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CONVERGENT EVOLUTION OF DARWIN'S FINCHES CAUSED BY INTROGRESSIVE HYBRIDIZATION AND SELECTION

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Abstract.—Between 1973 and 2003 mean morphological features of the cactus finch, *Geospiza scandens*, and the medium ground finch, *G. fortis*, populations on the Galápagos island of Daphne Major were subject to fluctuating directional selection. An increase in bluntness or robustness in the beak of *G. scandens* after 1990 can only partly be explained by selection. We use 16 microsatellite loci to test predictions of the previously proposed hypothesis that introgressive hybridization contributed to the trend, resulting in genes flowing predominantly from *G. fortis* to *G. scandens*. To identify F_1 hybrids and backcrosses we use pedigrees where known, supplemented by the results of assignment tests based on 14 autosomal loci when parents were not known. We analyze changes in morphology and allelic composition in the two populations over a period of 15–20 years. With samples that included F_1 hybrids and backcrosses, the *G. scandens* population became more similar to the *G. fortis* population both genetically and morphologically. Gene flow between species was estimated to be three times greater from *G. fortis* to *G. scandens* than in the opposite direction, resulting in a 20% reduction in the genetic difference between the species. Nevertheless, removing identified F_1 hybrids and backcrosses from the total sample and reanalyzing the traits did not eliminate the convergence. The two species also converged in beak shape by 22.2% and in body size by 45.5%. A combination of introgressive hybridization and selection jointly provide the best explanation of convergence in morphology and genetic constitution under the changed ecological conditions following a major El Niño event in 1983. The study illustrates how species without postmating barriers to gene exchange can alternate between convergence and divergence when environmental conditions oscillate.

Key words.—Assignment tests, Darwin's finches, introgression, microsatellites, selection.

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Microevolutionary dynamics of populations are governed by the gain of alleles from mutation, immigration, and introgressive hybridization and the loss of alleles through selection (natural and sexual), drift, and emigration. These processes vary in both situation- and taxon-specific ways. It is a considerable challenge to explain changes in the genetic composition of populations and variation among them in terms of all six processes (Hartl and Jones 1998; Hedrick 2000). One solution is to exploit the convenience of particular systems that allow direct measures of one or more of the processes and to use inferences based on indirect measures for the rest. In this article we present the results of a long-term study of two populations that provide measures of selection and observations of hybridization. The species are the cactus finch, *Geospiza scandens*, and the medium ground finch, *G. fortis*, on the Galápagos island of Daphne Major.

Geospiza scandens, a cactus-feeding specialist with an average weight of 21 g, is larger than the generalist granivore *G. fortis* (~17 g) and has a more pointed beak. From 1973 to 2001 mean morphological features of both species were subject to fluctuating directional selection (Grant and Grant 2002). One morphological trend could only partly be explained by selection. This was an increase in bluntness or robustness in the beak of *G. scandens* after 1990. *Geospiza scandens* became more *fortis*-like in this trait, as well as in overall body size. We hypothesized that the trend was caused partly by introgressive hybridization, predominantly from *G. fortis* to *G. scandens*. Rare hybridization between the species, occurring partly as a result of misimprinting on song (Grant and Grant 1998), was documented by observation from 1976 onward (Grant et al. 2003). Backcrossing according to song type was first recorded in 1984, when F_1 hybrids produced in 1983 backcrossed to the *G. fortis* population. After 1987 no additional backcrossing of F_1 hybrids to *G. fortis* was observed, but backcrossing to the *G. scandens* population, first observed in 1991, continued in subsequent years (Grant and Grant 1997a,b, 1998).

There has been no direct study of gene transfer between these species to confirm hybridization and to estimate its effects. The only relevant genetic data have been quantitative genetic parameters of morphological variation estimated from parent-offspring regressions. The quantitative genetic study demonstrated a high degree of heritable variation in *G. fortis* and *G. scandens* and, consistent with the hybridization hypothesis, morphological intermediacy of individuals identified from pedigrees as F_1 hybrids and backcrosses (Grant and Grant 1994, 2000). Microsatellite DNA markers are useful for detecting and measuring gene exchange between populations of the same or different species (McDonald and Potts 1997; Rannala and Mountain 1997; Hansson et al. 2003, Hutchinson et al. 2003). In this paper we use microsatellite markers to test the hypothesis of introgressive hybridization and to quantify some of its effects.

