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

Luis Fernando de León, Eldredge Bermingham, Jeffrey Podos and Andrew P. Hendry

Supplementary data	"Data Supplement" http://rstb.royalsocietypublishing.org/content/suppl/2010/02/22/365.1543.1041.DC1.html
References	**This article cites 85 articles, 20 of which can be accessed free** http://rstb.royalsocietypublishing.org/content/365/1543/1041.full.html#ref-list-1
Rapid response	Respond to this article http://rstb.royalsocietypublishing.org/letters/submit/royptb;365/1543/1041
Subject collections	Articles on similar topics can be found in the following collections evolution (1458 articles)
Email alerting service	Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

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

Luis Fernando de León[1,2,*]**, Eldredge Bermingham**[2]**, Jeffrey Podos**[3]
and Andrew P. Hendry[1]

[1]*Redpath Museum and Department of Biology, McGill University, 859 Sherbrooke Street West, Montréal, QC, Canada H3A 2K6*
[2]*Smithsonian Tropical Research Institute, Apartado Postal 2072, Balboa, Panama*
[3]*Department of Biology, University of Massachusetts, Amherst, MA 01003, USA*

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 interspecific hybridization might contribute to the formation of beak-size morphs within *G. fortis*.

Keywords: sympatric speciation; ecological speciation; disruptive selection; reproductive isolation; gene flow; Darwin's finches

1. INTRODUCTION

Strict geographical isolation surely aids speciation in many cases (Mayr 1963; Felsenstein 1981; Coyne & Orr 2004), but there are several contexts where divergence can proceed despite (or even be enhanced by) a lack of geographical 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 2000b) 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 the longstanding (Smith 1966; Endler 1973; Felsenstein 1981) 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, 2001; Schluter 2000a; 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*a*; Grant & Grant 2006). In this three-phase model, the incipient species continue to diverge following secondary contact because allopatric divergence has led to assortative mating and selection against hybrids that is then manifest in sympatry (Grant & Grant 1993, 1996*a,b*, 1997*a*; Grant 1999).

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 towards this population. In the other case, Ford *et al.* (1973) described a population of *G. 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, 1997*a*, 1998, 2008; Grant 1999; Sato *et al.* 1999; Zink 2002; Grant *et al.* 2005), 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 1996*b*). 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*a*; 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.

(a) *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 (figure 1; Hendry *et al.* 2006). The two morphs (i) have beaks adapted for different food types (Herrel *et al.* 2005; Foster *et al.* 2008), (ii) produce distinctive vocal mating signals (Podos *et al.*

Figure 1. Typical small and large beak-size morphs in *G. fortis*. These two mature males were caught at the same time in the same mist net, and were photographed together. Photo by A. Hendry.

2004; Huber & Podos 2006; Herrel *et al.* 2009) and respond differently to those signals (J. Podos 2007, unpublished data), (iii) show higher survival than birds with intermediate-sized beaks (Hendry *et al.* 2009), (iv) pair assortatively by beak size (Huber *et al.* 2007), and (v) 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 intraspecific divergence to speciation. This last pattern would also be consistent with a possible role for hybridization.
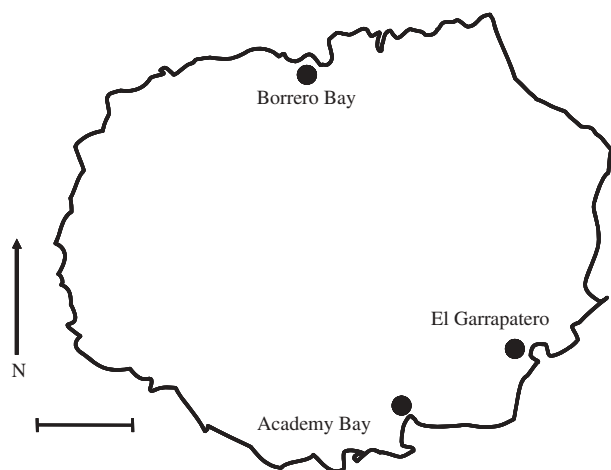
Figure 2. Collection sites on Santa Cruz Island, Galápagos. Scale bar, 10 km.

We test for the above signatures of ecological speciation by using microsatellite loci to analyse population structure on Santa Cruz Island, specifically in relation to (i) the small and large *G. fortis* beak-size morphs (figure 1), (ii) the different sampling sites (figure 2), and (iii) the different granivorous ground finch species (*G. fuliginosa*, *G. fortis* and *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; figure 3). 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; figure 3). At a third site (Borrero Bay), the large morph is rare (Grant *et al.* 1976; Hendry *et al.* 2006; figure 3).

2. MATERIAL AND METHODS
(a) *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 ethylenediamine tetraacetic acid (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 *G. fuliginosa* (small ground finch) from El Garrapatero, six *G. 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 analyse 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 was assigned either to the small or to the 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 (i) consider the intermediate-size class as an additional group (electronic supplementary material, appendix S1) or (ii) divide the birds into small and large beak-size categories based strictly on the above dividing point—i.e. without excluding any *G. fortis* (electronic supplementary material, appendix S2).

