PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2010) **365**, 1065–1076
doi:10.1098/rstb.2009.0283

Conspecific versus heterospecific gene exchange between populations of Darwin's finches

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This study addresses the extent and consequences of gene exchange between populations of Darwin's finches. Four species of ground finches (*Geospiza*) inhabit the small island of Daphne Major in the centre of the Galápagos archipelago. We undertook a study of microsatellite DNA variation at 16 loci in order to quantify gene flow within species owing to immigration and between species owing to hybridization. A combination of pedigrees of observed breeders and assignments of individuals to populations by the program STRUCTURE enabled us to determine the frequency of gene exchange and the island of origin of immigrants in some cases. The relatively large populations of *Geospiza fortis* and *G. scandens* receive conspecific immigrants at a rate of less than one per generation. They exchange genes more frequently by rare but repeated hybridization. Effects of heterospecific gene flow from hybridization are not counteracted by lower fitness of the offspring. As a result, the standing genetic variation of the two main resident populations on Daphne Major is enhanced to a greater extent by introgressive hybridization than through breeding with conspecific immigrants. Immigrant *G. fuliginosa* also breeds with *G. fortis*. Conspecific immigration was highest in the fourth species, *G. magnirostris*. This species is much larger than the other three and perhaps for this reason it has not bred with any of them. The source island of most immigrants is probably the neighbouring island of Santa Cruz. Evolutionary change may be inhibited in *G. magnirostris* by continuing gene flow, but enhanced in *G. fortis* and *G. scandens* by introgressive hybridization.

Keywords: immigration; introgression; microsatellites; pedigrees; selection

1. INTRODUCTION

A well-known result of population genetics theory is that one breeding immigrant per generation (N_m) is sufficient to counteract the loss of genetic variation owing to drift (Crow & Kimura 1970; Miles & Allendorf 1996). Recent interest has focused on the question of how immigration affects the genetic structure of populations and, in particular, the outcome of selection and local adaptation in the face of immigration, referred to as gene flow (Slatkin 1985, 1987*a*). In the absence of selection, the effect of migration on the frequency of an allele at a locus is proportional to the difference in frequency between donor (d) and recipient (r) populations. The effect of selection on the frequency of an allele (a) at the locus is proportional to its relative fitness, that is the difference in fitness (W) between the donated allele and other alleles at that locus. Thus, the total effect of immigration (Hedrick 2000) is a change in allele frequency (Δ_p) as a function of the migration rate (m), the difference in allele frequency ($p_d - p_r$), and the relative fitness of the introduced allele ($\bar{W}_a/\bar{W}$)

$$\Delta_p = m(p_d - p_r)\left(\frac{\bar{W}_a}{\bar{W}}\right).$$

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One contribution of 13 to a Theme Issue 'Darwin's Galápagos finches in modern evolutionary biology'.

Immigrants (Hendry 2004; Bolnick & Nosil 2007; Harper & Pfennig 2008) or their descendants (Verhulst & van Eck 1996; Marr 2006) might be at a selective disadvantage, but if common enough they could counteract the effects of selection and local adaptation (García-Ramos & Kirkpatrick 1997; Kirkpatrick & Barton 1997; Hedrick 2000; Lenormand 2002; Hendry & Taylor 2004; Moore *et al.* 2007). Alternatively, they could introduce advantageous genes and thereby enhance local adaptation (Ebert *et al.* 2002; Saccheri & Brakefield 2002). A steady state may be reached at a point of balance between tendencies to diverge as a result of selection and drift (fission) and converge (fusion) as a result of immigration (Slatkin 1985, 1987*b*; Hendry *et al.* 2002; Cheviron & Brumfield 2009; reviewed in Bolnick *et al.* 2008).

This framework for understanding the dynamics of populations open to immigration should be extended to include interspecific hybridization and introgression. There is a rapidly increasing body of evidence that introgressive hybridization is widespread in a variety of animal taxa (Mallet 2005; Arnold 2008; Schwenk *et al.* 2008) and plant taxa (Arnold 1997, 2006; Rieseberg 1997), not to mention prokaryotes and their tendencies to exchange genes by horizontal transfer. Introgression is particularly prevalent in the early stages of adaptive radiations (Grant & Grant 2008*a*), as exemplified by cichlid fish of the African Great Lakes (Seehausen 2004, 2006) and the

silversword alliance of plants (Compositae) in Hawaii (Barrier *et al.* 1999). Introgressive hybridization can have positive or negative effects upon the recipient population. It can inhibit divergence, but, on the other hand, it has the potential to increase standing genetic variation and to introduce new, selectively advantageous, alleles to a greater extent than is possible with conspecific gene flow. As such it may be especially important in evolution by creating selectively advantageous combinations of genes (Lewontin & Birch 1966; Svärdson 1970; Grant & Grant 1989, 2008*b*).

Attempts have rarely been made to quantify the effects of both introgression and immigration in the same study. Three components could affect the outcome in contrasting ways. First, conspecific gene flow might occur more often than heterospecific gene flow, even when conspecific and heterospecific encounter rates are equal, because responses to conspecific mating signals are likely to be stronger than responses to heterospecific ones: $m_c > m_h$, where subscripts c and h refer to conspecific and heterospecific, respectively. Second, on the other hand, the difference in allele frequencies between donors and recipients should be greater when the populations are heterospecific than when they are conspecific: $(p_{cd} - p_{cr}) < (p_{hd} - p_{hr})$. Third, genetic effects of heterospecific alleles may be beneficial with weakly differentiated species, yet disadvantageous with more strongly differentiated species. At equilibrium:

$$m_c(p_{cd} - p_{cr})\left(\frac{\bar{W}_{ca}}{\bar{W}}\right) = m_h(p_{hd} - p_{hr})\left(\frac{\bar{W}_{ha}}{\bar{W}}\right).$$

Departures from equilibrium, and the relative contribution of the three components on each side of the equation, have rarely been investigated. A few studies have found greater conspecific than heterospecific effects on allele frequencies (Alexandrino *et al.* 2006; Lorenzen *et al.* 2006; Harper *et al.* 2007), but they have relied on indirect estimates of gene flow from genetic data, and these are well known to be imprecise (Wilson & Rannala 2003; Faubert *et al.* 2007; Peery *et al.* 2008). In the study reported here, we use more direct methods involving genetic assignments of individuals to populations in order to estimate the two forms of gene flow. Estimating gene flow by direct observations of breeders is difficult but can be done with small populations of closely related species living in archipelagos (Keller *et al.* 2001*b*) or archipelago-like situations, such as clusters of ponds (Ebert *et al.* 2002) or fragmented patches of terrestrial habitat (Hanski *et al.* 1994).

