Molecular Ecology (2005) **14**, 2943–2957 doi: 10.1111/j.1365-294X.2005.02632.x

Comparative landscape genetics and the adaptive radiation of Darwin's finches: the role of peripheral isolation

K. PETREN,* P. R. GRANT,† B. R. GRANT† and L. F. KELLER‡
*Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221-0006, USA, †Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544-1003, USA, ‡Zoologisches Museum, Universität Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland

Abstract

We use genetic divergence at 16 microsatellite loci to investigate how geographical features of the Galápagos landscape structure island populations of Darwin's finches. We compare the three most genetically divergent groups of Darwin's finches comprising morphologically and ecologically similar allopatric populations: the cactus finches (*Geospiza scandens* and *Geospiza conirostris*), the sharp-beaked ground finches (*Geospiza difficilis*) and the warbler finches (*Certhidea olivacea* and *Certhidea fusca*). Evidence of reduced genetic diversity due to drift was limited to warbler finches on small, peripheral islands. Evidence of low levels of recent interisland migration was widespread throughout all three groups. The hypothesis of distance-limited dispersal received the strongest support in cactus and sharp-beaked ground finches as evidenced by patterns of isolation by distance, while warbler finches showed a weaker relationship. Support for the hypothesis that gene flow constrains morphological divergence was only found in one of eight comparisons within these groups. Among warbler finches, genetic divergence was relatively high while phenotypic divergence was low, implicating stabilizing selection rather than constraint due to gene flow. We conclude that the adaptive radiation of Darwin's finches has occurred in the presence of ongoing but low levels of gene flow caused by distance-dependent interisland dispersal. Gene flow does not constrain phenotypic divergence, but may augment genetic variation and facilitate evolution due to natural selection. Both microsatellites and mtDNA agree in that subsets of peripheral populations of two older groups are genetically more similar to other species that underwent dramatic morphological change. The apparent decoupling of morphological and molecular evolution may be accounted for by a modification of Lack's two-stage model of speciation: relative ecological stasis in allopatry followed by secondary contact, ecological interactions and asymmetric phenotypic divergence.

Keywords: drift, gene flow, Geospizinae, mtDNA, peripheral isolate, SSR

Received 9 February 2005; revision accepted 28 April 2005

Introduction

Growing empirical evidence indicates that a complete lack of gene flow is not required for divergence and speciation to occur (e.g. Smith *et al*. 1997; Irwin *et al*. 2001, 2005). Incomplete isolation lies at the core of some classical models of speciation and divergence (Wright 1931; Mayr 1963). Low levels of gene exchange may facilitate selection by increasing genetic variation in fragmented populations (Gavrilets *et al*. 1998; Church & Taylor 2002). Under some conditions, selection and divergence can proceed faster in small populations that receive occasional immigrants (Wade & Goodnight 1998; Whitlock *et al*. 2000; Gavrilets & Gibson 2002). Thus, small, partially isolated, peripheral populations may play an important role in speciation and divergence.

Peripheral populations are typically viewed as incipient species that may continue to diverge gradually into phenotypically distinct species over time (Mayr 1963). However,

Correspondence: Kenneth Petren, Fax: 513-556-5299; E-mail: ken.petren@uc.edu

© 2005 Blackwell Publishing Ltd

few empirical studies have been able to reconstruct the progression from peripheral populations to species. Neutral molecular genetic variation can be used to reconstruct population history, but only recently have methods become available that can span the continuum from populations to species. At timescales of less than a dozen generations, it is now possible to infer recent demographic history (Cornuet & Luikart 1996) or recent admixture due to migration or hybridization (Pritchard *et al*. 2000; Anderson & Thompson 2002; Wilson & Rannala 2003). Fewer methods are available to infer population history at intermediate levels of divergence that are critical for understanding speciation processes, in the range of tens to thousands of generations. Methods based on coalescent (Beerli & Felsenstein 1999) or cladistic (Templeton 2004) models are currently available to reconstruct the deeper history of populations, but they must rely on sometimes tenuous assumptions (Knowles & Maddison 2002; Abdo *et al*. 2004).

A different approach to inferring the intermediate stages of population history is to focus less on individual populations, and instead increase the geographical scope of comparison. Varied landscapes with naturally fragmented populations can be used to directly assess the role of geographical factors in population divergence (Epperson 2003; Manel *et al*. 2003). This landscape approach can be made even more powerful by comparing different molecular loci (e.g. Scribner *et al*. 2001; Shaw 2002), or by extending comparisons among species. The advantage of a comparative approach is that general patterns may be revealed even if stochastic events have affected a subset of populations. Comparative landscape genetics, like comparative phylogeography (Avise 2000), is based on the premise that populations and species occurring in the same landscape will be subject to similar geographical constraints. Habitat islands are especially suitable for comparative landscape genetics. Islands are discrete, and their attributes provide an indirect way to estimate relative population size and levels of immigration: island size limits population size and island locations provide fixed dispersal distances among populations.

Adaptive radiations are ideal systems for the comparative landscape genetics approach. Adaptive radiations also tend to occur in fragmented landscapes, which in itself suggests that geographical structure may enhance divergence (Givnish & Sytsma 1997; Schluter 2000). Darwin's finches occur in the fragmented and varied landscape of the Galápagos. All but one species occur in broad sympatry, often with seven or more species inhabiting a single island (Fig. 1). A question that remains largely unanswered in this adaptive radiation is how differentiation in allopatry, of which there are many examples, leads to replicate communities of species in sympatry.

Darwin's finches differ primarily in morphology, ecology and song, and many species differences are comparable

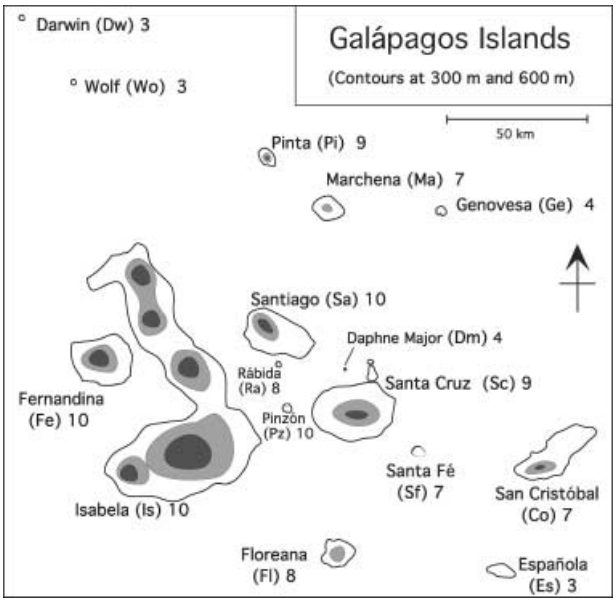

Fig. 1 A map of the Galápagos Islands with island abbreviations used in the text and the number of Darwin's finch species known to breed on each island (Grant 1999).

to family-level differences in other birds (Grant 1999). The adaptive radiation occurred very recently, within the last 3 million years (Myr) (Yang & Patton 1981; Stern & Grant 1996; Sato *et al*. 1999). Differentiation in allopatry alone appeared to be insufficient to account for such rapid morphological evolution, thus Lack (1947) proposed a two-stage model of divergence. Initial divergence occurs first in allopatry on different islands, followed by a rare colonization event to establish sympatry and further divergence in sympatry due to ecological competition for food. Alternative models have also been proposed (Hamilton & Rubinoff 1963; Grant & Grant 1989a; Cox 1990), but Lack's two-stage model has remained largely untested and unaltered for more than half a century, largely because few studies have quantified molecular genetic variation among populations.

Recent attempts to reconstruct the evolutionary history of Darwin's finches with molecular markers have met with limited success. Studies of mitochondrial (Freeland & Boag 1999a, b; Sato *et al*. 1999) and nuclear (Freeland & Boag 1999a) sequence variation have been unable to resolve relationships among most species. The poor resolving ability of mtDNA can be attributed to the compressed time frame of divergence, geographical structure among island populations (Petren *et al*. 1999a; Grant *et al*. 2000a) and low levels of hybridization (Grant & Grant 1994). Microsatellites have provided resolution of many species relationships (Petren *et al*. 1999a). Some aspects of interisland population structure were investigated with allozymes (Yang & Patton 1981), and microsatellites (Grant *et al*. 2000a), but geographical

variation among populations of Darwin's finches has not been systematically studied with DNA markers.

Here we attempt to connect geographical processes of population divergence with phenotypic divergence of Darwin's finches. Using a comparative landscape genetic approach, we focus on three groups of allopatric populations that show the most pronounced interisland population structure: the sharp-beaked ground finches (*Geospiza difficilis*), the cactus finches (*Geospiza scandens* and *Geospiza conirostris*) and the warbler finches (*Certhidea olivacea* and *Certhidea fusca*). Thus, two of these groups include populations that have diverged enough in allopatry to be considered distinct species.