(b) *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-labelled primers (FAM, HEX, PET and TET) were used to label the 5′ tail of the different loci. A multicapillary ABI 3100-base station was used to score the microsatellite fragments.

Raw genotypes were imported into GENALEX v. 6 (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.

(c) *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
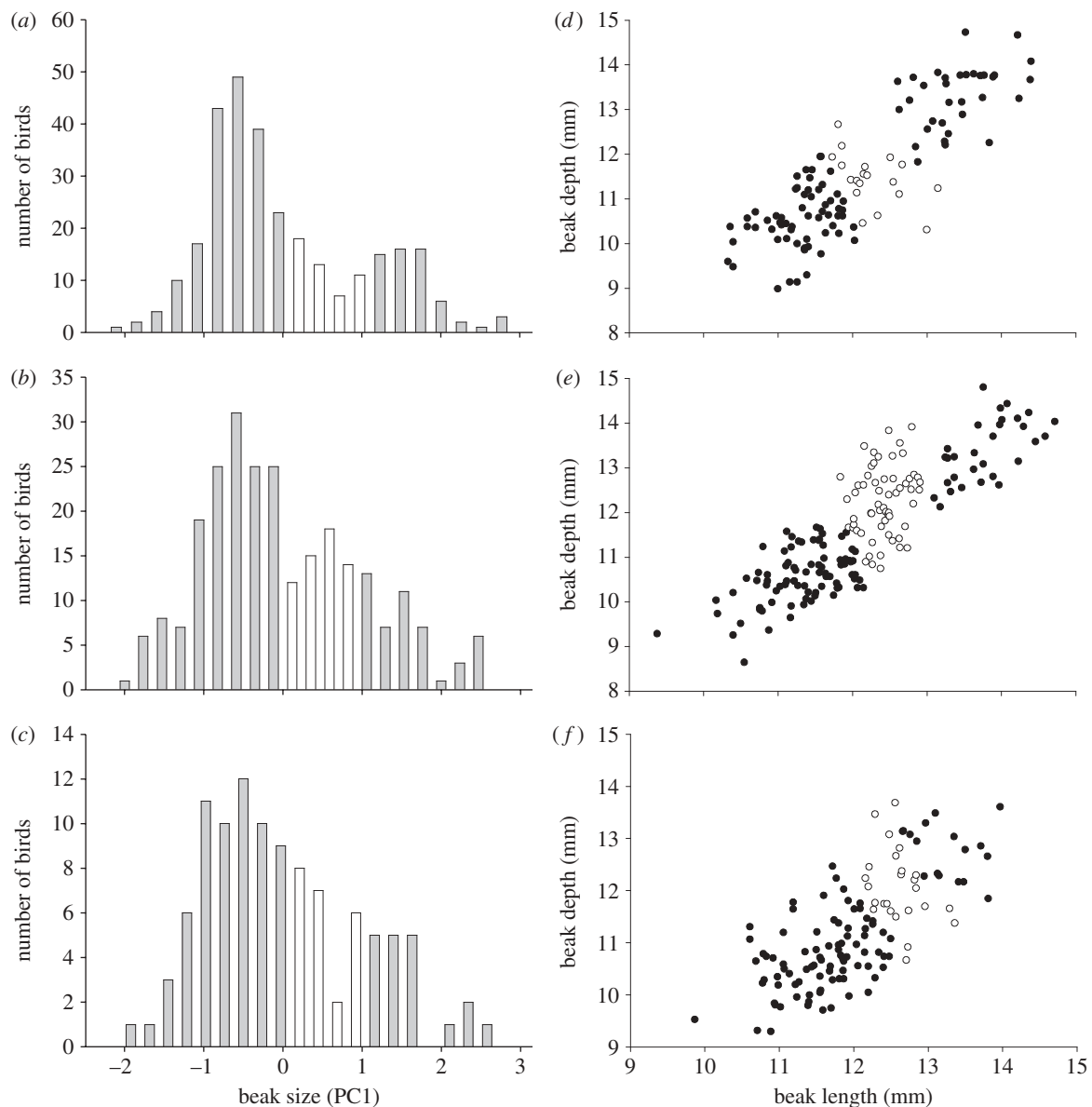
Figure 3. Distribution of beak sizes in *G. fortis* at three different sites on Santa Cruz Island in 2004. At El Garrapatero, (*d*) small and large morphs are both common and (*a,d*) intermediates are relatively few. At Academy Bay, (*e*) small and large morphs are both common and (*b,e*) intermediates are less rare. (*c,f*) At Borrero Bay, the large morph is rare. These data are from Hendry *et al.* (2006). (*a–c*) The white sections on the histograms and (*d–f*) the open circles on the graphs represent the 'intermediate beak-size class' omitted from the genetic analyses (§2).

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 (i) beak-size classes nested within sites and (ii) sites nested within beak-size classes.

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

Table 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**

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.

(d) *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 10 000 simulations. This method (i) 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 (ii) 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 GENE-CLASS 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 that it performs poorly when estimating gene flow (Abdo *et al.* 2004; Slatkin 2005; Chapuis *et al.* 2009).