This paper describes a study of Darwin's finch populations on Daphne Major Island designed in part to elucidate the role of introgression in young adaptive radiations. There are several advantages of studying this system. First, the island is only moderately isolated, by 8 km from the nearest large islands of Santa Cruz, Baltra and Seymour (Grant 1999), and immigration is known to occur (Grant *et al.* 2001). Second, finches are observable, parentage can be determined by genotyping and fitness can be quantified in small populations because the survival and reproductive fates of offspring can be documented. Finally, introgression between sympatric species occurs in the entirely natural environment of Daphne Major (Grant 1993) and neither is restricted to a hybrid zone as in many other taxa (Barton & Hewitt 1985; Harrison 1993; Barton 2001), nor is it restricted to sister species. This paper provides a new assessment of introgression on Daphne from genetic analysis of an expanded dataset, in conjunction with the first direct estimate of conspecific gene flow that results from immigration and breeding in the same species that hybridize. We provide additional information on relative fitness and genetic differences at microsatellite loci to assess the relative magnitude of conspecific and heterospecific gene exchange.

2. FINCHES AND HYBRIDIZATION

Daphne Major is a small island, approximately 34 ha in area, near the centre of the Galápagos archipelago. Four species of ground finches breed on the island. *Geospiza fortis* (approx. 17 g), the medium ground finch, is a granivorous bird with a short and blunt beak; *G. scandens* (approx. 21 g), the cactus finch, which feeds on *Opuntia* cactus seeds, pollen and nectar in the dry season, has a long pointed beak; *G. magnirostris* (approx. 30 g), the large ground finch, feeds on large and hard seeds; and *G. fuliginosa* (approx. 12 g), the small ground finch, feeds on small seeds. Depending on environmental conditions, the population of *G. fortis* ranges from well over 1500 to less than 100 individuals, whereas the *G. scandens* population ranges from approximately 600 to less than 60 individuals. *Geospiza magnirostris* established a breeding population on Daphne in 1982–1983 and its numbers gradually increased to a maximum of approximately 350 in 2003, then fell during a severe drought (Grant & Grant 2006) and increased afterwards. *Geospiza fuliginosa* is a frequent immigrant that occasionally breeds on the island in numbers of less than 10.

Geospiza fortis occasionally breeds with *G. scandens* and *G. fuliginosa* (Grant 1993). The latter two species have not been observed to breed with each other, and none of the species has bred with *G. magnirostris* on Daphne. *Geospiza fortis* hybridizes at a low frequency in each year of full breeding (1–3% of pairs are mixed), more frequently with *G. fuliginosa* than with *G. scandens*. Interbreeding results in introgression because hybrids are viable and fertile (Grant & Grant 1992*a*; Grant *et al.* 2004). The direction of introgression is determined by the mating pattern of the F_1 offspring: offspring choose mates on the basis of paternal song, with very few exceptions (Grant & Grant 2008*a*). The relative fitness of hybrids depends upon the availability of an appropriate food (seed) supply (Grant & Grant 1993). Seed composition varies according to environmental conditions that fluctuate from droughts to extremely wet conditions associated with periodic El Niño events (Gibbs & Grant 1987; Grant & Grant 2002).

A pair of fourth-generation offspring of an immigrant *G. fortis* bred on Daphne in 2005, and their

offspring bred with each other (Grant & Grant 2008*c*, 2009). This endogamous group has not been included in the analyses reported here because they did not breed with residents.

3. MATERIAL AND METHODS

Beginning in 1973, we captured finches in mist nets, measured them and gave them a unique combination of coloured leg bands and a numbered metal band before releasing them. Six body size and beak traits were measured, described in Boag & Grant (1984) and illustrated in Grant & Grant (2008*a*). From 1988 onwards, we took a small drop of blood from the brachial vein, transferred it to EDTA-soaked filter paper and stored it in Drierite for later analysis of allelic variation at 16 microsatellite loci (Petren *et al.* 1999). This method bypassed the need for a buffer without sacrifice of DNA quality. For pedigree analysis when genotypes were not available, we identified social parents at nests. An attempt was made to find most nests in 1976 and 1992–1997, and all nests on the island in the years 1978–1991 and 1998. Nestlings ($n = 7496$) were banded at day 8; 802 of them were hybrids. From 1990 onwards, a drop of blood was taken from them at this time. Many were captured in nets as adults and measured. Genotyping was performed by Petren and colleagues (Grant *et al.* 2004), and by Ecogenics GmbH, Switzerland, and results were standardized (Grant & Grant 2008*c*). For an assessment of parentage, we allowed 2bp differences at two loci to be within the range of scoring variation and declared a mismatch when more differences or a single difference of at least 4bp were found (Keller *et al.* 2001*a*). Almost all offspring matched both parents at all loci. Extra-pair paternity was found in approximately 10 per cent of the 1794 offspring checked. The biological father was identified in 60–80% of the cases, depending on the year, where the social father was excluded as the biological father. To examine how well the hybrids and conspecific immigrants survived in comparison with the pure species hatched at the same time and living under the same conditions, we followed the survival of banded nestlings of the seven largest cohorts produced in the years of average or abundant rainfall (1978, 1981, 1983, 1984, 1987, 1991 and 1998).

Birds that lacked leg bands when captured in nets, and therefore not known to have hatched on the island, could be immigrants or residents. Prior to 1988, we used beak measurements to determine their identity from reference samples of non-overlapping distributions of measurements of the species at Borrero Bay, Santa Cruz Island (Grant 1993). From 1988 onwards, we used genotypic information from blood samples to identify hybrids and backcrosses, and island of origin of suspected immigrants, with v. 2.2 (Pritchard *et al.* 2007) of the program STRUCTURE (Pritchard *et al.* 2000; Falush *et al.* 2003). An attempt to use the alternative program *NewHybrids* (Anderson & Thompson 2002; Anderson 2008) was abandoned because it failed to identify known F_1 hybrids from the pedigrees.