We use microsatellite variation at 16 nuclear loci to address a series of geographical hypotheses. We first test for the presence of genetic structure among island populations. Many populations of Darwin's finches are confined to small islands that are prone to drought and demographic bottlenecks that can cause genetic drift (Grant *et al*. 2000b). We evaluate the evidence for drift by testing for the predicted positive correlation of genetic diversity and population size, using island size as an approximation of population size. We then test for historical evidence of interisland migration, and obtain general estimates of interisland gene flow for each group. Dispersal among populations is often limited by geographical distance, and we test for the predicted isolation-by-distance patterns. Gene flow may restrict local adaptation and morphological divergence among populations, and we test for the predicted correlation of morphological and genetic divergence among populations. Finally, we place the landscape patterns of genetic divergence into the context of adaptive radiation by comparing microsatellite divergence and mtDNA (cytochrome *b*) sequence divergence among populations and species.

Materials and methods

Field sampling and laboratory methods

Microsatellite variation was analysed in 1428 birds from 74 populations at 16 loci. This sample represents more than 70% of all populations of Darwin's finches known from major islands separated by at least 1 km. Approximately half of the microsatellite genotypes analysed here (from 43 populations) were available from previous studies (Petren *et al*. 1999a; Grant *et al*. 2000a). This is the first molecular study to include the endangered mangrove finch (*Cactospiza heliobates*; Grant & Grant 1997). Blood was collected and dried in the field, and DNA was extracted using previously published methods (Petren 1998). Laboratory methods for microsatellite genotyping, including initial development in *Geospiza fortis*, have been described elsewhere (Petren 1998; Petren *et al*. 1999b). All 16 microsatellite loci are pure dinucleotide repeats selected to be at least 12 repeat units long (Petren 1998).

Our mtDNA sampling effort favoured previously unstudied populations that were likely to yield unique haplotypes based on substantial microsatellite divergence. Mitochondrial DNA sequences for an 864-bp segment of the cytochrome *b* gene were obtained from 18 individual birds including *Geospiza difficilis* from Darwin ($N = 2$), Fernandina, Pinta, Santiago and Wolf ($N = 2$); *Geospiza conirostris* from Española; and *Platyspiza crassirostris* from Pinta. Polymerase chain reaction (PCR) primers were developed from previously published sequences (Sato *et al*. 1999) and used for PCR and direct sequencing using standard laboratory procedures (Tonnis *et al*. in press). Seventeen complete haplotypes were unique and were deposited in GenBank (accession numbers AY700033–AY700049). These sequences were combined with previously published sequences from other species ($N = 40$; Sato *et al*. 1999) and other *Certhidea* populations ($N = 23$; Tonnis *et al*. in press) to yield a set of 89 complete haplotypes. Only complete, unique haplotypes ($N = 57$) were used for the current analysis.

For comparative analyses, allopatric island populations were initially grouped into species according to taxonomic classification and morphological, ecological and behavioural similarity. Thus, morphologically similar warbler finch species (*Certhidea olivacea* and *Certhidea fusca*) were considered a single group because they are completely allopatric at the scale of islands. Subgroups were analysed separately where appropriate. Small sample sizes of birds from the satellite islands of Santiago, Albany and Rábida, were combined for all analyses.

Genetic diversity and structure

We report observed (H_O) and unbiased expected (H_E; Nei 1987) heterozygosities at 14 autosomal microsatellite loci. Populations were tested for deviations from Hardy–Weinberg equilibrium using approximations of an exact test (Guo & Thompson 1992; Raymond & Rousset 1995a). We tested for significant structuring among island populations using the unbiased approximation of Raymond & Rousset (1995a). Two loci (Gf2 and Gf10) were excluded from heterozygosity and migration analyses because they are sex-linked (Z-linked in birds), as determined by parentage studies (Petren *et al*. 1999b; Grant *et al*. 2001; Keller *et al*. 2001). Sample sizes of six or more birds were used for population level analyses. To assess the effect of small sample size, we resampled (with replacement) 20 times from four *Geospiza scandens* populations with large sample sizes. Variation among samples of six individuals does not differ dramatically from samples of 16 individuals for estimates of heterozygosity ($N = 6$: SD = 5.2%; $N = 16$: SD = 2.4%) or 16-locus genetic distances among populations ($N = 6$: SD = 8.0%; $N = 16$: SD = 3.6%).

Migration

We used a method based on coalescent theory to estimate population size and migration rates based on microsatellite variation (Beerli & Felsenstein 1999; Beerli 2004a). This maximum-likelihood method (implemented in MIGRATE) allows for mutation, but assumes equal mutation rates and constant population size. These assumptions may not be satisfied in Darwin's finches that are known to fluctuate in size (e.g. Grant *et al.* 2000b; Grant & Grant 2002a). Computer processing time is also a limitation for large data sets. For these reasons, our strategy was to gain information on overall patterns rather than obtain precise estimates for specific populations. We used a Brownian motion approximation that is appropriate for microsatellites, with starting conditions based on F_{ST} and a UPGMA tree. Unknown alleles were excluded, and searches included 10 short chains and three long chains, all with increments of 20 and 2000 steps. The burn-in was 10 000, and profile likelihoods were calculated using the 'quick' method. Analyses repeated with subsets of populations, different mutation models, and different starting conditions yielded similar results.

Interisland population structure

To estimate patterns of divergence among populations, we used Nei's standard genetic distance, D_S (Nei 1972; Takezaki & Nei 1996), because it allows for mutation, which is likely to be a factor over the timescales considered. Nei's distance does not assume a specific model of mutation and has performed well with microsatellite data (Estoup *et al.* 1995; Takezaki & Nei 1996; Paetkau *et al.* 1997; Rao *et al.* 1997; Petren *et al.* 1999a). Genetic distances were computed with PHYLIP (Felsenstein 1993). Other distance measures were calculated for comparison (e.g. F_{ST}; Weir & Cocherham 1984; $\delta\mu^2$; Goldstein *et al.* 1995). We conducted population-level analyses only for groups that showed levels of population divergence that approached or exceeded differences among species.

We assessed correlations of genetic distances and geographical features of islands with Mantel correlations (r_M, Mantel 1967) and partial Mantel correlations (Castellano & Balletto 2002). Statistical significance was assessed by permutation ($N = 1000$; Legendre & Vaudor 1991). Geographical distance among populations was the nearest shore-to-shore distance among islands (Hamilton & Rubinoff 1967; Wiggins & Porter 1971). We used differences in adult male beak length and depth ($\log_{10}$ transformed) to quantify morphological divergence among populations. These traits capture a large proportion of the morphological variation among Darwin's finches (Grant *et al.* 1985). We used beak length to represent variation in size, and the ratio of beak length to depth to represent shape variation. Measurements for the Daphne Major population of *G. scandens* were from Grant (1999) and corrected for comparison (Grant *et al.* 1985).

We used multidimensional scaling for graphical display of distance matrices (Young & Hamer 1994). This method makes few assumptions about the distribution of data and provides two (or more) dimensional axes. Statistical analyses were performed with GENEPOP (Raymond & Rousset 1995b), and SPSS (version 10.0, SPSS Inc.). Data were visually inspected for normality and transformed where appropriate. We used an alpha level of 0.05 for hypothesis testing. Significance levels for multiple tests were adjusted using the Bonferroni procedure (Rice 1989).

Phylogeographical reconstruction: microsatellites

We used Nei's distance (D_S) to directly compare microsatellite divergence among populations and species. Populations within species were combined for analysis unless they showed substantial divergence as noted above. We constructed evolutionary trees using UPGMA (Sokal & Sneath 1963; Nei & Takezaki 1996; Rao *et al.* 1997) and report 1000 bootstrap resamplings among loci. Previous analyses showed similar topologies are obtained using a wide variety of methods (Petren *et al.* 1999a). *Tiaris bicolor* was used as the outgroup for microsatellite analysis because it was the only one among several candidate outgroup species (Burns *et al.* 2002) for which sufficient numbers of individuals were available.

Phylogeographical reconstruction: mtDNA

We used unweighted parsimony with an equal transition/transversion ratio and full heuristic search on unlimited numbers of trees using the tree-bisection–reconnection (TBR) algorithm (Swofford 1998). Each parsimony bootstrap replicate ($N = 1000$) had a 200-tree limit and 10 stepwise addition replicates. We used MODELTEST (Posada & Crandall 1998) to determine that the HKY with gamma model was most appropriate for our data. Bayesian analysis was used to calculate probabilities of support for individual nodes. The transition/transversion ratio ($nst = 2$), base frequencies and gamma shape parameter were estimated from the data. We sampled four chains over 1×10^6 generations. After the first 4×10^5 generations, we saved one tree every 100 generations, and nodal probabilities were calculated from these 600 trees. This process was repeated and yielded the exact same probabilities for nodes with > 50% support. A neighbour-joining topology was used for graphical display (Swofford 1998). We tested four different outgroup species from South America and the Caribbean that are among the closest mainland relatives of Darwin's finches: *Tiaris obscura*, *Tiaris bicolor*, *Tiaris olivacea* and *Melanospiza richardsoni* (Sato *et al.* 2001; Burns *et al.* 2002).