MATERIALS AND METHODS

Field Data Collection

Daphne Major is a small island (0.34 km^2) centrally placed in the main part of the Galápagos archipelago. Beginning in

1973 (Abbott et al. 1977), in most years finches have been captured in mist nets, uniquely banded, measured, and released. Measurements of mass, wing length, tarsus length, beak length, beak depth, and beak width were made. Details, including repeatabilities of the measurements, have been given in Boag and Grant (1984) and Grant and Grant (1994). Annual survival has been determined by observation in the first three months of each year. Breeding of finches was studied intensively in the years 1976–1991, when average breeding population sizes were approximately 50 pairs of *G. scandens* and 100 pairs of *G. fortis* (Grant and Grant 1992a). It was again studied intensively in 1998. Uniquely in this year the banding of nestlings was largely restricted to families with at least one banded parent. Breeding was studied less intensively in the intervening years 1992–1997. Pairs were identified, their offspring were banded at day 8, and many were recaptured when fully grown after day 60. In 1977, 1982, 1985, 1988, 1989, 1994, 1996, 1999, and 2003 there was little or no rain or breeding. From 1988 onward a blood sample was taken by venipuncture (Petren 1998) from all nestlings and birds captured in nets for DNA extraction and characterization.

Identification of Species, Hybrids, and Immigrants

A combination of morphology and song features was used to assign individuals to species (Grant 1993; Grant and Grant 1998), that is, to the breeding population of *G. fortis* or to the breeding population of *G. scandens*. F_1 hybrids and backcrosses were identified in the first instance from pedigrees when the apparent parents were known from observations of adults that were incubating eggs (females only) or feeding nestlings and fledglings (both social parents). All birds, that is F_1 hybrids and backcrosses as well as nonhybrids, mate according to paternal song type (Grant and Grant 1997a,b, 1998); therefore, F_1 hybrids and backcrosses, including those that failed to breed, could be assigned to one population or to the other.

Fourteen unlinked autosomal and two sex-linked loci have previously been used in studies of parentage and heritability of morphological traits (Keller et al. 2001) as well as inbreeding in these populations (Keller et al. 2002; Grant et al. 2003; Markert et al. 2004). There are no known instances of egg dumping, but extrapair paternity has been estimated at 8% for *G. scandens* (Petren et al. 1999a) and 20% for *G. fortis* (Keller et al. 2001). Therefore we used microsatellite markers to check the parentage of F_1 hybrids and backcrosses identified from the observed pedigrees. A single pair of backcrosses was found to be misidentified through incorrect paternity and deleted from the records. When parents were not known, microsatellite data were used in assignment tests (Pritchard et al. 2000) that employ Bayesian analysis to assess the probability of each individual being correctly assigned to *G. fortis* or to *G. scandens*. The criterion for recognizing an F_1 hybrids or backcross individual from genetic data was a probability of correct assignment to one species or the other of less than 90%. Because this criterion is arbitrary, we determine if results change qualitatively when alternative criteria of 85% or 95% are used instead. A model-based method to identify F_1 hybrids and backcrosses (Anderson and Thompson 2002) gave very similar results to the assignment tests, and we report only the results of the latter here.

Immigrants cannot be reliably identified morphologically or by song, although some have been suspected on the basis of subtle plumage features (Boag and Grant 1984) and size (Grant and Grant 1996). We applied assignment tests to genetic data from birds captured for the first time as adults in mist nets and not banded as nestlings, hence of unknown origin (see also Grant et al. 2001). For *G. fortis* the tests did not give stable and interpretable results owing to strong genetic similarities among island samples (Petren et al. 1999b), and therefore the results are not reported. Tests with *G. scandens* of unknown origin were successful, probably because populations of this species are genetically more differentiated than *G. fortis* populations (Petren et al. 1999b, unpubl. ms.). We used the following groups for assigning *G. scandens* individuals to source populations, with number of birds in each group given in parentheses: Daphne (44), Santa Cruz (37), Santiago (4), Rábida (12), Floreana (5), San Cristóbal (23), and Pinta and Marchena (6), which were combined due to their proximity and small sample sizes.

Interbreeding of *G. fortis* with rare immigrant *G. fuliginosa* occurred in the first half of the study (Grant 1993). The few F_1 hybrids and backcrosses surviving to 1988 (the first year of blood sampling) and their descendants have been excluded.