STRUCTURE employs a Bayesian analysis to assign individuals to specified groups with a probability estimated from frequencies of microsatellite alleles. We applied the majority rule ($p > 0.500$) to assign individuals to groups. The immigration problem involves each species, whereas hybridization involves *G. fuliginosa*, *G. fortis* and *G. scandens* but not *G. magnirostris*. Following the authors' recommendations, we used a burn-in of 50 000 iterations and a run length of 100 000. For each new analysis, we repeated the procedure once to make sure results were consistent. We used the Popinfo option to select a no-admixture model and chose the correlated allele option. For the immigration problem, the birds to be identified that lacked bands were given a value of zero in the Popflag column, and all individuals from the candidate source islands were given a value of unity. This allowed a repeated updating of allele frequencies of all groups except the target Daphne group. The number of previous generations was set at zero.

For the hybridization problem, an ancestry model with prior generations is appropriate. We set the number of previous generations at two. In this analysis, a given individual may be genetically identified with an estimated probability of belonging to another species (generation 0), having a parent (generation 1) or having a grandparent (generation 2) from another species. These last two are equivalent to F_1 and first-generation backcross (B_1) classes in most circumstances. Analyses with two prior generations performed better than those with either one or three prior generations. Those with one prior generation yielded fewer identified hybrids, and those with three yielded no more hybrids than did the two prior generation analyses, and typically at lower probabilities. Admixture and non-admixture models generally give similar results (Pritchard *et al.* 2007). We found the same, but when tested against pedigree information admixture models performed somewhat less well than no-admixture models; results were sometimes unrealistic and are not reported here.

We split the birds into an early (before 1998) and a late groups (1999–2008) for two reasons: (i) pedigree information was available up to 1998 but not afterwards and (ii) allele frequencies of the species changed as a result of introgressive hybridization. Hybrids in the early part of the study were detected in relation to contemporary allele frequencies of the species better than in relation to the total sample for the species.

To provide a methodological check on the ability of STRUCTURE to assign hybrids and backcrosses correctly, we constructed 25 artificial interspecific pairs from contemporaneous *G. fortis* and *G. scandens* individuals with assignment probabilities greater than 0.99. The male was a *G. fortis* individual in 13 pairs and a *G. scandens* in 12 pairs. To generate artificial offspring ($n = 50$), two per pair, we randomly drew alleles from the parents. The F_1 hybrids were then backcrossed to each parental species, 15 families per species, to generate the B_1 generation ($n = 60$) by the same procedure. F_1s and B_1s were assigned to one species or the other based on the father because paternal song determines mate choice (Grant & Grant 1997*a*,*b*). We ran the no-admixture model with the second-generation back option. Forty-eight

of the 50 F_1 assignments (0.96) were significantly different from the parental species, but only 24 (0.48) were correctly assigned to the F_1 category. For the backcrosses, 46/60 B_1 assignments (0.77) were significantly different from the parental species, but only 41 (0.68) were correctly assigned to the B_1 category. The overall success rate is higher for identifying hybrids (0.85) than for the particular class of hybrids (0.68). Therefore, in reporting the results, we attach greater confidence to the identification of hybrids than to the particular class of hybrids. Oliveira *et al.* (2008) had a higher rate of success in a similar simulation with fewer microsatellite loci (12) but a larger sample of pairs of parents (40) and offspring (100).

Species are identified by song and morphology (Grant 1993, 1999). Gene exchange between populations is defined as the breeding of a member of one population with a member of the other. The F_1 offspring in the pedigree were assigned to species according to the song sung by their father (Grant & Grant 2008*a*); paternal song indicates the direction of gene flow through backcrossing.

4. INTRASPECIFIC GENE FLOW

(a) G. fortis

Sixty-seven genotyped birds lacking bands when captured in the years 1981–1998 were possible immigrants. They were identified as *G. fortis* by their measurements. STRUCTURE was run in order to assign them to the following defined populations: Santa Cruz ($n = 39$ genotyped individuals), Santiago ($n = 9$), Rábida ($n = 3$), Marchena ($n = 17$), San Cristóbal ($n = 4$), Pinta ($n = 12$), Isabela ($n = 11$) and Daphne ($n = 969$). The sample of 67 birds to be assigned was not defined. The defined Daphne population comprised all contemporaneous *G. fortis* known to have hatched on the island during the same period, and no hybrids. The small samples from most islands were not well characterized genetically, so the program was rerun with only the sample from the neighbouring Santa Cruz Island as a potential source. All birds lacking bands when captured on Daphne were assigned to the Daphne population except for four. These were assigned to Santa Cruz with high probabilities ($p > 0.900$), and two of them bred with resident *G. fortis*. Given an average generation length of 4.5 years for *G. fortis* on Daphne (Grant & Grant 1992*b*), two conspecific immigrants (N_c) in 18 years represent 0.50 per generation. They produced 15 and 10 fledglings (corrected for extra-pair young) and contributed five and one recruit to the next generation, respectively. One additional male that bred may have been an immigrant. It had unusually large measurements but was not genotyped. If it is included the number of breeding immigrants becomes three or 0.75 per generation.

(b) G. scandens

Sixty-four birds identified by measurements as *G. scandens* and lacking bands when captured in the years 1976–1998 were possible immigrants. STRUCTURE was run in order to assign them to the following defined populations: Santa Cruz ($n = 23$), Santiago ($n = 4$), Rábida ($n = 12$), Marchena and Pinta combined ($n = 6$), San Cristóbal ($n = 6$) and Daphne ($n = 403$). The defined Daphne population comprised all contemporaneous *G. scandens* known to have hatched on the island. All 64 candidate immigrants were assigned to the Daphne population except for three: one male was assigned to Santa Cruz ($p = 0.734$) and the other two were not assigned to any one population by the majority rule. Results remained unchanged when islands with the lowest assignment probabilities to any island were serially deleted until only Santa Cruz remained. The mean generation length of *G. scandens* is 5.5 years (Grant & Grant 1992*b*). One immigrant (N_c) in 24 years represents 0.23 per generation. It bred with a resident *G. scandens* female (F_1 hybrid) in 1997 and produced at least three fledglings (all confirmed within-pair young), one of which bred successfully the following year.

(c) G. fuliginosa

At least two, and a maximum of 16, pairs of *G. fuliginosa* bred on the island, but only in the years 1976–1984. Most individuals lacked leg bands when captured, and therefore their identity and origin (Daphne resident or immigrant) could not be confirmed. Immigration is strongly suspected. Since identification poses a special problem, they are considered together with hybrids in §5*a*(iii). Assuming the two breeding pairs were immigrants, and assuming a generation length of 4.5 years like *G. fortis*, the number of immigrants is 0.78 when calculated over 23 years (1976–1998) or 2.0 over the period 1976–1984.