Results

Microsatellite variation

Microsatellite loci developed in one species tend to produce smaller and less variable alleles when used to genotype other species. This indicates the assumption of equal mutation rates among lineages may be violated (Primmer *et al*. 1996; Ellegren *et al*. 1997). In our data set, genetic variation in the larger populations of *Certhidea* and in the outgroup *Tiaris bicolor* is not substantially reduced compared to the species used for microsatellite development, *Geospiza fortis* (Table 1). Each species has on average less than 62% of the total number alleles found in all Darwin's finches (mean A_N = 23.3), suggesting that homoplasy due to range constraints (Nauta & Weissing 1996) is minimal.

Tests of Hardy–Weinberg equilibrium

Population level analysis of microsatellite variation revealed a trend toward heterozygote deficiency ($P < 0.01$), and no trend toward heterozygote excess ($P = 1.0$), which suggests that any effects of recent bottlenecks are limited (Luikart & Cornuet 1999). When corrected for multiple tests ($N = 22$), two populations of *Certhidea* (Santiago and Genovesa) had significantly fewer heterozygotes than expected ($P < 0.05$), and two loci were implicated in a deficiency of heterozygotes (Gf09 and Gf11, $P < 0.05$). There are many potential causes of heterozygote deficiency, including recent immigration, cryptic population structure, recent selection at linked loci, nonamplifying or 'null' alleles (Callen *et al*. 1993), and chance. Null alleles are unlikely to be a general explanation because they are rare, as indicated by Mendelian inheritance in more than 350 parent–offspring comparisons in four different species (Petren *et al*. 1999a; Grant *et al*. 2001; Keller *et al*. 2001).

Tests of population substructure

Most species showed relatively minor interpopulation differentiation (mean $D_S \leq 0.35$; Table 2); however, three groups showed significant differentiation among island populations and were subjected to further population analyses. This includes the warbler finches (*Certhidea*, $D_S = 1.23$; $P < 0.001$), the sharp-beaked ground finch (*Geospiza difficilis*, $D_S = 0.73$; $P < 0.001$), and the cactus finches (*Geospiza scandens* and *Geospiza conirostris*, $D_S = 0.44$; $P < 0.001$). The small and large cactus finches were treated as a single group because, like the warbler finches, populations are entirely allopatric and they are morphologically, ecologically and genetically similar. The two island populations of *G. conirostris* (Genovesa and Española) are on average more genetically similar to *G. scandens* populations ($D_S = 0.54$), than they are to each other ($D_S = 0.63$). Genovesa *G. conirostris* is most closely related to Santa Cruz *G. scandens* ($D_S = 0.38$), and Española *G. conirostris* is most closely related to San Cristóbal

Table 1 Microsatellite genetic diversity of Darwin's finch species (island abbreviations as in Fig. 1)

			Islands	Birds	Genetic diversity*		
Species	Abbrev.	Island populations	N	N	A_N	H_O	D_S
Geospiza							
G. fuliginosa	*(Gfu)*	Dm, Es, Fl, Is, Pi, Sa, Sc, Co	8	145	14.4	76%	0.20
G. fortis	*(Gfo)*	Dm, Is, Ma, Pi, Sa, Sc, Co	7	272	13.6	68%	0.25
G. magnirostris	*(Gma)*	Dm, Ge, Is, Ma, Sc, Sa,	6	262	9.9	65%	0.25
G. scandens	*(Gsc)*	Dm, Ma, Pi, Sa, Sc, Co	6	119	11.2	68%	0.35
G. conirostris	*(Gco)*	Es, Ge	2	72	7.9	61%	0.63
G. difficilis	*(Gdi)*	Fe, Ge, Pi, Co, Dw, Wo	6	97	10.3	51%	0.73
Camarhynchus							
C. parvulus	*(Cpa)*	Sc, Fl, Fe, Is	4	48	8.1	54%	0.12
C. psittacula	*(Cps)*	Sc, Ma, Pi, Fe, Is	5	27	5.9	46%	0.17
C. pauper	*(Cpp)*	Fl	1	19	5.2	53%	—
Cactospiza							
C. pallida	*(Cpd)*	Sc, Sa, So, Fe, Is	5	51	6.4	42%	0.18
C. heliobates	*(Che)*	Is	1	12	2.8	35%	—
Platyspiza crassirostris	*(Pcr)*	Sc, Sa, Ma, Pi, Fe, Is	6	57	7.6	44%	0.34
Pinaroloxias inornata	*(Pin)*	Cocos Is.	1	30	4.6	51%	—
Certhidea	*(Col/Cfu)*	Ge, Es, Fe, Is, Ma, Pi, Sa, Sc, Sf, Co	10	198	17.0	47%	1.23
Tiaris bicolor	*(Tbi)*	Panama	1	19	9.7	68%	—

*The mean number of alleles (A_N) at 16 loci, the mean observed heterozygosity (H_O) at 14 autosomal loci, and the mean standard genetic distance (D_S Nei 1972) among populations.

Table 2 Microsatellite variation* among populations of three groups of Darwin's finches that show substantial interisland population structure

Group/population	N	H_O (%)	H_E (%)	A_N
1. Warbler finches (*Certhidea*)				
Green warbler finch (*C. olivacea; Col*)				
Fernandina, Fe	19	53	59	5.7
Isabela, Is	25	58	63	7.9
Santa Cruz, Sc	15	65	64	7.2
Santiago, Sa	31	62	65	7.1
Gray warbler finch (*C. fusca; Cfu*)				
Espanola, Es	30	36	37	3.8
Genovesa, Ge	23	20	25	2.2
Marchena, Ma	8	25	30	2.2
Pinta, Pi	19	42	41	3.4
San Crisrobal, So	19	53	58	5.8
Santa Fé, Sf	9	41	36	2.4
2. Sharp-beaked ground finches (*Geospiza difficilis; Gdi*)				
Darwin, Da	12	46	45	2.8
Fernandina, Fe	8	55	54	4.2
Genovesa, Ge	30	63	61	5.4
Pinta, Pi	23	36	37	3.1
Santiago, Sa	14	55	56	5.2
Wolf, Wo	10	44	39	2.2
3. Cactus ground finches				
Small cactus finch (*G. scandens*†; *Gsc*)				
Daphne Major, Dm	81	67	65	7.1
Santa Cruz, Sc	19	66	71	8.2
Santiago, Sa	9	74	72	5.9
San Cristobal, So	6	81	75	4.9
Large cactus finch (*G. conirostris; Gco*)				
Española, Es	23	53	59	4.8
Genovesa, Ge	49	64	64	6.4

*The mean number of alleles (A_N) among 16 loci, the mean observed heterozygosity (H_O), the mean expected heterozygosity (H_E; Nei 1987), and the mean genetic distance (D_S Nei 1972) among populations.
†*G. scandens* from Marchena (N = 2) and Pinta (N = 2) are not included in population analyses.

G. scandens (D_S = 0.43). Among warbler finch populations, all pairwise D_S distances between the two main groups ($\bar{X}$ = 1.86, range 1.21–2.95) were larger than all distances within groups (*Certhidea fusca* $\bar{X}$ = 0.44, range 0.11–0.80; *Certhidea olivacea*, $\bar{X}$ = 0.64, range 0.08–0.90), even though interisland geographical distances within and between these groups were similar (within groups $\bar{X}$ = 100 km, range 5–245; between groups $\bar{X}$ = 111, range 18–217). These groups were therefore considered separately for interisland contrasts.

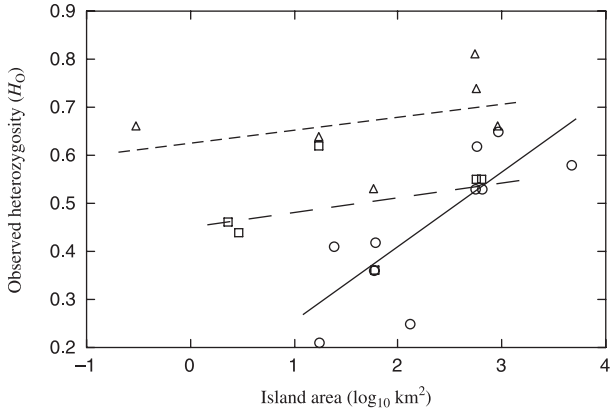

Fig. 2 Genetic diversity in terms of heterozygosity (H_O) generally increases with island area in three groups that show extensive interpopulation divergence. *Geospiza scandens/Geospiza conirostris* (triangles, small dashed line), *Geospiza difficilis* (boxes, wide dashed line), *Certhidea fusca* and *Certhidea olivacea* (open circles, solid line). Lines are the best fit according to the least squares.

Migration and Drift

The estimated number of immigrants per generation among all island populations ranged between 0.0002 and 0.008 (Table 3). The lower 99.5% confidence limit for every migration rate estimate ($M = m/\mu$) was greater than zero for all population pairs within each species group. Although some assumptions may not be met, the analysis leaves little doubt that even the more remote populations of all species occasionally receive genetic immigrants.

As predicted, there was a general trend for smaller islands to support populations with reduced genetic diversity (Fig. 2), however this pattern is only statistically significant in warbler finches (Table 4).