3. RESULTS

(a) *Variation within G. fortis*

All of the loci showed moderate to high levels of variation (table 1). One locus (Gf10) turns out to be Z-linked (Petren *et al.* 2005) and was therefore omitted from subsequent analyses. Of the 10 remaining loci, six (Gf03, Gf04, Gf09, Gf11, Gf12 and Gf16)

showed significant heterozygote deficits when all *G. fortis* were pooled together (four after sequential Bonferroni correction; table 1). Five pairings of loci in this pooled sample showed significant linkage disequilibrium (two pairs after sequential Bonferroni correction, results not shown). These deviations from Hardy–Weinberg and linkage equilibria probably reflect population structure within *G. fortis*—because these loci apparently lack null alleles (Petren 1998; Keller *et al.* 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 versus large) and collection sites (Academy Bay, El Garrapatero and Borrero Bay) based on standard population genetic tests. We then examine the groupings revealed by analysing multilocus genotypes, whether defined *a priori* or not.

Some subtle genetic differences were evident among the sites (tables 2–4), 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 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 pairwise 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 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 3).

The above findings were mirrored in analyses of multilocus genotypes. Bayesian analyses in STRUCTURE recovered two clusters (figure 4) that roughly corresponded to the small and large beak-size classes. For example, 63 per cent of birds in the large beak-size class were assigned with the highest probability to one cluster, whereas 60 per cent 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 2006). Similar to STRUCTURE, BAPS largely recovered the two beak-size classes when using the entire dataset, but not when each site was analysed separately (results not shown). Finally, factorial correspondence analysis revealed that the differences between morphs were

Table 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 10 polymorphic microsatellite loci (i.e. excluding Gf10). Levels of significance were extracted after 10 000 permutations, as implemented by ARLEQUIN v. 3.1.

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 3. Genetic differentiation in *G. fortis* between beak-size classes (S, small; L, large) from different collection sites (AB, Academy Bay; BB, Borrero Bay; and 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 (*) indicating 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	

greatest at El Garrapatero, lower at Academy Bay and absent at Borrero Bay (figure 5).

(b) *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 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 5). These results were supported in analyses of multilocus genotypes. When STRUCTURE (figure 6) and BAPS (results not shown) were asked to assign birds to only two clusters, these programs found (i) a strong separation between *G. fuliginosa* and *G. magnirostris* and (ii) 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.

(c) *Gene flow*

Estimates of contemporary gene flow from GENECLASS were consistent with key results from the above analyses of population structure: (i) gene flow was lower between beak-size morphs than within them, both within and between sites and (ii) gene flow was lower between the beak-size classes at El Garrapatero than at other sites (tables 6 and 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 (electronic supplementary material, appendices S3 and S4).

4. 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 m.a.s.l.) and ecological

Table 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 (*) indicating 95% confidence intervals that do not overlap with zero. R_{ST} values are below the lower diagonal.

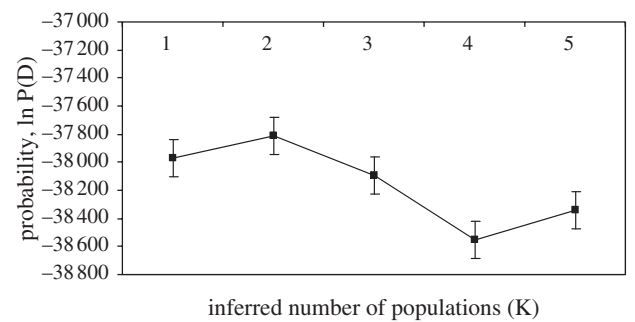|	Academy Bay	Borrero Bay	El Garrapatero
Academy Bay		**0.010**	**<0.001**
Borrero Bay	0.002*		**<0.001**
El Garrapatero	0.003*	0.002*	
Academy Bay			
Borrero Bay	0.001		
El Garrapatero	0.003	0.003	

Figure 4. Two clusters are most likely when combining all *G. fortis* in STRUCTURE v. 2.1. This conclusion holds when using the ad hoc criterion of Evanno *et al.* (2005). The consensus of five simulations following the parameters described in §2 is shown. Error bars show the variation in the probability of assignment.

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.

(a) 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 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 2009). 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 3). Indeed, birds