(d) G. magnirostris

The following populations were included in an analysis of 117 birds captured on Daphne without bands in the years 1988–1998 and treated as an undefined population: Santa Cruz ($n = 12$), Santiago ($n = 10$), Genovesa ($n = 32$), Rábida ($n = 5$), Marchena ($n = 10$), Pinta ($n = 7$), Fernandina ($n = 9$) and Isabela ($n = 6$). We serially deleted potential source populations with the lowest set of probability values without changing the results. Three islands were identified as sources of birds on Daphne: Santa Cruz (85), Santiago (7) and Pinta (3). None of the remaining 22 birds were assigned to an island by the majority rule. Twenty-two of the 117 bred on Daphne. Eighteen of them were identified by their assignments as coming from Santa Cruz, one was from Santiago and three were unassigned. These results differ from a previous analysis (Grant *et al.* 2001) in which Rábida and Marchena were identified as the most frequent sources of immigrants. The previous analysis lacked samples from Santiago and Pinta, however.

The analysis was repeated with 159 birds captured after 1998, the same non-Daphne populations as before, and a user-defined Daphne population comprising 11 residents that bred on Daphne in 1998 and their offspring ($n = 100$). Assignments of these were as follows: 117 to Daphne, 20 to Santa Cruz, three to Santiago and three to Pinta. Sixteen were unassigned.

Up to 1998, 22/117 = 0.118 bred. If the unknown generation length of *G. magnirostris* is the same as that of *G. fortis*, namely 4.5 years, the number of immigrants is 9.0 per generation. In the 10 years after 1998, a minimal estimate of the proportion of immigrants is 26/159 = 0.163 in 10 years. At least two immigrants bred, but the total number of breeders is not known. If all of them bred, which is highly unlikely, the number of immigrants is 7.3 per generation. If only two bred, the number of immigrants is 0.9 per generation. In both cases, it is lower than the earlier rate.

5. INTERSPECIFIC GENE FLOW

(a) G. fortis: *interbreeding with* G. fuliginosa *immigrants*

(i) *Identification of* G. fuliginosa *and hybrids*

Geospiza fortis and *G. fuliginosa* hybridize on Daphne (Grant & Price 1981; Boag & Grant 1984). Estimating the frequency is difficult because some *G. fuliginosa* individuals are morphologically indistinguishable from hybrids produced by *G. fuliginosa* × *G. fortis* pairs. In an earlier study (Grant 1993), we used measurements of *G. fuliginosa* from Borrero Bay on the north shore of the neighbouring island of Santa Cruz to define limits to the species. This is possible to do because at that locality there is a gap of 1.0 mm between the largest beak depth of *G. fuliginosa* (small ground finch: $n = 109$) and the smallest *G. fortis* (medium ground finch: $n = 137$), and a gap of 0.6 mm in beak width between the species. There are no such gaps within the frequency distributions of either species.

A total of 302 finches lacking leg bands when captured in mist nets on Daphne were classified as *G. fuliginosa* by their beak measurements. For the small sample genotyped ($n = 39$), we ran STRUCTURE twice, separately for the early and for the late samples. Two populations were specified: *G. fuliginosa* (Santa Cruz only) and *G. fortis* (*G. fortis* and *G. fuliginosa* on Daphne combined). The second-generation back option was chosen to allow identification of hybrid categories.

Nineteen of the 29 birds we originally identified as *G. fuliginosa* in the early sample on Daphne were assigned to *G. fuliginosa* (Santa Cruz) by applying the majority rule. Of the remaining 10, two were assigned to *G. fortis*, one to the F_1 category, three to the first-generation backcross category, and four were not assigned to any category by the rule. Of the late sample of seven *G. fuliginosa*, three were assigned to *G. fuliginosa* (all $p = 1$), two were assigned to backcrosses ($p = 0.620$, 0.821), and two were assigned to *G. fortis* ($p = 0.971$, 0.988). Altogether 22/36 were confirmed genetically as *G. fuliginosa*. These results were not altered when the sample of *G. fuliginosa* from Santiago, the next closest island to Daphne, was substituted for the Santa Cruz sample in the analysis.

For the remaining birds on Daphne without genotypic or pedigree information we used morphological measurements to identify *G. fuliginosa* as follows. The smallest Daphne hybrid has beak depth and width measurements of 6.9 mm. It was assigned to the first-generation backcross class. All birds with smaller beak dimensions are considered to be unambiguously *G. fuliginosa*. The largest *G. fuliginosa* at Borrero Bay had a beak depth of 7.8 mm, and the largest beak width in the sample was 7.4 mm. Identities of birds on Daphne with measurements in the intervening range between these two limits of 6.9/6.9 and 7.8/7.4 ($n = 116$) are ambiguous. Thirteen are known hybrids (10 F_1s, and three backcrosses: two B_1 and one B_2). The proportion of Borrero Bay *G. fuliginosa* in this ambiguous range of measurements is 36/109 = 0.33. The proportion of Daphne birds smaller than this maximum size and not known to be hybrids ($n = 285$) that are in this range is almost the same, 0.36. On these grounds, almost all birds classified as *G. fuliginosa* on Daphne are indeed likely to be *G. fuliginosa*.

(ii) *Source of immigrants*

We ran STRUCTURE to assign *G. fuliginosa* on Daphne (undefined) to the following user-defined populations: Santa Cruz ($n = 24$), Santiago ($n = 19$), Rábida ($n = 10$), Española ($n = 10$), Floreana ($n = 10$), San Cristóbal ($n = 21$), Pinta ($n = 10$) and Isabela ($n = 13$). None of the Daphne birds was assigned to a population with a probability exceeding 0.500. All were assigned to Santa Cruz and Santiago with about equal probabilities (0.337–0.478), and also when only Santa Cruz and Santiago populations were included as possible source populations. Therefore, Santa Cruz and Santiago *G. fuliginosa* are not different enough genetically at the 16 loci to make possible the identification of the source island of birds that immigrated to Daphne. On geographical grounds, Santa Cruz is the more likely, but both may have contributed immigrants to Daphne. Morphologically, the populations on Santa Cruz and Santiago are almost identical (Lack 1947; Grant *et al.* 1985).