Table 3 Migration and population size parameters estimated for populations of each species (mean and range) using the maximum likelihood method of Beerli (2004a)

	Populations N	Θ ($4N_e\mu$)	Lower 95.5% CI M (m/μ)	Proportion immigrants (m)*
C. olivacea	4	2.2 (1.1–3.9)	9.0 (2.4–26.5)	0.0027 (0.0010–0.0060)
C. fusca	6	0.8 (0.3–1.6)	3.5 (0.7–8.4)	0.0013 (0.00027–0.0034)
G. difficilis	6	0.8 (0.2–2.1)	4.2 (0.4–12.9)	0.0016 (0.0007–0.0024)
G. scandens	6	0.7 (0.3–1.4)	11.8 (3.5–50.2)	0.0050 (0.0029–0.0074)

*Summed per generation immigration rates from all other populations. The assumed mutation rate for nuclear microsatellites is 10^{-4}.

Table 4 Correlations of genetic diversity of populations and genetic distances between islands and island size†

Species	N	Genetic diversity‡ & area (r_P)	Genetic distance§ & area difference (r_M)	Genetic distance§ & geographical distance (r_M)	Genetic distance§ & geographical distance (r_M, partial w/area)
G. scandens	6	0.367	−0.042	0.720**	0.745**
G. difficilis	6	0.331	0.180	0.507*	0.533*
Certhidea (all)	10	0.800**	0.391*	0.056	0.099
C. fusca	6	0.584	−0.687***	0.366	0.260
C. olivacea	4	−0.085	−0.510	0.534	0.292

†Statistical significance: *indicates $P \leq 0.05$, **indicates $P \leq 0.01$, ***indicates $P \leq 0.001$.
‡The Pearson correlation coefficient (r_P) between observed heterozygosity and log-transformed island area.
§Mantel matrix correlations (r_M) of genetic distances and differences in island area or geographical distances among populations, and partial Mantel correlations with area included as a third matrix.

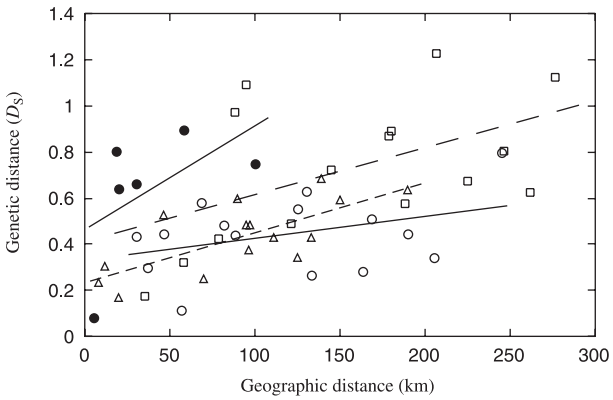

Fig. 3 Genetic distance between populations increases with geographical distance among islands in the three groups that show extensive interpopulation divergence. Symbols are as in Fig. 2, but with *Certhidea fusca* (open circles, solid line) and *Certhidea olivacea* (filled circles, solid line) plotted separately.

Table 5 Correlations (r_M) of genetic distances (D_S) and morphological differences† among populations

Group	(N)	Size	Shape
All populations	22	0.32**	0.49***
G. scandens	6	−0.18	0.78**
G. difficilis	6	0.43	−0.31
C. fusca	6	−0.53*	−0.01
C. olivacea	4	−0.98*	−0.61*

†Sizes were approximated by log-transformed beak length, and shape was the ratio of log-transformed beak length to beak depth.
‡Statistical significance: *indicates $P \leq 0.05$, **indicates $P \leq 0.01$, ***indicates $P \leq 0.001$.

The hypothesis of distance-limited dispersal

Genetic distance is positively correlated with geographical distance among the three most structured groups (Fig. 3). Mantel correlations revealed a significant positive association of geographical and genetic distance in *G. scandens* and *G. difficilis* (Table 4). When the two groups of *Certhidea* were analysed separately, isolation-by-distance patterns emerge, as indicated by higher correlation coefficients, but they are only marginally significant (for *C. fusca* $P = 0.07$; for *C. olivacea* $P = 0.11$). Including island area in a partial Mantel test did not disrupt the isolation-by-distance patterns in *G. scandens* and *G. difficilis*, suggesting that reduced gene flow rather than reduced population size drives these correlations.

Morphological variation

The predicted positive correlation between microsatellite genetic distances and morphological differences among populations is evident when all populations are analysed together (Table 5). These correlations are likely to be inflated due to shared ancestry among subsets of populations (Harvey & Pagel 1991). Within groups, only one of six correlations is significant in the predicted positive direction, while six of eight correlations were negative, and three were significantly negative. The hypothesized role of gene flow in constraining morphological divergence was only supported for beak shape variation among *G. scandens*. Large genetic differences among allopatric *Certhidea* populations with comparatively little morphological differentiation (Fig. 4) appears to indicate ecological constraint due to stabilizing selection, rather than constraint due to gene flow.

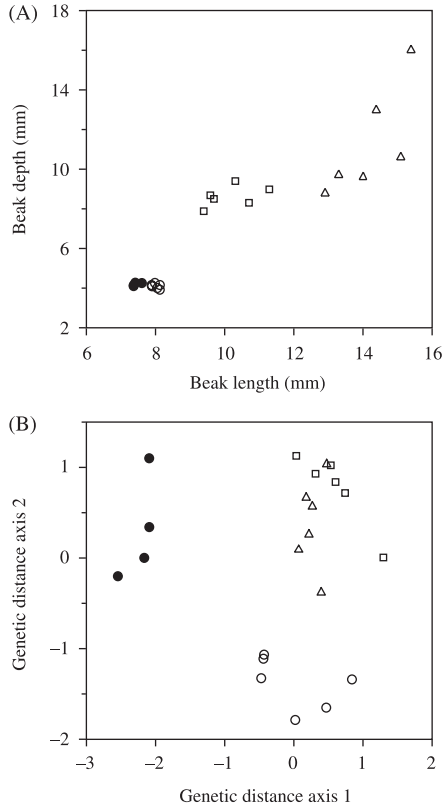

Fig. 4 (A) Morphological variation in beak traits of male birds among populations of the three focal groups (Lack 1947; Grant *et al*. 1985). (B) Genetic variation among populations based on pairwise microsatellite distances (D_S), represented by two multidimensional scaling axes. Symbols: *Geospiza scandens*/*Geospiza conirostris* (triangles), *Geospiza difficilis* (boxes), *Certhidea fusca* (open circles) and *Certhidea olivacea* (filled circles).

Comparison of microsatellite and mtDNA distances

Among populations and species of Darwin's finches, Nei's standard genetic distance (D_S) is correlated with uncorrected (p) cytochrome *b* distances (D_S: $r_M = 0.71$, $P = 0.001$; $N = 28$ taxa or 364 pairs). Nei's distance appears to be more linear and therefore more appropriate for species comparisons when compared to alternative distance measures which showed a weaker correlation with mtDNA distances, including F_{ST} ($r_M = 0.34$, $P = 0.001$) and $\delta\mu^2$ ($r_M = 0.23$, $t = 2.46$: $P = 0.001$). Although the outgroup was excluded from these correlations, genetic distances to the outgroup appear to be linear for D_S (Fig. 5A), nonlinear for F_{ST} (Fig. 5B) and extremely nonlinear for $\delta\mu^2$ (Fig. 5C).

Phylogeographical variation among species: microsatellites

An evolutionary tree based on microsatellite variation revealed a deep division between *C. olivacea* and *C. fusca* (Fig. 6A). The microsatellite tree also revealed deep genetic divisions among *G. difficilis* populations. Five island populations of *G. difficilis* are monophyletic and branch off before the divergence of 11 other species including the ground finches, tree finches, and the vegetarian finch, while the