Analysis

All F_1 hybrids and backcrosses were included in one species or the other. Gene frequencies for each species were computed and distances calculated with the program PHYLIP (Felsenstein 1993). Unbiased heterozygosities were also calculated with PHYLIP, assignment tests were performed with STRUCTURE (Pritchard 2000), and other statistical analyses were conducted in Statview 5.0. Individuals in their first year of life were excluded from analyses except for the few individuals that bred in their first year under the exceptional El Niño conditions in 1983 and 1987 (Grant et al. 2000). Genetic differences between samples of *G. fortis* and *G. scandens* or between samples of the same species in different years, were measured with Nei's *D* (Nei 1972), a distance based on the frequency of shared alleles. It is more appropriate than diversity-based distances such as F_{ST}, especially when there has been recent admixture between species (Hedrick 1999). Statistical analyses of temporal heterogeneity and linear trends with time were performed only with independent samples, that is, samples of birds hatched in different years. Principal components analysis was performed with the correlation matrix of untransformed data and used to obtain a single body size factor (PC1) from an analysis of mass, wing length, and tarsus length. In a separate analysis of beak length, depth, and width, beak size (PC1) and shape (PC2) factors were obtained (Grant and Grant 2002). Beak shape, a measure of pointedness, is not correlated with body size or beak size in either species. Beak size and body size are strongly correlated, with r^2 values of 0.55 for *G. scandens* and 0.53 for *G. fortis*, and therefore are only partly independent traits.

Even though blood sampling started in 1988, we could genotype birds hatched more than a decade earlier because they live for up to 16 years (Grant and Grant 1996). For the

direct determination of gene exchange, we defined introgression as the appearance of an allele in a population for the first time after an adequate period of sampling, and present in the other population at that time. We considered the cumulative samples of each species adequate to detect rare alleles when the sum of alleles across all the studied loci had reached 100. This level was reached in the *G. fortis* samples in 1982 and in the *G. scandens* samples in 1984. The method fails to detect introgression of an allele already present in the recipient population, and falsely includes previously overlooked very rare alleles in the recipient population. Identification of introgressed alleles by this method is therefore supplemented by data from pedigrees of observed instances of interbreeding.

The allelic diversity of each species was analyzed as a function of cumulative sample size (Fig. 1). Cumulative sample sizes increased faster in *G. fortis* than in *G. scandens*. For statistical testing we compared the mean number of alleles per locus in early and late samples of comparable size in each species.

Results

Immigrants

Seven of 33 *G. scandens* of unknown origin in the samples up to and including 1998, the last year in which nestlings were banded, could not be assigned to the Daphne population of *G. scandens* with probabilities greater than 0.1. These seven were therefore considered to be possible immigrants from other islands, although some may have been F_1 hybrids and backcrosses. Their sources were identified as Pinta or Marchena (4) and Floreana (3), but only one individual was assigned to an island (Pinta or Marchena) with a probability greater than 0.90. The seven constitute 2.4% of the total *G. scandens* sample (291) in this period, or approximately one per generation (Grant and Grant 1992a), all others having been known to hatch on the island. At this low frequency, immigrants could have counteracted the effects of drift but beyond that are unlikely to have altered the genetic composition of the *G. scandens* population to any marked degree. Three of them introduced one new allele each. Deleting these birds and their known descendants and reanalyzing the data does not alter any of the results below, therefore they have been included as Daphne residents because they all bred on Daphne with no more than average success.

Effects of Introgression on Genetic Diversity

Total allelic diversity is slightly greater in *G. fortis*, the larger population, than in *G. scandens* (Table 1). Even though *G. scandens* sample sizes were generally smaller, the population was proportionally better sampled than *G. fortis* during most of the study (Fig. 1). Accumulation of alleles with increasing cumulative sample sizes and time shows signs of saturation in both species but somewhat more pronounced in *G. fortis* than in *G. scandens* (Fig. 1).

The introgression hypothesis predicts an increase in allelic diversity in *G. scandens* independent of sample size effects. As expected from observations, the average number of alleles per locus in *G. scandens* is lower in the period up to 1993 than afterward, whereas there is no such temporal heterogeneity in *G. fortis*. Because data in successive years are not independent, a paired *t*-test of equal mean numbers of alleles at each locus was made with early and late samples of approximately equal sizes. The test for *G. fortis* was made with 1984 ($n = 69$ birds) and 1999 ($n = 75$) samples, and for *G. scandens* it was made with 1988 ($n = 79$) and 1999 ($n = 81$) samples. No individual was present in both early and late samples. Results were $t_{15} = 0.312$, $P = 0.7590$ for *G. fortis* and $t_{15} = 4.490$, $P = 0.0004$ for *G. scandens*. In agreement with expectation, *G. scandens* had on average two more al-