with similar beak sizes were more genetically similar across sites than were birds of different beak sizes within sites (table 3). These patterns probably 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 3 and figure 5), where the population is currently bimodal for beak size (figure 3; Hendry *et al.* 2006). Genetic differentiation is weaker at Academy Bay (table 3 and figure 5), 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 3 and figure 5), 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
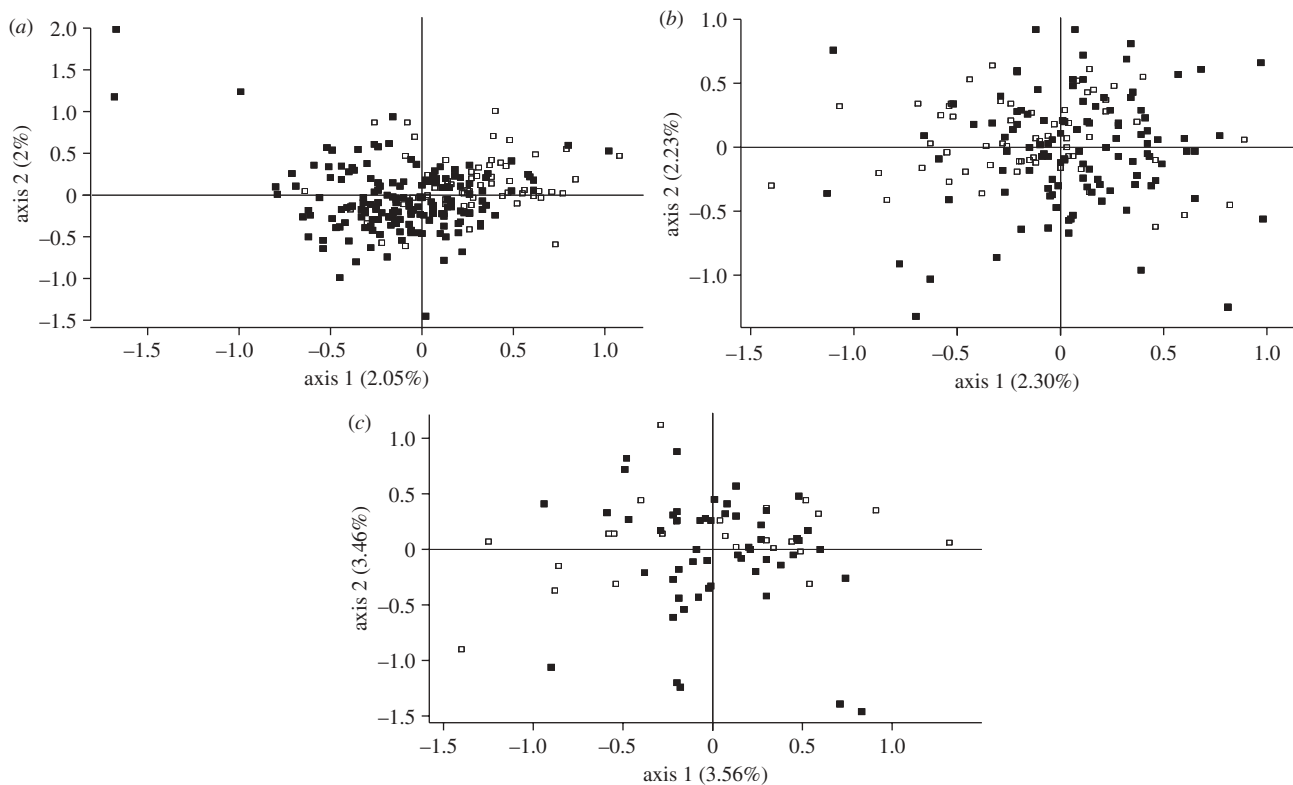
Figure 5. Population structure in *G. fortis* as visualized through factorial correspondence analysis of multilocus genotypes (GENETIX v. 3.1). The black and white squares are small and large beak-size classes, respectively. (*a*) At El Garrapatero, the two morphs occupy different, although partly overlapping distributions. (*b*) At Academy Bay and (*c*) Borrero Bay, the two morphs occupy similar distributions, although some slight separation is evident, particularly for Academy Bay.

Table 5. Genetic differentiation within and between the three granivorous ground finch species. *Geospiza fortis* is represented by the small and large beak-size classes and by all size classes (including intermediates) pooled. *p*-values for genetic 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 (*) indicating 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. magnirostris*	*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. magnirostris	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. magnirostris	0.014	0.001	0.054		
G. fortis (all)	—	—	0.011	0.001	

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.

(**b**) **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
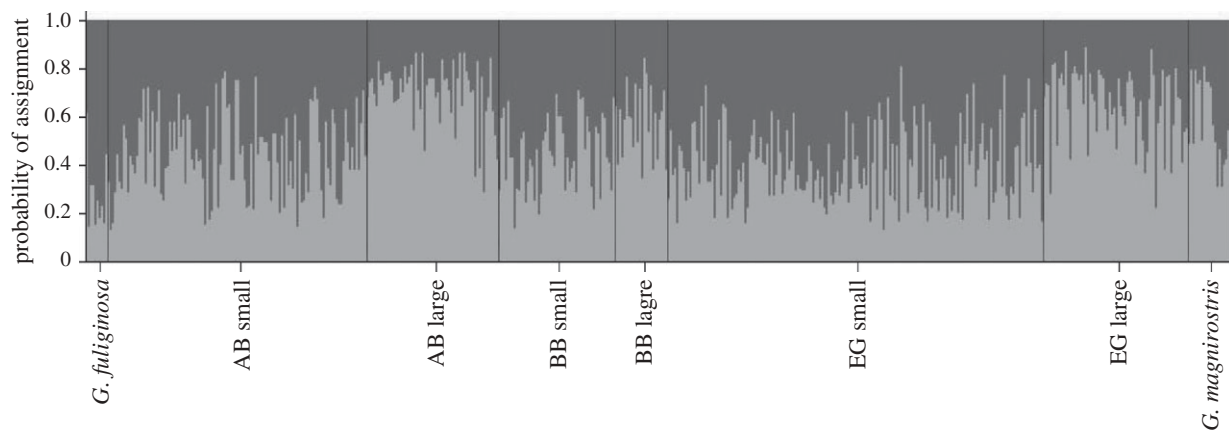
Figure 6. Bayesian clustering analysis of population structure performed in STRUCTURE v. 2.1. Black bars represent the separation between different groups: *G. fuliginosa*, *G. fortis* (AB small, AB large, BB small, BB large, EG small and EG large) and *G. magnirostris*, AB, Academy Bay; BB, Borerro Bay; EG, El Garrapatero.