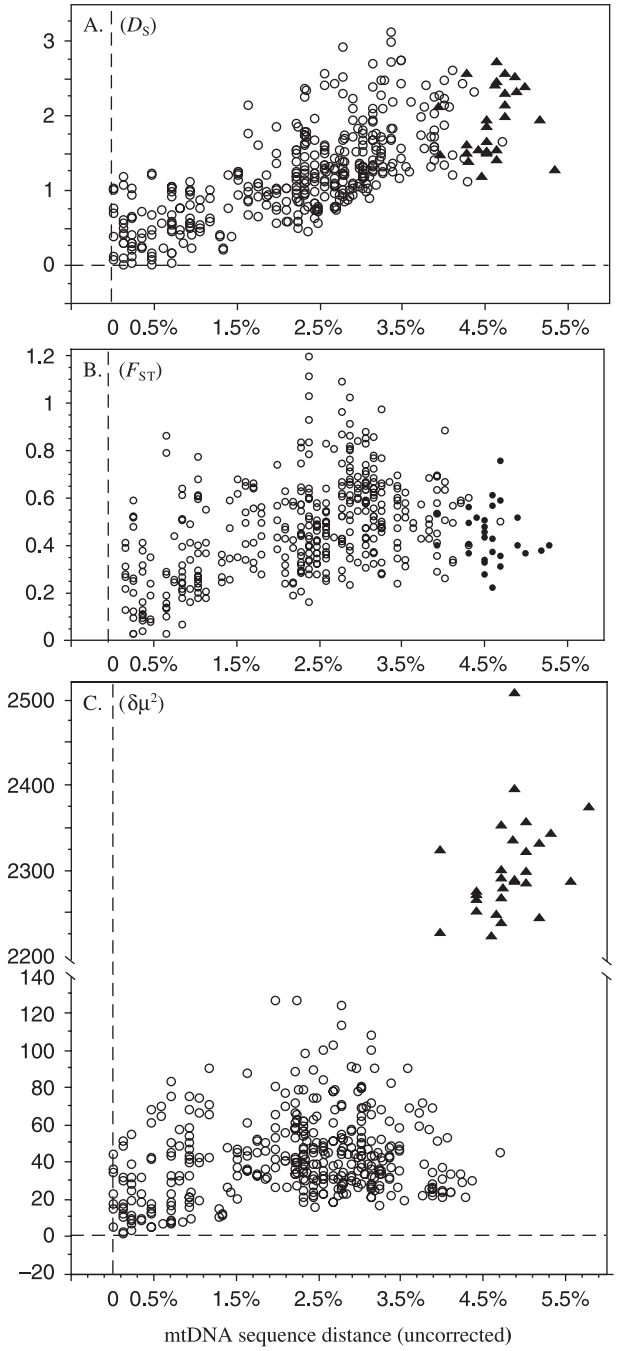

Fig. 5 Pairwise genetic distances among Darwin's finches (circles) and between Darwin's finches and *Tiaris bicolor* (triangles) based on mitochondrial sequence divergence (uncorrected) and microsatellites. Microsatellite distances along the *y*-axis are calculated using (A) D_S (Nei 1972), (B) F_{ST} (Weir & Cockerham 1984) and (C) $\delta\mu^2$ (Goldstein *et al*. 1995).

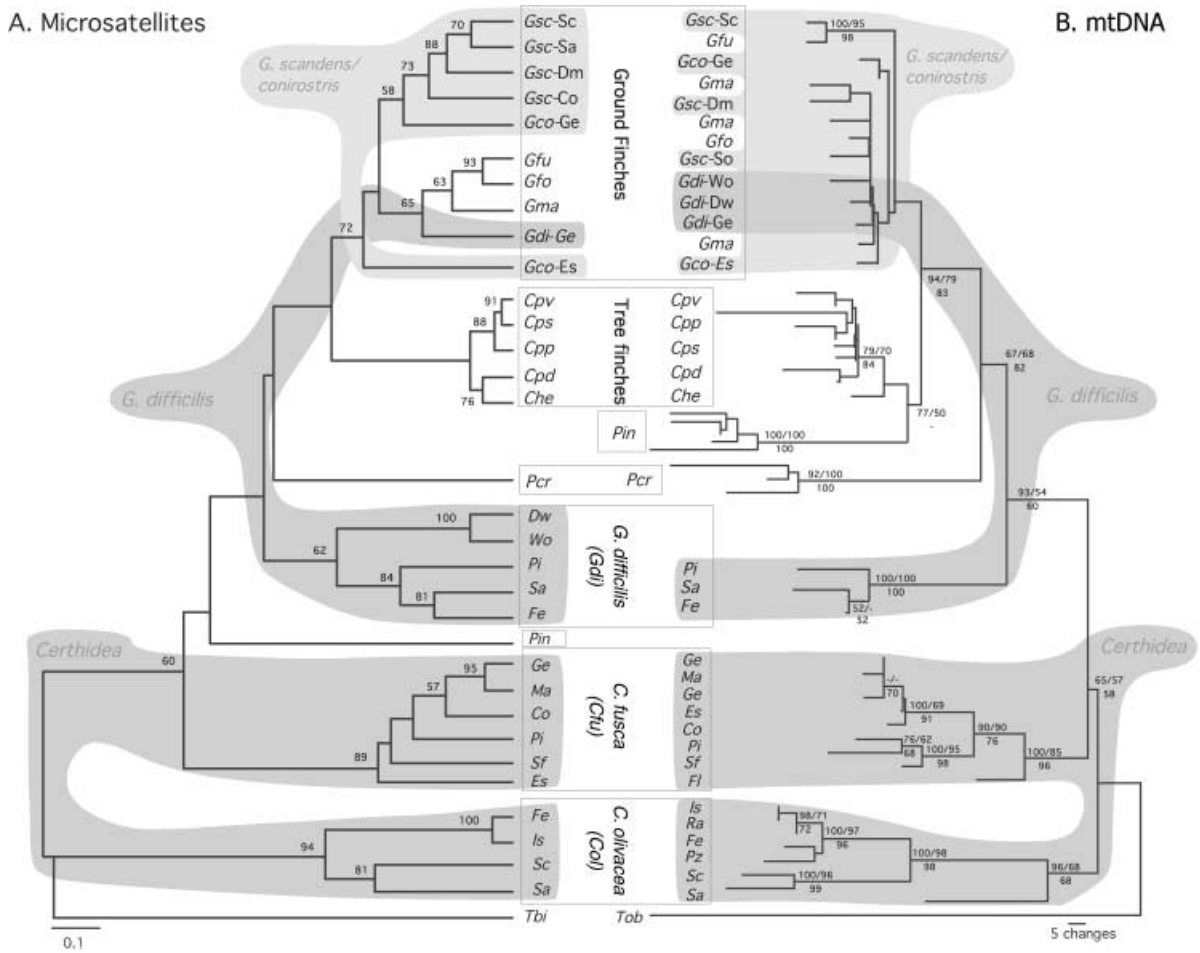

Fig. 6 Evolutionary relationships among populations and species of Darwin's finches. Microsatellite genotypes at 16 loci were obtained from 1428 birds from 74 populations, and D_S and UPGMA was used to produce the topology shown (A). mtDNA (cyt *b*) sequence variation among 57 unique haplotypes was used to construct a single neighbour-joining (NJ) tree (B). Abbreviations for taxa are species names (from Table 1), followed in some cases by abbreviations of island names (from Fig. 1). Shaded areas connect populations of each of the three main groups: warbler finches (*Certhidea fusca* and *Certhidea olivacea*), sharp-beaked ground finches (*Geospiza difficilis*); and cactus finches (*Geospiza scandens* and *Geospiza conirostris*). Numbers indicate support for nodes based on bootstraps among loci for microsatellites, and for mtDNA according to parsimony bootstraps (above left), Bayesian probabilities (above right) and NJ bootstraps (below).

Genovesa population is nested among the other ground finches. Among the cactus finches (*G. scandens* and *G. conirostris*), the single population from Española is distinct, while other populations are more closely related to other species. Bootstrap support for many nodes within the tree finch and ground finch clades is high, indicating consistent genetic relationships across different loci. The microsatellite tree depicts the mangrove finch (*Cactospiza heliobates*) as the sister taxon to its congener, the woodpecker finch (*C. pallida*).

Phylogeographical variation among species: mtDNA

Uncorrected (*p*) genetic distances between *C. olivacea* and *C. fusca* averaged 3.0%. Distances among island populations of *G. difficilis* were on average 2.1% (min: 0.0; max: 2.28%). There was no evidence of deep genetic divisions among *G. conirostris*/*G. scandens* populations, or within any other Galápagos species based on mtDNA variation (all pairwise distances < 1%). Phylogenetic reconstructions based on mtDNA variation (Fig. 6A) revealed that many species relationships were poorly resolved as reported previously (Freeland & Boag 1999a). All methods of analysis revealed a deep division among *Certhidea* corresponding to peripheral island *C. fusca* and central island *C. olivacea*. All methods also support a deep genetic division among populations of *G. difficilis*. Three island populations of *G. difficilis*, Santiago, Pinta and Fernandina, formed a well-supported clade that diverged before 11 other Galápagos species including the ground finches, tree finches, and the vegetarian finch. *Geospiza difficilis* haplotypes from Darwin and Wolf clustered with all other *Geospiza* haplotypes including the Genovesa population.

Comparison of microsatellite and mtDNA tree topologies

The microsatellite tree is not completely congruent with the cytochrome *b* phylogeny. One difference is that the Cocos Island finch occupies a more basal position in the microsatellite tree. Another contrast occurs with respect to the Darwin and Wolf populations of the sharp-beaked ground finch (*G. difficilis*), which are quite distinct from the Genovesa population and other species based on microsatellites, but are nearly identical to the Genovesa population based on cytochrome *b*. Despite these differences, both tree topologies reflect the same two distinct clades of warbler finches (*C. olivacea* and *C. fusca*). Both markers also reflect deep genetic relationships among *G. difficilis* populations, though in this case the exact division differs.