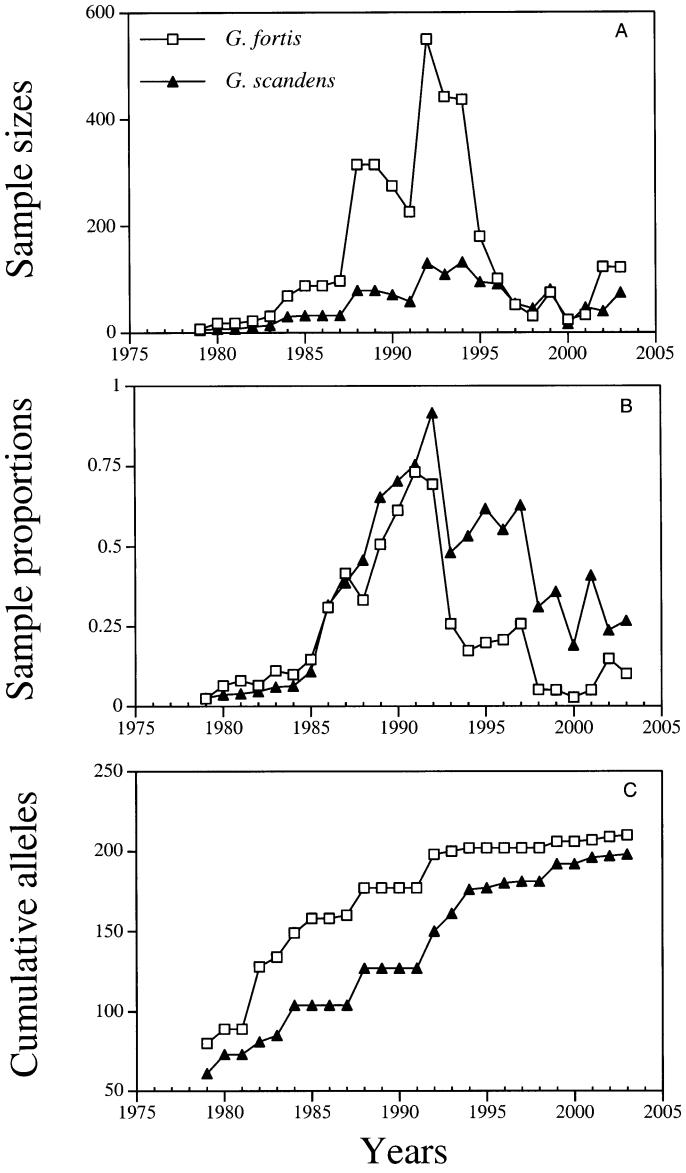

FIG. 1. Samples for genetic analysis. (A) Annual samples of genotyped *Geospiza fortis* and *G. scandens*. (B) Proportions of each population that were sampled. (C) Evidence for saturation of allelic composition, as shown by a decreasing rate of accumulation of number of different alleles with time: the squared term in polynomial regressions is significant for both *G. fortis* ($b = -0.3171 \pm 0.031$, $t = 10.168$, $P < 0.001$) and *G. scandens* ($b = -0.078 \pm 0.033$, $t = 2.351$, $P = 0.0281$).

TABLE 1. Diversity of alleles at 16 microsatellite loci (an asterisk indicates sex linked). Com, common alleles that are present at a frequency of 10% in at least one year; Priv, private alleles (Slatkin 1985), present only in that population; H_e, unbiased expected heterozygosity.

	Geospiza scandens					*Geospiza fortis*				
Locus	*n*	Total	Com	Priv	H_e	*n*	Total	Com	Priv	H_e
Gf-1	346	12	4	3	0.80	938	14	5	5	0.75
Gf-2*	340	12	5	0	0.82	951	17	7	5	0.71
Gf-3	257	8	7	1	0.76	710	10	4	3	0.75
Gf-4	322	4	3	0	0.49	757	7	2	3	0.50
Gf-5	329	9	4	4	0.43	832	8	3	3	0.64
Gf-6	274	7	4	2	0.56	567	6	3	1	0.72
Gf-7	315	19	5	8	0.80	855	13	4	2	0.81
Gf-8	342	17	7	1	0.85	952	22	5	4	0.87
Gf-9	328	11	2	2	0.82	918	13	5	4	0.31
Gf-10*	333	9	5	0	0.78	779	14	6	5	0.75
Gf-11	320	22	9	5	0.85	933	24	5	7	0.91
Gf-12	332	17	7	3	0.86	742	16	7	2	0.86
Gf-13	332	17	4	1	0.85	757	18	8	2	0.70
Gf-14	302	10	6	2	0.52	780	10	3	2	0.81
Gf-15	311	13	4	3	0.62	747	14	4	4	0.39
Gf-16	331	12	8	2	0.75	936	12	4	2	0.88
Total	349	199	83	37	0.71	960	220	75	54	0.71