Table 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	

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, 2001; Schluter 2000a; 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). 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 favour 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, 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 interspecific hybridization leading to the origin of one of the morphs. The most probable 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 (i) the small *G. fortis* morph is widespread, whereas the large *G. fortis* morph is not (Grant *et al.* 1976; Hendry *et al.* 2006), (ii) the large *G. fortis* morph is morphologically and genetically intermediate between the small *G. fortis* morph and *G. magnirostris*, and (iii) 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.

Table 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	

5. 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 led 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 where 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.

REFERENCES

Abdo, Z., Crandall, K. A. & Joyce, P. 2004 Evaluating the performance of likelihood methods for detecting population structure and migration. *Mol. Ecol.* **13**, 837–851. (doi:10.1111/j.1365-294X.2004.02132.x)

Abzhanov, A., Protas, M., Grant, B. R., Grant, P. R. & Tabin, C. J. 2004 Bmp4 and morphological variation of beaks in Darwin's finches. *Science* **305**, 1462–1465. (doi:10.1126/science.1098095)

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. & Bonhomme, F. 2004 GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. In *Laboratoire génome, populations, interactions, CNRS UMR 5171*. Montpellier, France: Université de Montpellier II.

Berner, D., Grandchamp, A., Hendry, A. P. & Feder, J. 2009 Variable progress toward ecological speciation in parapatry: stickleback across eight lake–stream transitions. *Evolution* **63**, 1740–1753. (doi:10.1111/j.1558-5646.2009.00665.x)

Berry, O., Mandy, D. T. & Stephen, D. S. 2004 Can assignment tests measure dispersal? *Mol. Ecol.* **13**, 551–561. (doi:10.1046/j.1365-294X.2004.2081.x)

Blondel, J., Dias, P. C., Perret, P., Maistre, M. & Lambrechts, M. M. 1999 Selection-based biodiversity at a small spatial scale in a low-dispersing insular bird. *Science* **285**, 1399–1402. (doi:10.1126/science.285.5432.1399)

Bolnick, D. I. & Fitzpatrick, B. 2007 Sympatric speciation: theory and empirical data. *Annu. Rev. Ecol. Syst.* **38**, 459–487. (doi:10.1146/annurev.ecolsys.38.091206.095804)

Bowman, R. I. 1961 Morphological differentiation and adaptation in the Galápagos finches. *Univ. Calif. Publ. Zool.* **48**, 1–326.

Brown Jr, W. L. & Wilson, E. O. 1956 Character displacement. *Syst. Zool.* **5**, 49–64. (doi:10.2307/2411924)

Chapuis, M. P., Lecoq, M., Michalakis, Y., Loiseau, A., Sword, G. A., Piry, S. & Estoup, A. 2009 Do outbreaks affect genetic population structure? A worldwide survey in *Locusta migratoria*, a pest plagued by microsatellite null alleles. *Mol. Ecol.* **17**, 3640–3653. (doi:10.1111/j.1365-294X.2008.03869.x)

Christensen, R. & Kleindorfer, S. 2009 Jack-of-all-trades or master of one? Variation in foraging specialisation across years in Darwin's Tree finches (*Camarhynchus spp.*). *J. Ornithol.* **150**, 383–391. (doi:10.1007/s10336-008-0358-y)

Corander, J., Waldmann, P., Marttinen, P. & Sillanpää, M. J. 2004 BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics* **20**, 2363–2369. (doi:10.1093/bioinformatics/bth250)

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

Coyne, J. A. & Orr, H. A. 2004 *Speciation*. Sunderland, MA: Sinauer Associates, Inc.

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

Dieckmann, U. & Doebeli, M. 1999 On the origin of species by sympatric speciation. *Nature* **400**, 354–357. (doi:10.1038/22521)

Doebeli, M. & Dieckmann, U. 2003 Speciation along environmental gradients. *Nature* **421**, 259–264. (doi:10.1038/nature01274)

Doebeli, M., Blok, H. J., Leimar, O. & Dieckmann, U. 2007 Multimodal pattern formation in phenotype distributions of sexual populations. *Proc. R. Soc. B* **274**, 347–357. (doi:10.1098/rspb.2006.3725)

Eldridge, M. D. B., Kinnear, J. E. & Onus, M. L. 2001 Source population of dispersing rock-wallabies (*Petrogale lateralis*) identified by assignment tests on multilocus genotypic data. *Mol. Ecol.* **1310**, 2867–2876.