(iii) *Breeders*

Most immigrant *G. fuliginosa* died without breeding or emigrated. To estimate the numbers that bred on Daphne, we applied criteria for inclusion at three levels of strictness. The strictest method requires genotype and/or unambiguous measurements. By this method, the total is 10: three males and seven females. Two of them were genotyped, six were measured and two more, although not measured, were the parents of an offspring with very small beak measurements (6.4 and 6.3 mm). When birds with measurements in the ambiguous zone are included the total rises to 15: five males and 10 females. When birds identified by observation alone are included, the total rises yet further. In the years 1976–1997, 44 breeding birds were identified by observation as *G. fuliginosa*. As noted above, two were the parents of a phenotypically confirmed *G. fuliginosa* and are already included in the estimates. The number remaining, 42, should be reduced to about half (21) to allow for errors in classification revealed by genotyping (above). In addition, three genotyped *G. fuliginosa* were suspected of breeding in 2002. When all these are included (the least strict criteria) roughly 40 are identified as breeding immigrant *G. fuliginosa*.

(iv) *Number of immigrants*

Four *G. fuliginosa* bred intraspecifically. More were observed but not confirmed because they were not captured. A maximum of 14 pairs of *G. fuliginosa* lacking leg bands bred in the years 1976–1984. The remainder of the known immigrants and at least two of the *G. fuliginosa* hatched on the island bred with *G. fortis*, as did all of the F_1 offspring that survived to breed, i.e. they backcrossed to *G. fortis*. Depending on the criteria adopted for inclusion, the number of immigrants that bred with *G. fortis* is six, 11 or 18. In terms of *G. fortis* generation lengths (4.5 years on average: Grant & Grant 1992*b*) N_h, the number of migrants (heterospecific) per generation over 36 years is 0.75, 1.375 or 2.25. We believe the middle estimate is the most realistic.

The estimate of the magnitude of migration is an average over eight generations. Immigration was not uniform across time, however, but was at an apparent maximum at the beginning of the study, declined markedly after 1986 according to observations and mist-net captures (Grant & Grant 1995) and remained low thereafter. For the last 6 years of the study, there was only one confirmed *G. fuliginosa* breeding (with *G. fortis*) on the island. The decline in immigration is reflected in the change in proportion of *G. fortis* identified as hybrids with *G. fuliginosa* by assignment tests (figure 1), from 14.0 per cent in 1981–1998 to 4.7 per cent in 1999–2008.

(b) G. fortis*: interbreeding with* G. scandens *residents*

(i) *Detection by genotypes*

After excluding hybrids between *G. fortis* and *G. fuliginosa* ($F_1 + B_1$), we ran STRUCTURE with two prior generations to assign individuals in two defined populations, *G. fortis* and *G. scandens*, separately in early and late samples. Hybrids were found to be more common in the *G. scandens* than in the *G. fortis* samples, and more common in the late than in the early samples. By the majority rule, 40 (2.81%) of the early *G. fortis* sample ($n = 1423$) and nine (2.58%) of the late *G. fortis* sample ($n = 349$) were assigned to hybrids. Twenty-six (5.16%) of the early *G. scandens* sample ($n = 504$) and 17 (9.29%) of the late *G. scandens* sample ($n = 183$) were assigned to hybrids. The pattern is consistent with asymmetric gene exchange between the species, as reported before (Grant *et al.* 2004; Grant & Grant 2006), and a recent intensification of the asymmetry. The increase in gene flow is reflected in the change in the distribution of assignment probabilities from early to late samples (figure 2). Twenty-seven of the 92 hybrids in total (29.3%) were identified as F_1s: note the percentage is subject to error (see §3).

(ii) *Detection by pedigrees*

In the 21 years from 1978 to 1998, there were 13 observed cases of interbreeding. Some of the offspring were observed to breed with either *G. fortis* or *G. scandens*. Others were assigned to species according to the song sung by their social father. Combining observed and potential breeding of the F_1 offspring,

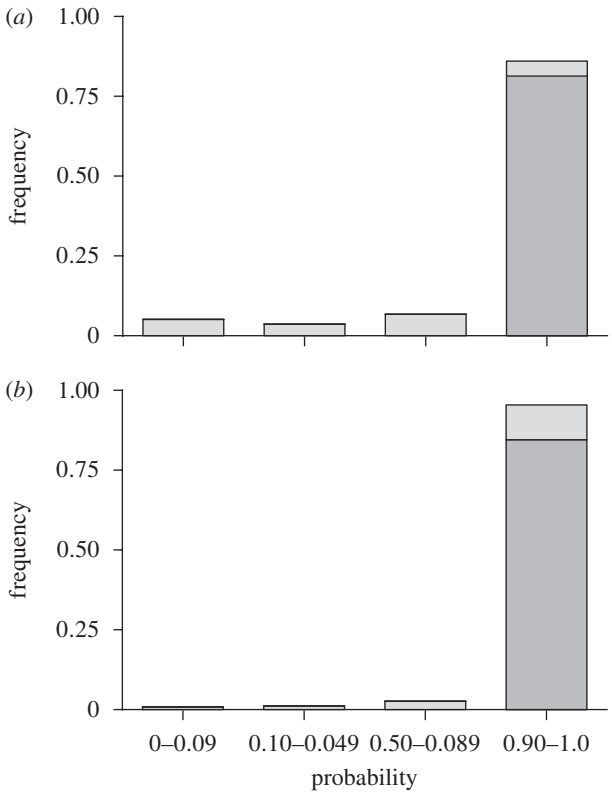

Figure 1. Probabilities of assignment of *G. fortis* individuals in (*a*) early (1976–98; $n = 1382$) and (*b*) late (1998–2008; $n = 344$) samples. Probabilities of less than 0.900 indicate hybrids and backcrosses with mixtures of *G. fortis* and *G. fuliginosa* genes. Probabilities of greater than 0.99 are indicated in grey. *Geospiza fortis* × *G. scandens* hybrids and backcrosses are not included.

we identified three as members of the *G. fortis* population and six as members of the *G. scandens* population. Two more did not breed and their fathers were not known. One was morphologically more like *G. fortis* than *G. scandens*, and therefore considered part of the *G. fortis* population, while the other more closely resembled *G. scandens* and was added to that population. Thus, the *G. fortis* population received genes from four *G. scandens* individuals in 21 years, or 0.86 per generation. *Geospiza scandens* received genes from at least seven *G. fortis* individuals, or 2.19 individuals per generation. Offspring without genotypes or measurements of the remaining two pairs have not been included. If they were included, *G. scandens* received genes from nine *G. fortis* individuals, or 2.36 individuals per generation.