Discussion

*Population divergence among cactus finches (*Geospiza scandens *and* Geospiza conirostris*)*

The two populations of the large cactus finch, *Geospiza conirostris*, do not form a natural group according to the loci we surveyed. Their previous classification as the same species is evidence of convergence. A close affinity to *Geospiza scandens* had been suspected previously for the Genovesa population of *G. conirostris* based on morphology and song. The Española population was previously thought to be sufficiently phenotypically and behaviourally distinct to warrant preservation of species status (Lack 1947; Bowman 1961; Grant 1999). Microsatellite variation supports the notion that this population is distinct from all other *G. scandens* populations (Fig. 6). The Daphne Major population of *G. scandens* harbours exceptionally high levels of genetic variation (H_O = 67%) for such a small island (*c.* 0.34 km^2), which may be partly attributable to introgressive hybridization (Grant *et al*. 2004). There is general evidence of hybridization among species on some islands, but levels of introgression are low and are not likely to have dramatically affected our main results (Grant 1999; Grant *et al*. in press).

*Population divergence among sharp-beaked ground finches (*Geospiza difficilis*)*

Historically, the sharp-beaked ground finch *Geospiza difficilis* has been difficult to classify taxonomically, hence the species name *difficilis* (Lack 1947). All populations generally have sharp beaks and sing similar songs, but they differ greatly in body size, beak shape, ecology and specific song components (Schluter & Grant 1984; Grant *et al*. 2000a). The more intense sampling of populations undertaken here yielded a result fundamentally different from earlier genetic studies (Freeland & Boag 1999a, b; Petren *et al*. 1999a; Sato *et al*. 1999). Tree topologies derived from microsatellites and mtDNA reveal that the earliest division among populations of *G. difficilis* occurred after the warbler finches diverged, but before all other species began to differentiate, with the exception of the ambiguous origin of the Cocos Island finch.

*Population divergence among the warbler finches (*Certhidea olivacea *and* Certhidea fusca*)*

The completely allopatric warbler finches (*Certhidea olivacea* and *Certhidea fusca*) are more genetically distinct than any other species of Darwin's finches. Their occupation of islands that place them in close proximity suggests that geographical distance does not currently account for this deep genetic divergence, and raises the question of how these groups remain genetically distinct. Song playback responses in the field reveal little evidence of reproductive isolation (Grant & Grant 2002b). Some of the geographical pattern may be due to recent habitat specific colonization, as females of each species appear to preferentially disperse to islands that are ecologically similar to their natal environment (Tonnis *et al*. in press). The divergence of *Certhidea* species predates the rest of the adaptive radiation. Based on 3.3% average divergence between *C. olivacea* and all other species, and using a standard avian mtDNA clock of 2% per Myr (Shields & Wilson 1987; Garcia-Moreno 2004), the timescale of the earliest divergence within the Galápagos is pushed back from 1.40 million years ago (Ma) to roughly 1.65 Ma (Sato *et al*. 1999)

Genetic drift

Island area, our approximate indicator of population size, is positively associated with standing genetic diversity only in the warbler finches (*Certhidea*). A previous allozyme study found a similar relationship among five populations of warbler finches (Yang & Patton 1981). Area was less associated with genetic variation in *G. scandens* and *G. difficilis*, although both showed a slight trend in the same direction. The effects of drift may only be evident in *Certhidea* because of their unique ecology. *Certhidea* rely primarily on gleaned insects as a food source, whereas *Geospiza* rely primarily on seeds, and these resources respond differently to drought (Grant 1999). Ecological differences may affect how closely island size approximates population size for each species, and this is a limitation of the comparative approach. Populations of *G. difficilis* and *C. olivacea* on larger islands also tend to be restricted to upland habitat (Grant 1999; Grant *et al*. 2001; Tonnis *et al*. in press). Although drift does not have a detectable general effect in the *Geospiza* populations studied here, it may be important for specific populations under specific conditions.

Gene flow and peripheral isolation

Migration rate estimates support the conclusion that gene flow occurs among all populations of the three most highly structured groups. Migration rates were higher than those recently reported for pond- and stream-dwelling stickleback fish using the same method (Hendry & Taylor 2004). Our estimates of the number of immigrants per generation ranged between 2 and 80 for every 10 000 residents. These numbers are higher than expected based on field evidence, where a singing male from another population can generally be easily detected (Grant & Grant 1996; Grant 1999). The assumption of the method that is most likely to be violated is that of constant population size, which on many islands is linked to oscillating droughts (Grant & Grant 2002a) and El Niño events (Grant *et al*. 2000b). Population size estimates may be inflated by unaccounted for populations, but migration rates are not similarly inflated by missing populations (Beerli 2004b), thus, introgression is not likely to have influenced migration rate estimates. Some populations may have been recently established and therefore not in equilibrium, but recent colonization is not a more likely alternative than ongoing gene flow for 22 populations from three different species groups.

The inference of ongoing gene flow is consistent with other evidence. Large ground finches (*Geospiza magnirostris*) that founded a population on Daphne Major were a non-random subset of potential founders, many of which likely continued to travel to other islands (Grant *et al*. 2001). A recent analysis of *Certhidea* revealed evidence of recent long-distance dispersal of females (Tonnis *et al*. in press). Our results also show that peripheral populations of Darwin's finches are more distinct genetically, a pattern attributable to distance-limited dispersal (Epperson 2003). In birds, patterns of isolation-by-distance are sometimes absent across large areas of contiguous habitat, but are more commonly observed in discontinuous habitat (McDonald *et al*. 1999; Gibbs *et al*. 2000), such as an archipelago.

Contrary to earlier views that suggested interisland movements were very rare (Lack 1947), the adaptive radiation of Darwin's finches has very likely occurred in the presence of gene flow. Individual populations, especially small populations on the periphery, may receive immigrants only rarely. Immigration may also be episodic and therefore difficult to detect in the field. However, even rare immigrants can affect evolution by infusing populations with novel alleles, in a similar manner to that envisioned for hybridization (Grant & Grant 1994; Morjan & Rieseberg 2004).

Potential interactions of selection, limited gene flow and drift

The classical role envisioned for drift in small populations (Wright 1931) has recently been revitalized by models with low levels of immigration, which can infuse small, drift-prone populations with genetic variation. Natural selection may then act upon this variation to cause change to occur faster than in large populations (Whitlock *et al*. 2000; Church & Taylor 2002; Gavrilets & Gibson 2002). Our results show that some populations of Darwin's finches may experience genetic drift, low levels of gene flow as well as natural selection. However, it is not possible to directly test for a synergistic interaction among these processes, partly because size and isolation are correlated among the islands we sampled (Hamilton & Rubinoff 1967).

Morphological divergence and natural selection

The notion that gene flow constrains phenotypic divergence in Darwin's finches received some support from a previous study showing that endemism at the level of subspecies is generally associated with geographical isolation (Hamilton & Rubinoff 1967). Our direct estimates of genetic divergence do not support the hypothesis that gene flow constrains local adaptation in Darwin's finches. Only *G. scandens* beak shape differences were correlated with genetic divergence, and in this case, we cannot rule out differing selection pressures on the smaller peripheral islands inhabited by *G. conirostris* as a cause (Grant & Grant 1989b). Warbler finches are morphologically constrained in allopatry compared to many other species of Darwin's finches. This constraint is not related to gene flow, but is more likely caused by stabilizing selection due to similar ecological circumstances. The lack of positive correlation between differences in beak dimensions and genetic divergence in warbler finches emphasizes how local processes outweigh the effects of gene flow among populations. The unexpected finding of significant negative correlations may be due to the tendency for ecologically similar islands to be geographically widely dispersed (Tonnis *et al*. in press). The ecological stasis among warbler finches contrasts markedly with the rapid phenotypic divergence of other species. This combination of stasis in the midst of change is unusual compared to other adaptive radiations.

Phylogenetic relationships among populations and species

Support for a single tree topology was not particularly strong according to either marker (Fig. 6). Mitochondrial DNA resolution was poor among many closely related species, and some species share identical haplotypes. These patterns can be attributable to rapid speciation, stochastic lineage sorting among highly structured island populations, and hybridization. Based on the low levels of variation observed among haplotypes from 61 additional individuals (Tonnis *et al*. in press), more intensive sampling of individuals or populations is unlikely to alter these results or improve resolution. Sequencing of additional

nuclear loci may help to overcome problems associated with stochastic lineage sorting (Hudson & Coyne 2002). Microsatellites were somewhat complementary to mtDNA by providing better resolution among the more closely related species, where mtDNA resolution was practically absent.

In spite of these limitations, two types of molecular markers with different mutation mechanisms, modes of inheritance and methods of analysis are in general agreement: populations of some species (*Certhidea*, *G. difficilis*), are more closely related to other species. In both instances, it is generally the more peripherally located populations that are closer to other species. All *C. fusca*, or more precisely the common ancestor of this peripheral species, are more closely related to all other species of Darwin's finches than they are to more centrally located *C. olivacea*. The Genovesa population of *G. difficilis* (or the populations of Genovesa, Darwin and Wolf according to mtDNA) is more closely related to many other species of Darwin's finches than it is to more centrally located populations of *G. difficilis*. One interpretation of the phylogeographical patterns is that peripheral populations may have been differentially and profoundly affected by past introgression, causing them to become more closely related to other species. It is very difficult to rule out past introgression as a cause of current genetic relationships among species. However, peripheral populations generally conform to expectations of isolation by distance according to microsatellites, and studies of recent hybridization do not suggest peripheral populations are differentially affected relative to central populations (Grant 1999; Grant *et al.* in press; unpublished). We conclude that the current genetic relationships among morphologically similar populations that are not likely to have been profoundly affected by past introgression with other species.