Endler, J. A. 1973 Gene flow and population differentiation: studies of clines suggest that differentiation along environmental gradients may be independent of gene flow. *Science* **179**, 243–250. (doi:10.1126/science.179.4070.243)

Evanno, G., Regnaut, S. & Goudet, J. 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620. (doi:10.1111/j.1365-294X.2005.02553.x)

Excoffier, L., Laval, G. & Schneider, S. 2005 ARLEQUIN version 3.0: an integrated software package for population genetics data analysis. *Evol. Bioinfo. Online* **1**, 47–50.

Felsenstein, J. 1981 Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution* **35**, 124–138. (doi:10.2307/2407946)

Ford, H. A., Parkin, D. T. & Ewing, A. W. 1973 Divergence and evolution in Darwin's finches. *Biol. J. Linn. Soc.* **5**, 289–295. (doi:10.1111/j.1095-8312.1973.tb00707.x)

Foster, D. J., Podos, J. & Hendry, A. P. 2008 A geometric morphometric appraisal of beaks shape in Darwin's finches. *J. Evol. Biol.* **21**, 263–275.

Fry, J. D. 2003 Multilocus models of sympatric speciation: Bush versus Rice versus Felsenstein. *Evolution* **57**, 1735–1746.

Gavrilets, S. 2004 *Fitness landscapes and the origin of species.* Princeton, NJ: Princeton University Press.

Gavrilets, S. & Vose, A. 2005 Dynamic patterns of adaptive radiation. *Proc. Natl Acad. Sci. USA* **102**, 18 040–18 045. (doi:10.1073/pnas.0506330102)

Gavrilets, S., Vose, A., Barluenga, M., Salzburger, W. & Meyer, A. 2007 Case studies and mathematical models of ecological speciation. 1. Cichlids in a crater lake. *Mol. Ecol.* **16**, 2893–2909. (doi:10.1111/j.1365-294X.2007.03305.x)

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

Goudet, J. 2005 HIERFSTAT, a package for R to compute and test hierarchical *F*-statistics. *Mol. Ecol. Notes* **5**, 184–186. (doi:10.1111/j.1471-8286.2004.00828.x)

Grant, P. R. 1972 Convergent and divergent character displacement. *Biol. J. Linn. Soc.* **4**, 39–68. (doi:10.1111/j.1095-8312.1972.tb00690.x)

Grant, P. R. 1999 *Ecology and evolution of Darwin's finches.* Princeton, NJ: Princeton University Press.

Grant, P. R. 2001 Reconstructing the evolution of birds on islands: 100 years of research. *Oikos* **92**, 385–403. (doi:10.1034/j.1600-0706.2001.920301.x)

Grant, B. R. & Grant, P. R. 1979 Darwin's finches: population variation and sympatric speciation. *Proc. Natl Acad. Sci. USA* **76**, 2359–2363. (doi:10.1073/pnas.76.5.2359)

Grant, P. R. & Grant, B. R. 1989 Sympatric speciation in Darwin's finches. In *Speciation and its consequences* (eds D. Otte & J. A. Endler). Sunderland, MA: Sinauer Associates.

Grant, B. R. & Grant, P. R. 1993 Evolution of Darwin's finches caused by a rare climatic event. *Proc. R. Soc. Lond. B.* **251**, 111–117. (doi:10.1098/rspb.1993.0016)

Grant, P. R. & Grant, B. R. 1994 Phenotypic and genetic effects of hybridization in Darwin's finches. *Evolution* **48**, 297–316. (doi:10.2307/2410094)

Grant, B. R. & Grant, P. R. 1996a Cultural inheritance of song and its role in the evolution of Darwin's finches. *Evolution* **50**, 2471–2487. (doi:10.2307/2410714)

Grant, B. R. & Grant, P. R. 1996b High survival of Darwin's finch hybrids: effects of beak morphology and diets. *Ecology* **77**, 500–509. (doi:10.2307/2265625)

Grant, P. R. & Grant, B. R. 1997a Hybridization, sexual imprinting, and mate choice. *Am. Nat.* **149**, 1–28. (doi:10.1086/285976)

Grant, P. R. & Grant, B. R. 1997b Mating patterns of Darwin's finch hybrids determined by song and morphology. *Biol. J. Linn. Soc.* **60**, 317–343. (doi:10.1111/j.1095-8312.1997.tb01499.x)

Grant, B. R. & Grant, P. R. 1998 Hybridization and speciation in Darwin's finches: the role of sexual imprinting on a culturally transmitted trait. In *Endless forms: species and speciation* (eds D. J. Howard & S. H. Berlocher). Oxford, UK: Oxford University Press.

Grant, P. R. & Grant, B. R. 2002 Unpredictable evolution in a 30-year study of Darwin's finches. *Science* **296**, 707–711. (doi:10.1126/science.1070315)

Grant, P. R. & Grant, B. R. 2006 Evolution of character displacement in Darwin's finches. *Science* **313**, 224–226. (doi:10.1126/science.1128374)

Grant, P. R. & Grant, B. R. 2008 *How and why species multiply: the radiation of Darwin's finches.* Princeton, NJ: Princeton University Press.