6. GENE EXCHANGE THROUGH HYBRIDIZATION

Immigrant *G. fuliginosa* that bred with *G. fortis* brought to the island 21 alleles at the 16 microsatellite loci not detected in the *G. fortis* population at the time of their arrival. Three of the 21 alleles (14.7%) appeared in later samples of *G. fortis* or hybrids, presumably as a result of introgression. This indicates a slow addition of new alleles. Most introduced alleles gave rise to minor alterations in the frequencies of pre-existing alleles. Similarly, the interbreeding populations of *G. fortis* and *G. scandens* gained alleles from each

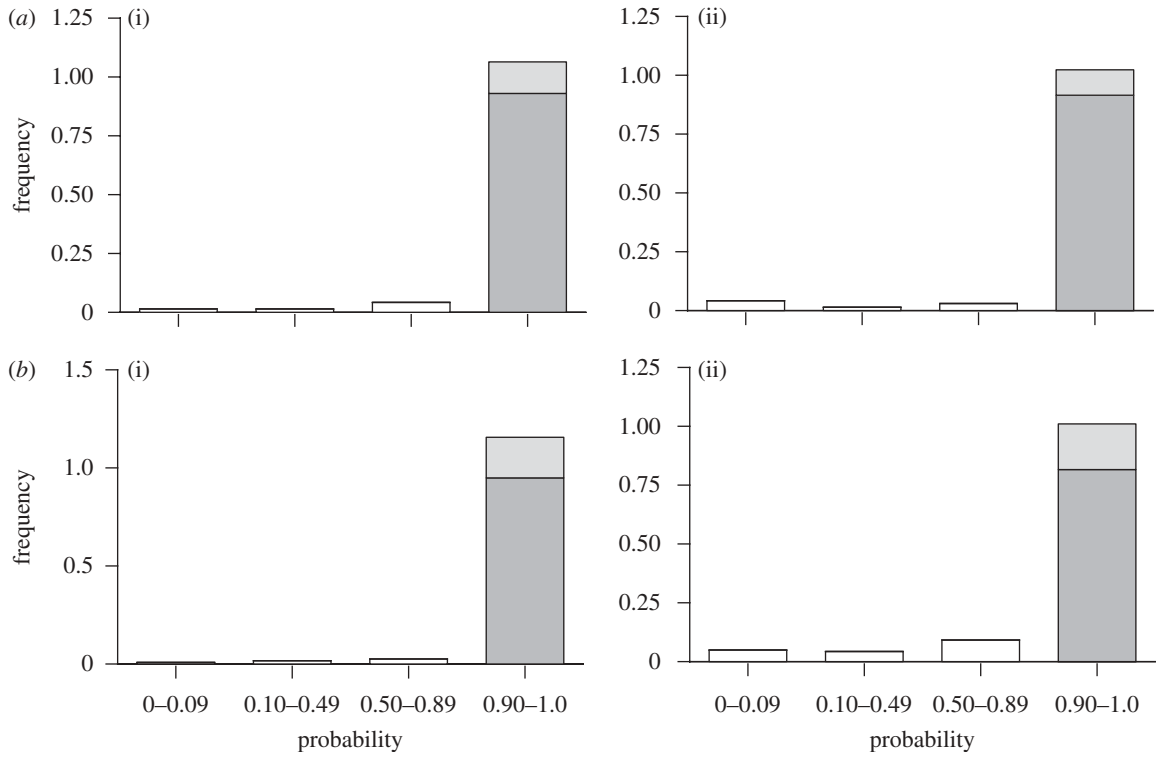

Figure 2. Probabilities of assignment of individuals to (i) *G. fortis* ($n = 1642$) or (ii) *G. scandens* ($n = 689$) in (*a*) early (1976–1998) and (*b*) late (1998–2008) samples. Probabilities of less than 0.900 reflect mixtures of *G. fortis* and *G. scandens* genes. Probabilities of greater than 0.99 are indicated in grey. *Geospiza fortis* × *G. fuliginosa* hybrids and backcrosses are not included.

other. Although most remained at low frequencies as with introgression of *fuliginosa* alleles into the *G. fortis* population, two increased substantially in the *G. scandens* population (Grant *et al*. 2004).

7. GENETIC EFFECTS OF GENE FLOW

The flow of genes into the two main study populations on Daphne from conspecific and heterospecific sources is rare. Although the numbers of migrants per generation are low and are point estimates without confidence limits, they permit the conclusion that interbreeding is less frequent with conspecific immigrants ($N_c = 0.50,\ 0.23$) than with heterospecific immigrants (*G. fortis* × *G. fuliginosa*; $N_h = 1.37$) or residents (*G. fortis* × *G. scandens*; $N_h = 0.86,\ 2.19$). These contrasts are summarized in figure 3.

The frequency of migrants is not a reliable index to the genetic effects of interbreeding when the source of the migrants, as here, is heterogeneous. For a given number of migrants per generation, the genetic effect of interbreeding is proportional to the mean of the absolute differences in allele frequencies between donor (Daphne or Santa Cruz) and recipient populations. A useful quantitative index for comparative purposes is the product of the number of migrants and the mean difference in allele frequencies (Nei's *d*) between the interbreeding populations, either conspecific or heterospecific. Summing intraspecific ($N_c d_c$) and interspecific ($N_h d_h$) gene inputs gives a value for this index of 1.6807 for *G. fortis* and 2.0456 for *G. scandens*. The interspecific contribution to total genetic input is 77.3 per cent for *G. fortis* and 95.9 per cent for *G. scandens*. Heterospecific sources do not contribute equally. The genetic effect on *G. fortis* of breeding with *G. scandens* (59.2%) is greater than the effect of breeding with *G. fuliginosa* (40.8%). All these refer to calculations based on data up to 1998. The contribution made by *G. fuliginosa* must have declined after this time because immigration declined.

8. RELATIVE FITNESS

The greater genetic effect of heterospecific gene flow on Daphne populations is not counterbalanced by low relative fitness. On the contrary, F_1 hybrid and conspecific resident individuals survived at least as well as the parental species on average, if not better (figure 4), over the same environmental conditions. Approximately half of the individuals in the seven major cohorts died in their first year. Mean survival over the first year was almost the same among *G. fortis* × *G. fuliginosa* F_1s (0.52 ± 0.059 s.e.; $n = 83$) and *G. fortis* (0.51 ± 0.048; $n = 3860$) and was higher among the *G. fortis* × *G. scandens* F_1s (0.679 ± 0.161; $n = 10$) than among *G. scandens* (0.404 ± 0.054; $n = 1771$) and *G. fortis*.