Although the patterns of molecular variation do not support monophyletic groupings that correspond to phenotypes, the tree topologies may shed light on the speciation process (Avise 2000; Funk & Omland 2003). The implication according to both topologies is that peripheral populations are the source of new species that have undergone rapid phenotypic change, while peripheral populations themselves have remained phenotypically similar to centrally located populations. Adaptive radiations are known for rapid phenotypic evolution with comparative little genetic divergence (Givnish & Sytsma 1997). Darwin's finches seem to take this relationship a step farther. Phenotypic evolution appears to be largely decoupled from genetic evolution at presumably neutral loci in Darwin's finches. This is true within species, as shown by the lack of genotype/phenotype correlations, and among species as shown by patterns of relative phenotypic stasis and rapid asymmetric phenotypic divergence of a subset of lineages.

Peripheral isolation and adaptive radiation

These results have implications for general models of speciation and adaptive radiation in Darwin's finches and other groups. Evidence of phenotypic stasis in allopatry supports the notion that allopatric processes alone are unlikely to account for this adaptive radiation (Lack 1947). The evidence of interisland gene flow seems to increase the possibility of sympatric speciation. However, empirical data suggest that sympatric speciation is not a likely occurrence in Darwin's finches (Grant & Grant 1989a), or in other island birds (Coyne & Price 2000). We suggest Lack's two-stage model of speciation is more parsimonious than sympatric models, and it requires only minor modification to account for our results.

Lack's model states that ecological character displacement drives morphological change in sympatry, but only after colonization establishes secondary contact between two populations that initially diverged in allopatry. Our results suggest the first stage of divergence in allopatry occurs with gene flow, which does not preclude divergence in allopatry as shown by our results and other studies (Smith *et al.* 1997; Irwin *et al.* 2001, 2005). Establishment of the second part of Lack's two-stage model, colonization and secondary contact, is very feasible in light of the widespread evidence of interisland migration. Most interisland movement results in gene flow, while on rare occasions conditions may permit two populations to coexist in sympatry where ecological processes drive them apart. The first stage in allopatry allows for correlations to build among traits that affect ecology and mate choice thereby circumventing the weakest aspect of sympatric models (Bolnick 2004). The second stage retains an important aspect of sympatric models: the possibility of rapid phenotypic divergence due to ecological interactions.

The aspects of the two-stage model that require most modification are the timing and pattern of major phenotypic change. Our results suggest that initial differentiation in allopatry is relatively minor, while the second stage in sympatry may lead to rapid and asymmetric phenotypic change. The evidence for isolation-by-distance patterns implies that peripheral populations are more likely to become reproductively isolated (Mayr 1963; Garcia-Ramos & Kirkpatrick 1997). The phylogeographical analysis suggests that some peripheral populations are more closely related to other species. Thus our results suggest peripheral populations are sources of colonists that establish new species. Peripheral and central populations remain relatively unchanged, while the founders of a new species change rapidly in secondary contact. Ecological models support the possibility of asymmetric divergence due to resource competition in sympatry (Doebeli & Dieckmann 2000). Asymmetric divergence may be even more likely

when a small newly founded population is competing with a large, locally adapted, resident population.

The applicability of this revised two-step model to other systems will depend on the nature and degree of divergence occurring in allopatry, the frequency of dispersal and the conditions under which colonists establish a sympatric, reproductively isolated population rather than simply interbreeding with residents. Thus the model hinges on infrequent dispersal, which is difficult to detect and is perhaps underestimated in many systems. The two-stage model should be investigated further because it implies a different role for partially isolated populations in generating future biodiversity. Peripheral isolates may represent potential sources of colonists that give rise to rapidly diverging new species, and geographical isolates may remain relatively unchanged phenotypically even after generating new species. This model will be difficult to test, as is the case for many other models of speciation. One potential source of evidence is when subsets of populations are found to be more closely related to other species.

Comparative landscape genetics

Comparative landscape genetics offers promise for distinguishing among complex speciation models, but it must overcome three limitations. The first is finding suitable molecular markers to resolve very recent speciation events. In this study, two different markers were informative and complementary across species and a wide range of temporal divergence. Mitochondrial DNA was unable to resolve divergence among most populations and species, but gave valuable support for deeper nodes. This study shows that microsatellites may be informative when compared among species that are less that 4% divergent based on mtDNA sequence, and Nei's distance (D_S) may be the most appropriate distance measure at these timescales. A second limitation of comparative landscape genetics is the lack of quantitative methods for comparing different molecular markers. This need may be met with further development of system-specific models that contrast specific alternative historical hypotheses (e.g. Piertney *et al*. 2000; Knowles & Maddison 2002). A third limitation is finding suitable natural systems where closely related species occur in sympatry. The greatest impact of comparative landscape genetics may come from studies of recent adaptive radiations in fragmented landscapes.

Acknowledgements

We thank the Galápagos National Park Service for permission to conduct this research, the Charles Darwin Research Station and the Charles Darwin Foundation for logistical support. We thank E. Monson, S. Tebbich, B. Fessl, E. Bermingham, P. Boag, H. Vargas, M. Wikelski and M. Hau for assistance with obtaining tissue samples. We thank J. Markert, J. Niedzwiecki, A. Clack, S. Bensch and two anonymous reviewers for comments that improved the manuscript. Financial support was provided by the National Science Foundation.

References

Abdo Z, Crandall KA, Joyce P (2004) Evaluating performance of likelihood methods for detecting population structure and migration. *Molecular Ecology*, **13**, 837–851.

Anderson EC, Thompson EA (2002) A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*, **160**, 1217–1229.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, Massachusetts.

Beerli P (2004a) *MIGRATE version 2.0: documentation and program. Revised 23 December 2004*. Distributed over the Internet, http://evolution.gs.washington.edu/lamarc.html.

Beerli P (2004b) Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. *Molecular Ecology*, **13**, 827–836.

Beerli P, Felsenstein J (1999) Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics*, **152**, 763–773.

Bolnick DI (2004) Waiting for sympatric speciation. *Evolution*, **58**, 895–899.

Bowman RI (1961) Morphological differentiation and adaptation in the Galápagos finches. *University of California Publications in Zoology*, **58**, 1–302.

Burns KJ, Hackett SJ, Klein NK (2002) Phylogenetic relationships and morphological diversity in Darwin's finches and their relatives. *Evolution*, **56**, 1240–1252.

Callen DF, Thompson AD, Shen Y (1993) Incidence and origin of 'null' alleles in the AC_N microsatellite markers. *American Journal of Human Genetics*, **52**, 922–927.

Castellano S, Balletto E (2002) Is the partial Mantel test inadequate? *Evolution*, **56**, 1871–1873.

Church SA, Taylor DR (2002) The evolution of reproductive isolation in spatially structured populations. *Evolution*, **56**, 1859–1862.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Cox GW (1990) Centres of speciation and ecological differentiation in the Galápagos land bird fauna. *Evolutionary Ecology*, **4**, 130–142.

Coyne JA, Price TD (2000) Little evidence for sympatric speciation in island birds. *Evolution*, **54**, 2166–2171.

Doebeli M, Dieckmann U (2000) Evolutionary Branching and sympatric speciation caused by different types of ecological interactions. *American Naturalist*, **156** (Suppl.), S77–S101.

Ellegren H, Moore S, Robinson N *et al*. (1997) Microsatellite evolution: a reciprocal study of repeat lengths at homologous loci in cattle and sheep. *Molecular Biology and Evolution*, **14**, 854–860.

Epperson BK (2003) *Geographical Genetics*. Princeton University Press, Princeton, New Jersey.

Estoup A, Garnery L, Solignac M, Cornuet J-M (1995) Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics*, **140**, 679–695.

Felsenstein J (1993) *PHYLIP: Phylogeny Inference Package version 3.5c*. Zoology Department, University of Washington, Seattle.

Freeland JR, Boag PT (1999a) The mitochondrial and nuclear genetic homogeneity of the phenotypically diverse Darwin's ground finches. *Evolution*, **53**, 1553–1563.

Freeland JR, Boag PT (1999b) Phylogenetics of Darwin's finches: paraphyly in the tree-finches and two divergent lineages in the warbler finch. *Auk*, **116**, 577–588.

Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology and Systematics*, **34**, 397–423.

Garcia-Moreno J (2004) Is there a universal mtDNA clock for birds? *Avian Biology*, **35**, 465–468.

Garcia-Ramos G, Kirkpatrick M (1997) Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, **51**, 21–28.

Gavrilets S, Gibson N (2002) Fixation probabilities in a spatially heterogeneous environment. *Population Ecology*, **44**, 51–58.

Gavrilets S, Li H, Vose MD (1998) Rapid parapatric speciation on holey adaptive landscapes. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **265**, 1–7.

Gibbs HL, Dawson RJG, Hobson KA (2000) Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? *Molecular Ecology*, **9**, 2137–2147.