Grant, P. R., Grant, B. R., Smith, J. N. M., Abbott, I. J. & Abbott, L. K. 1976 Darwin's finches: population variation and natural selection. *Proc. Natl Acad. Sci. USA* **73**, 257–261. (doi:10.1073/pnas.73.1.257)

Grant, P. R., Grant, B. R., Markert, J. A., Keller, L. F. & Petren, K. 2004 Convergent evolution of Darwin's finches caused by introgressive hybridization and selection. *Evolution* **58**, 1588–1599.

Grant, P. R., Grant, B. R. & Petren, K. 2005 Hybridization in the recent past. *Am. Nat.* **166**, 56–67. (doi:10.1086/430331)

Hendry, A. P., Grant, P. R., Grant, B. R., Ford, H. A., Brewer, M. J. & Podos, J. 2006 Possible human impacts on adaptive radiation: beak size bimodality in Darwin's finches. *Proc. R. Soc. B* **273**, 1887–1894. (doi:10.1098/rspb.2006.3534)

Hendry, A. P., Nosil, P. & Rieseberg, L. H. 2007 The speed of ecological speciation. *Func. Ecol.* **21**, 455–464. (doi:10.1111/j.1365-2435.2007.01240.x)

Hendry, A. P., Huber, S. K., de León, L. F., Herrel, A. & Podos, J. 2009 Disruptive selection in a bimodal population of Darwin's finches. *Proc. R. Soc. B* **276**, 753–759. (doi:10.1098/rspb.2008.1321)

Herrel, A., Podos, J., Huber, S. K. & Hendry, A. P. 2005 Bite performance and morphology in a population of Darwin's finches: implications for the evolution of beak shape. *Func. Ecol.* **19**, 43–48. (doi:10.1111/j.0269-8463.2005.00923.x)

Herrel, A., Podos, J., Vanhooydonck, B. & Hendry, A. P. 2009 Force–velocity trade-off in Darwin's finch jaw function: a biomechanical basis for ecological speciation? *Func. Ecol.* **23**, 119–125. (doi:10.1111/j.1365-2435.2008.01494.x)

Huber, S. K. & Podos, J. 2006 Beak morphology and song features covary in a population of Darwin's finches (*Geospiza fortis*). *Biol. J. Linn. Soc.* **88**, 489–498. (doi:10.1111/j.1095-8312.2006.00638.x)

Huber, S. K., de León, L. F., Hendry, A. P., Berminghan, E. & Podos, J. 2007 Reproductive isolation of sympatric morphs in a population of Darwin's finches. *Proc. R. Soc. B.* **274**, 1709–1714. (doi:10.1098/rspb.2007.0224)

Keller, L. F., Grant, P. R., Grant, B. R. & Petren, K. 2001 Heritability of morphological traits in Darwin's finches: misidentified paternity and maternal effects. *Heredity* **87**, 325–336. (doi:10.1046/j.1365-2540.2001.00900.x)

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

Lack, D. 1947 *Darwin's finches.* Cambridge, UK: Cambridge University Press.

Losos, J. B. & Schluter, D. 2000 Analysis of an evolutionary species-area relationship. *Nature* **408**, 847–850. (doi:10.1038/35048558)

Mallet, J. 2007 Hybrid speciation. *Nature* **446**, 279–283. (doi:10.1038/nature05706)

Mayr, E. 1963 *Animal species and evolution.* Cambridge, UK: Belknap Press of Harvard University Press.

Nosil, P., Funk, D. J. & Ortiz-Barrientos, D. 2009 Divergent selection and heterogeneous genomic divergence. *Mol.*

Ecol. **18**, 375–402. (doi:10.1111/j.1365-294X.2008.03946.x)

Paetkau, D., Slade, R., Burden, M. & Estoup, A. 2004 Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Mol. Ecol.* **13**, 55–65. (doi:10.1046/j.1365-294X.2004.02008.x)

Parent, C. E. & Crespi, B. J. 2006 Sequential colonization and diversification of Galápagos endemic land snail genus *Bulimulus* (Gastropoda, Stylommatophora). *Evolution* **60**, 2311–2328.

Peakall, R. & Smouse, P. E. 2006 GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**, 288–295. (doi:10.1111/j.1471-8286.2005.01155.x)

Petren, K. 1998 Microsatellites primers from *Geospiza fortis* and cross species amplification in Darwin's finches. *Mol. Ecol.* **12**, 1782–1784.