Hybrids do not experience a loss of fitness in acquiring mates (Grant & Grant 1997*a*,*b*) or in reproductive success (Grant & Grant 1992*a*). The same applies to conspecific immigrants that bred, although the numbers are too few for analysis. Therefore, overall, $(\bar{W}_{ca}/\bar{W}) \approx (\bar{W}_{ha}/\bar{W}) \approx 1$. As a consequence, backcrossing of F_1 hybrids and successive generations of offspring has given rise to a complex network of genetic relationships among the species (figure 5).

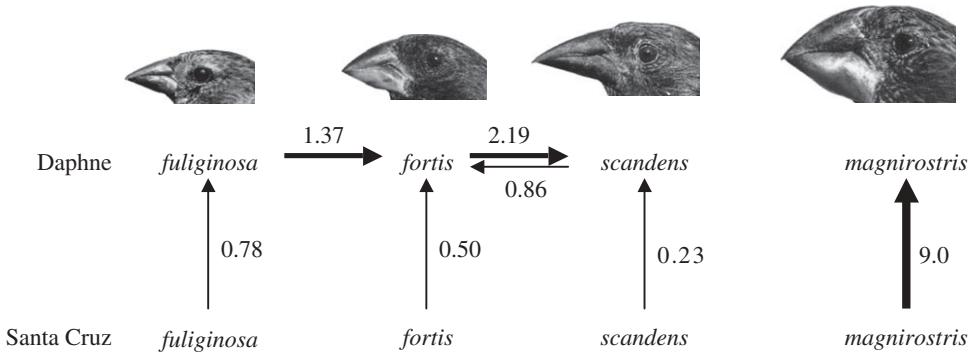

Figure 3. Summary of gene flow through immigration and introgression on Daphne Major Island. Numbers refer to immigrants or hybridizing individuals per generation (see §9 for migration rates). Genes flow from *G. fortis* to *G. scandens* when the father of an F_1 hybrid sings the *G. scandens* song, and vice versa for gene flow from *G. scandens* to *G. fortis*. The species hybridize on Santa Cruz Island to an unknown extent. *Geospiza magnirostris* hybridizes with *G. fortis* on Santa Cruz but not on Daphne.

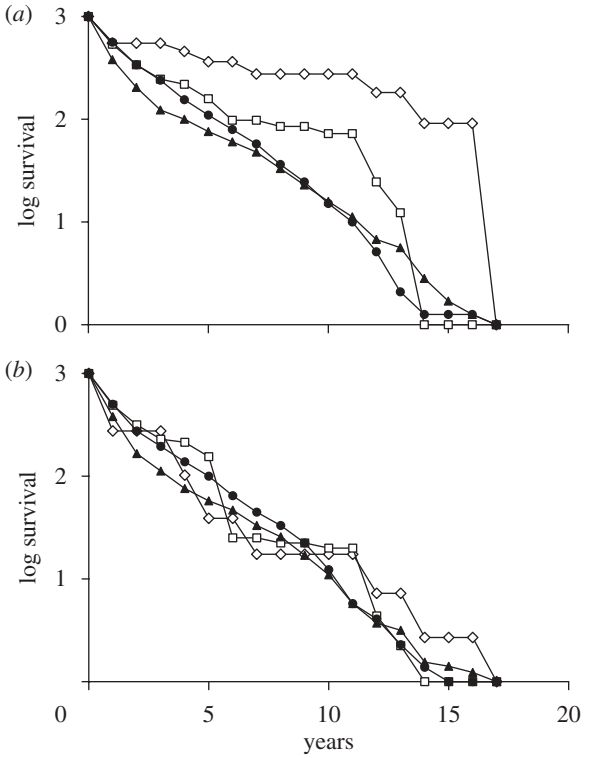

Figure 4. Composite survival curves of seven cohorts of finches, hatched in the years 1978, 1981, 1983, 1984, 1987, 1991 and 1998. (*a*) Numbers were summed across years, standardized to 1000 at fledging and converted to logs. (*b*) Numbers in each cohort were first standardized to 1000 at fledging and converted to logs, then summed across years and averaged. For variation among three of the cohorts see Grant & Grant (2008*a*,*b*). Filled circle, *G. fortis*; solid triangles, *G. scandens*; open squares, *G. fortis* × *G. fuliginosa*; open diamond, *G. fortis* × *G. scandens*.

9. SELECTION AND MIGRATION

Selection, to counteract the effect of migration, must exceed the migration rate (*m*), i.e. the proportion of the breeding population that are migrants. Over the relevant time periods, the average sizes of the breeding populations were approximately 80 *G. scandens* and 230 *G. fortis* individuals. Using numbers in figure 3, we calculate *m* from the combined heterospecific and conspecific sources to be 0.030 for *G. scandens* and 0.018 for *G. fortis*. Natural selection has been far stronger at times, on both species (Grant & Grant 2002, 2006), with selection coefficients as high as 0.5–1 standard deviations. Therefore, migration has been insufficient to counteract local adaptation. In contrast, natural selection has been scarcely detectable in *G. magnirostris* (Grant *et al.* 2001), whose average size of the breeding population was 25 individuals in 1988–1998. Migration rate, estimated at 0.360 and one order of magnitude greater than in *G. scandens* and *G. fortis*, has been more than sufficient to counteract local adaptation.

10. CONCLUSIONS

The two main study populations of Darwin's finches on Daphne Major Island receive genes by breeding with allopatric conspecific individuals that have immigrated, and from heterospecific individuals, both allopatric immigrants (*G. fuliginosa*) and sympatric residents (*G. fortis* and *G. scandens*). The flow of genes from conspecific and heterospecific sources is rare and unequal. Genes flow at a faster rate from heterospecific sources than from conspecific sources and have stronger effects because species differ genetically more than do populations of the same species. The effects of heterospecific gene flow are not counteracted by lower fitness of the offspring. As a result, the standing genetic variation of the two main resident populations on Daphne Major is enhanced to a greater extent by introgressive hybridization than through interbreeding with rare immigrants from another island. The situation may be exceptional because most species do not hybridize, but where hybridization does occur, as in the coexistence of closely related species, it can have a greater effect than conspecific gene flow upon gene dynamics. Approximately 10 per cent of all bird species are known to hybridize (Grant & Grant 1992*a*), which is not unusual among animal taxa but is low compared with plants (Mallet 2005).

