2001; Huber & Podos 2006), appear to have strengthened the association of some of the factor leading to reproductive isolation.

Magic traits can also promote “de-speciation”, as mentioned in the previous section and as described in Chapter 4 for Academy Bay *G. fortis*. The reason could be that a relaxation of divergent-disruptive selection causes a decrease in adaptive divergence which should then necessarily cause a reduction in assortative mating and an increase in gene flow between divergent populations. Assessments of the vulnerability of young species to fusion under environmental changes should therefore pay particular attention to whether the traits causing reproductive isolation fall into the “magic” category. Magic traits might thus be simultaneously conducive to speciation but also conducive to its loss, if natural conditions are altered.

6.6 Summary

Darwin’s finches are a young adaptive radiation and some parts of this radiation, such as the ground finches, are very recent. Work on differences among (Grant *et al.* 1976; Schluter & Grant 1984; Grant 1999) and within (present study) these species indicates that divergence can start with minor but important ecological differences. The coupling of these ecological differences with morphological, functional and genetic variation appears to be crucial in advancing these initial states of reproductive isolation. This coupling may be facilitated by the presence of a magic trait which is highly susceptible to speciation. This might be one of the mechanisms facilitating the journey of species through the adaptive landscape.

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