Petren, K., Grant, P. R., Grant, B. R. & Keller, L. F. 2005 Comparative landscape genetics and the adaptive radiation of Darwin's finches: the role of peripheral isolation. *Mol. Ecol.* **14**, 2943–2957. (doi:10.1111/j.1365-294X.2005.02632.x)

Phillimore, A. B., Orme, C. D. L., Thomas, G. H., Blackburn, T. M., Bennett, P. M., Gaston, K. J. & Owens, I. P. F. 2008 Sympatric speciation in birds is rare: insights from range data and simulations. *Am. Nat.* **171**, 646–657. (doi:10.1086/587074)

Piertney, S. B., Summers, R. & Marquiss, M. 2001 Microsatellite and mitochondrial DNA homogeneity among phenotypically diverse crossbill taxa in the UK. *Proc. R. Soc. Lond. B.* **268**, 1511–1517. (doi:10.1098/rspb.2001.1015)

Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L. & Estoup, A. 2004 GENECLASS2: a software for genetic assignment and first-generation migrant detection. *J. Heredity* **95**, 536–539. (doi:10.1093/jhered/esh074)

Podos, J. 2001 Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* **409**, 185–188. (doi:10.1038/35051570)

Podos, J., Southall, J. A. & Rossi-Santos, M. R. 2004 Vocal mechanics in Darwin's finches: correlation of beak gape and song frequency. *J. Exp. Biol.* **207**, 607–619. (doi:10.1242/jeb.00770)

Postma, E. & van Noordwijk, A. J. 2005 Gene flow maintains a large genetic difference in clutch size at a small spatial scale. *Nature* **433**, 65–68. (doi:10.1038/nature03083)

Pritchard, J. K., Stephens, M. & Donnelly, P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 45–959.

Räsänen, K. & Hendry, A. P. 2008 Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecol. Lett.* **11**, 624–636. (doi:10.1111/j.1461-0248.2008.01176.x)

Ratcliffe, L. M. & Grant, P. R. 1983 Species recognition in Darwin's finches (*Geospiza*, Gould). I: discrimination by morphological cues. *Anim. Behav.* **31**, 1139–1153. (doi:10.1016/S0003-3472(83)80021-9)

Raymond, M. & Rousset, F. 1995 GENEPOP (version 1.2) population genetics software for exact test and ecumenicism. *Heredity* **86**, 248–249.

Rice, W. R. & Hostert, E. E. 1993 Perspective: laboratory experiments on speciation: what have we learned in forty years? *Evolution* **47**, 1637–1653. (doi:10.2307/2410209)

Ricklefs, R. E. & Lovette, I. J. 1999 The roles of island area per se and habitat diversity in the species-area relationships of four Lesser Antillean faunal groups. *J. Anim. Ecol.* **68**, 1142–1160. (doi:10.1046/j.1365-2656.1999.00358.x)

Rundle, H. D. & Nosil, P. 2005 Ecological speciation. *Ecol. Lett.* **8**, 336–352. (doi:10.1111/j.1461-0248.2004.00715.x)

Ryan, P. G., Bloomer, P., Moloney, C. L., Grant, T. J. & Delport, W. 2007 Ecological speciation in South Atlantic Island finches. *Science* **315**, 1420–1423. (doi:10.1126/science.1138829)

Sambrook, J., Fritsch, E. F. & Maniatis, T. 1989 *Molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sato, A., O'hUigin, C., Figueroa, F., Grant, P. R., Grant, B. R., Tichy, H. & Klein, J. 1999 Phylogeny of Darwin's finches as revealed by mtDNA sequences. *Proc. Natl Acad. Sci. USA* **96**, 5101–5106. (doi:10.1073/pnas.96.9.5101)

Schluter, D. 2000a *The ecology of adaptive radiation*. Oxford, UK: Oxford University Press.

Schluter, D. 2000b Ecological character displacement in adaptive radiation. *Am. Nat.* **156**, S4–S16. (doi:10.1086/303412)

Schluter, D. & Grant, P. R. 1984 Determinants of morphological patterns in communities of Darwin's finches. *Am. Nat.* **123**, 175–196. (doi:10.1086/284196)

Schluter, D., Price, T. D. & Grant, P. R. 1985 Ecological character displacement in Darwin's finches. *Science* **227**, 1056–1059. (doi:10.1126/science.227.4690.1056)

Seehausen, O. 2004 Hybridization and adaptive radiation. *Trends Ecol. Evol.* **19**, 198–207. (doi:10.1016/j.tree.2004.01.003)

Servedio, M. R. & Noor, M. A. F. 2003 The role of reinforcement in speciation: theory and data meet. *Annu. Rev. Ecol. Syst.* **34**, 339–364. (doi:10.1146/annurev.ecolsys.34.011802.132412)

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

Slatkin, M. 2005 Seeing ghosts: the effect of unsampled populations on migration rates estimated for sampled populations. *Mol. Ecol.* **14**, 67–73. (doi:10.1111/j.1365-294X.2004.02393.x)

Smith, J. M. 1966 Sympatric speciation. *Am. Nat.* **100**, 637–650. (doi:10.1086/282457)

Waples, R. & Gaggiotti, O. 2006 What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol. Ecol.* **15**, 1419–1439. (doi:10.1111/j.1365-294X.2006.02890.x)

Weir, B. S. & Cockerham, C. C. 1984 Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370. (doi:10.2307/2408641)

Wiggins, I. L. & Porter, D. M. 1971 *Flora of the Galápagos Islands*. Stanford, CA: Stanford University Press.

Zink, R. 2002 A new perspective on the evolutionary history of Darwin's finches. *The Auk* **119**, 864–871. (doi:10.1642/0004-8038(2002)119[0864:ANPOTE]2.0.CO;2)