The study illustrates the dynamic nature of intraspecific and interspecific interactions in three ways. First, immigration (arrival) of *G. fuliginosa* declined after the mid-1980s. The unknown cause probably lies in the source islands (Grant & Grant 1995). Introgressive hybridization with *G. fortis* therefore declined,

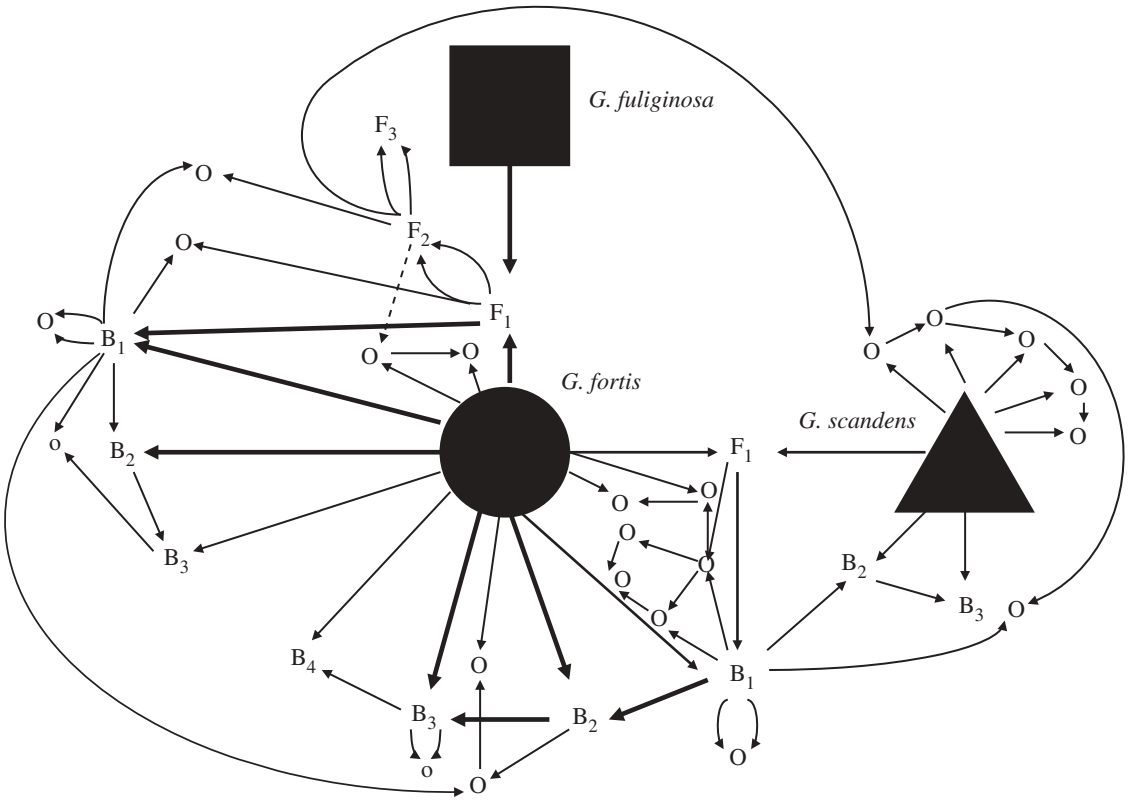

Figure 5. Gene flow network reflecting known and quantified introgressive hybridization. All three species are connected by exchanging genes, but the relatively rare *G. fuliginosa* has not hybridized with *G. scandens*, and *G. fortis* × *G. fuliginosa* hybrids have not backcrossed to *G. fuliginosa*. Thick lines indicate the primary pathways of genes from one population to another.

yet *G. fuliginosa* alleles persisted in the *G. fortis* population as a result of repeated backcrossing. The backcrossing was complex, resulting in a few cases in a combination of genes from three species in single individuals (figure 5). Second, introgressive hybridization between the resident species *G. fortis* and *G. scandens* increased at the same time as it decreased between *G. fuliginosa* and *G. fortis*. Here, the cause has been identified as an enduring transformation in the food supply resulting from a major, archipelago-wide, El Niño event in 1982–1983 (Grant & Grant 1996). Thus, the situation on Daphne is not equilibrial and is currently leading towards the fusion of *G. fortis* and *G. scandens* into a single panmictic population. Selection on *G. scandens* has not overridden effects of introgression on beak shape; instead selection may have augmented introgression (Grant *et al.* 2004). Nevertheless, the direction of change may be reversed if the climatic and floristic environment changes (Grant & Grant 2008*b*). Third, the El Niño event facilitated the establishment of a breeding population of *G. magnirostris* on Daphne in 1982–1983. The rate of immigration after the initial colonization was far higher than immigration of the other species, but showed signs of a density-related decline as population size increased. Immigration of this species is sufficiently frequent that it could overwhelm evolutionary change through natural selection on Daphne, but alternatively it might facilitate evolutionary change, as postulated for introgressive hybridization, by providing new genetic variation from as many as three source islands.

We conclude that conspecific gene flow as a result of immigration is insufficient to negate the strong effects of both hybridization and local selection on Daphne (Grant *et al.* 2004) and that conspecific and heterospecific gene flow in combination are sufficient to counteract random genetic drift. The chief implication of these findings is that gene exchange between populations is complex, heterogeneous and varies in time measured in decades. This dynamic perspective provides insight into population genetic structure, which is often used to infer average rates of gene flow at assumed steady state (Petren *et al.* 2005).

Additional tests of the relative importance of migration rate, genetic difference and relative fitness in hybridizing species could be conducted in hybrid zones in continental regions. Several avian hybrid zones are known to be moving northwards in the Northern Hemisphere (Cook 1975; Gill 1980, 2004; Rowher *et al.* 2001; Reudink *et al.* 2007), possibly influenced by climate warming (Cook 1975; Berthold *et al.* 1992). Their movement implies changing local dynamics of conspecific and heterospecific gene exchange. The local dynamics of the two sources of gene exchange are likely to vary wherever there are gradients in hybridization, as occurs in a variety of organisms, for example, cichlid fish (Seehausen & Magalhaes in press), *Daphnia* (Petrusek *et al.* 2008), *Heliconius* butterflies (Kronforst *et al.* 2006) and *Triturus* salamanders (Arntzen *et al.* 2009).

We thank the Galápagos National Parks Service and Charles Darwin Research Station for permission to carry out the fieldwork and for logistical support. We thank Arkhat Abzhanov for the invitation to contribute to this theme issue and many assistants over the years for help in the field. We thank Lukas Keller for help with assignment

tests. We are especially grateful to Ken Petren for initial genotyping, analysis and supplying us with blood samples from Santiago and Isabela. Two reviewers provided important clarification of some issues. The research has been supported by grants from the National Science and Engineering Research Council (Canada) and the National Science Foundation (USA).

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