Givnish TJ, Sytsma KJ (1997) *Molecular Evolution and Adaptive Radiation*. Cambridge University Press, Cambridge.

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use within microsatellite loci. *Genetics*, **139**, 463–471.

Grant PR (1999) *Ecology and Evolution in Darwin's Finches*, Second printing. Princeton University Press, Princeton, New Jersey.

Grant PR, Grant BR (1989a) Sympatric speciation in Darwin's finches. In: *Speciation and its Consequences* (eds Otte D, Endler JA). Sinauer Associates, Sunderland, Massachusetts.

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

Grant PR, Grant BR (1994) Phenotypic and genetic effects of hybridization in Darwin's finches. *Evolution*, **48**, 297–316.

Grant BR, Grant PR (1996) Cultural inheritance of song and its role in the evolution of Darwin's finches. *Evolution*, **50**, 2471–2487.

Grant PR, Grant BR (1997) The rarest of Darwin's finches. *Conservation Biology*, **11**, 119–126.

Grant PR, Grant BR (2002a) Unpredictable evolution in a 30-year study of Darwin's finches. *Science*, **296**, 707–711.

Grant BR, Grant PR (2002b) Lack of premating isolation at the base of a phylogenetic tree. *American Naturalist*, **160**, 1–19.

Grant PR, Abbott I, Schluter D, Curry RL, Abbott KL (1985) Variation in the size and shape of Darwin's finches. *Biological Journal of Linnean Society*, **25**, 1–39.

Grant PR, Grant BR, Petren K (2000a) The allopatric phase of speciation: the sharp-beaked ground finch (*Geospiza difficilis*) on the Galapagos islands. *Biological Journal of the Linnean Society*, **69**, 287–317.

Grant PR, Grant BR, Keller LF, Petren K (2000b) Effects of El Niño events on Darwin's finch productivity. *Ecology*, **81**, 2442–2457.

Grant PR, Grant BR, Petren K (2001) A population founded by a single pair of individuals: establishment, expansion, and evolution. *Genetica*, **112/113**, 359–382.

Grant PR, Grant BR, Keller LF, Markert JA, Petren K (2004) Convergent evolution of Darwin's finches caused by introgressive hybridization and selection. *Evolution*, **58**, 1588–1599.

Grant PR, Grant BR, Petren K (in press) Hybridization in the recent past. *American Naturalist*.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportions for multiple alleles. *Biometrics*, **48**, 361–372.

Hamilton TH, Rubinoff I (1963) Isolation, endemism, and multiplication of species in the Darwin's finches. *Evolution*, **17**, 388–403.

Hamilton TH, Rubinoff I (1967) On predicting insular variation in endemism and sympatry for the Darwin's finches in the Galápagos archipelago. *American Naturalist*, **101**, 161–167.

Harvey PH, Pagel MD (1991) *The Comparative Method in Evolutionary Biology*. Oxford University Press, New York.

Hendry AP, Taylor EB (2004) How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. *Evolution*, **58**, 2319–2331.

Hudson RR, Coyne JA (2002) Mathematical consequences of the genealogical species concept. *Evolution*, **56**, 1557–1565.

Irwin DE, Bensch S, Price TD (2001) Speciation in a ring. *Nature*, **409**, 333–337.

Irwin DE, Bensch S, Irwin JH, Price TD (2005) Speciation by distance in a ring species. *Science*, **307**, 414–415.

Keller LF, Grant PR, Grant BR, Petren K (2001) Heritability of morphological traits in Darwin's finches: misidentified paternity and maternal effects. *Heredity*, **87**, 325–336.

Knowles LL, Maddison WP (2002) Statistical phylogeography. *Molecular Ecology*, **11**, 2623–2635.

Lack D (1947) *Darwin's Finches*. Cambridge University Press, Cambridge.

Legendre P, Vaudor X (1991) *The R Package*. Université de Montréal.

Luikart G, Cornuet J-M (1999) Estimating the effective number of breeders from heterozygote excess in progeny. *Genetics*, **151**, 1211–1216.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology &Evolution*, **18**, 189–197.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Mayr E (1963) *Animal Species and Evolution*. Belknap Press, Cambridge, Massachusetts.

McDonald DB, Potts WK, Fitzpatrick JW, Woolfenden GE (1999) Contrasting genetic structures in sister species of North American scrub-jays. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **266**, 1117–1125.

Morjan CL, Rieseberg LH (2004) How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. *Molecular Ecology*, **13**, 1341–1356.

Nauta MJ, Weissing FJ (1996) Constraints on allele size at microsatellite loci: implications for genetic differentiation. *Genetics*, **143**, 1021–1032.

Nei M (1972) Genetic distance between populations. *American Naturalist*, **106**, 283–292.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Nei M, Takezaki N (1996) Reconstruction of phylogenetic trees from microsatellite (STR) loci. *25th International Conference on Animal Genetics*, **27**, 1–2.

Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics*, **147**, 1943–1957.

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

Petren K, Grant BR, Grant PR (1999a) A phylogeny of Darwin's finches based on microsatellite DNA length variation. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **266**, 321–329.

Petren K, Grant BR, Grant PR (1999b) Low extrapair paternity in the cactus finch (*Geospiza scandens*). *Auk*, **116**, 252–256.

Piertney SB, MacColl ADC, Bacon PJ *et al.* (2000) Matrilineal genetic structure and female-mediated gene flow in red grouse (*Lagopus lagopus scoticus*): an analysis using mitochondrial DNA. *Evolution*, **54**, 279–289.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Primmer CR, Moller AP, Ellegren H (1996) A wide-range survey of cross-species microsatellite amplification in birds. *Molecular Ecology*, **5**, 365–378.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Rao KB, Sil SB, Majumder PP (1997) How useful are microsatellite loci in recovering short-term evolutionary history? *Journal of Genetics*, **76**, 181–188.

Raymond M, Rousset F (1995a) An exact test for population differentiation. *Evolution*, **49**, 1280–1283.

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

Rice WR (1989) Analysing tables of statistical tests. *Evolution*, **43**, 223–225.

Sato A, O'hUigin C, Figueroa F *et al.* (1999) Phylogeny of Darwin's finches as revealed by mtDNA sequences. *Proceedings of the National Academy of Sciences, USA*, **96**, 5101–5106.

Sato A, Tichy H, O'hUigin C *et al.* (2001) On the origin of Darwin's finches. *Molecular Biology and Evolution*, **18**, 299–311.

Schluter D (2000) *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.

Schluter D, Grant PR (1984) Ecological correlates of morphological evolution in a Darwin's finch *Geospiza difficilis*. *Evolution*, **38**, 856–869.

Scribner KT, Petersen MR, Fields RL *et al.* (2001) Sex-biased gene flow in spectacled eiders (Anatidae): inferences from molecular markers with contrasting modes of inheritance. *Evolution*, **55**, 2105–2115.

Shaw KL (2002) Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. *Proceedings of the National Academy of Sciences, USA*, **99**, 16122–16127.

Shields GF, Wilson AC (1987) Calibration of mitochondrial DNA evolution in geese. *Journal of Molecular Evolution*, **24**, 212–217.

Smith TB, Wayne RK, Girman DJ, Bruford MW (1997) A role for ecotones in generating rainforest biodiversity. *Science*, **276**, 1855–1857.

Sokal RR, Sneath PH (1963) *Principles of Numerical Taxonomy*. Freeman, San Francisco.

Stern DL, Grant PR (1996) A phylogenetic reanalysis of allozyme variation among populations of *Galápagos* finches. *Zoological Journal of the Linnean Society*, **118**, 119–134.

Swofford DL (1998) PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods). v. 4*. Sinauer Associates, Sunderland, Massachusetts.

Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, **144**, 389–399.

Templeton AR (2004) Statistical phylogeography: methods of evaluating and minimizing inference errors. *Molecular Ecology*, **13**, 789–809.

Tonnis B, Grant PR, Grant BR, Petren K (2005) Habitat selection and ecological speciation in Galápagos warbler finches (*Certhidea olivacea* and *C. fusca*). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **272**, 819–826.

Wade MJ, Goodnight CJ (1998) The theories of Fisher and Wright in the context of metapopulations: when nature does many small experiments. *Evolution*, **52**, 1537–1553.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Whitlock MC, Ingvarsson PK, Hatfield T (2000) Local drift load and the heterosis of interconnected populations. *Heredity*, **84**, 452–457.

Wiggins IL, Porter DM (1971) *The Flora of the Galápagos*. Stanford University Press, Stanford, California.

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, **163**, 1177–1191.

Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.

Yang SY, Patton JL (1981) Genic variability and differentiation of Galápagos finches. *Auk*, **98**, 230–242.

Young FW, Hamer RM (1994) *Theory and Applications of Multidimensional Scaling*. Eribaum Associates, Hillsdale, New Jersey.

The authors have collaborated since 1997 to bring a molecular genetic perspective to the study of Darwin's finches. They have published a series of articles on this natural system that address fundamental questions of parentage, inbreeding, hybridization, dispersal, habitat choice, speciation and phylogeny. Peter and Rosemary Grant have been studying Darwin's finches in the field for more than 30 years.