leles per locus (10.1 ± 0.98 SE) in 1999 than in 1988 (8.0 ± 0.75 SE).

A second expected effect of introgression is an elevation of heterozygosity. Unbiased expected heterozygosity was much lower in *G. scandens* than in *G. fortis* at the beginning of the study, but in agreement with the hypothesis, it increased and by the end was almost the same in the two species (Fig. 2).

Direct Detection of Introgression

By the criterion described in Materials and Methods, 41 putatively introgressed alleles appeared unequally in the recipient species: 30 in *G. scandens* at 10 loci and 11 in *G. fortis* at six loci. Two new alleles appeared in the same individual in seven cases. Allowing for this the introgressed alleles are the product of 25 independent transfer events to *G. scandens* and nine transfer events to *G. fortis*. The ratio of 25:9 is not expected under a hypothesis of random sampling from an approximately equal number of alleles (Table 1) in each species (Binomial test, $Z = 2.57$, two-tailed $P = 0.0102$). It is expected, however, from the hypothesis of differential introgression into the *G. scandens* population.

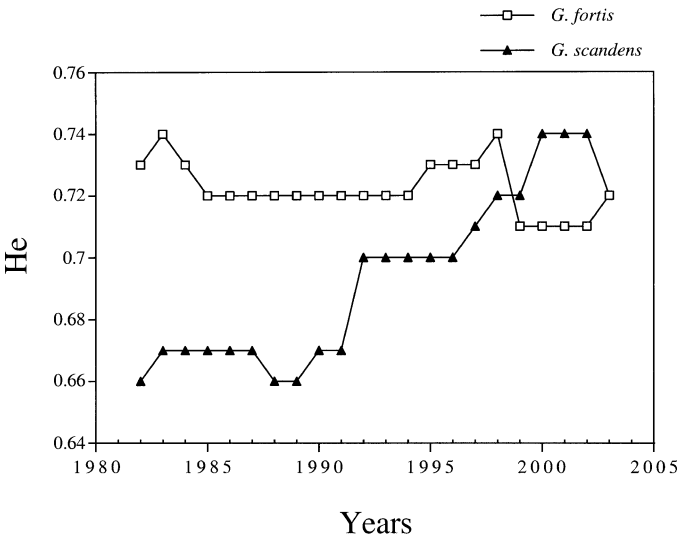

FIG. 2. Increase in expected heterozygosity (H_e) of the *Geospiza scandens* population contrasts with constancy of expected heterozygosity in *G. fortis*.

Some of the alleles may have been present in both species in the early years but not sampled until later years. For example, although observed hybridization was relatively frequent in 1987 it cannot fully account for the 24 new alleles in the two species combined that appeared in 1988, the most in any year. Some of the new alleles may have been previously present but undetected, although it is also possible that unobserved extrapair interspecific mating was responsible for them.

Several of the new alleles were present in individuals identified by observation as F_1 hybrids and backcrosses. Three of the 15 *G. fortis* recipients of *G. scandens* alleles and 15 of the 36 *G. scandens* recipients of *G. fortis* alleles were identified by observation as F_1 hybrids or backcrosses. Most of the alleles (32) never reached a frequency of more than 5%, and eight of these 32 were apparently lost from the *G. scandens* population. Of the remainder, one reached a maximum of 22% after 17 years and another reached the same frequency as in the *G. fortis* donor population (15%; Fig. 3).

Indirect Detection of Introgression

Assignment tests were used to classify individuals as *G. fortis* or *G. scandens*. The tests correctly classified 90.1% of individuals to the species identified by song and morphology, that is, with probability greater than 0.90. The remaining individuals were classified ambiguously and are treated as probable F_1 hybrids and backcrosses. As expected from the introgression hypothesis, members of the *G. scandens* population ($n = 373$) were more frequently classified ambiguously (16.7%) than were members of the *G. fortis* population

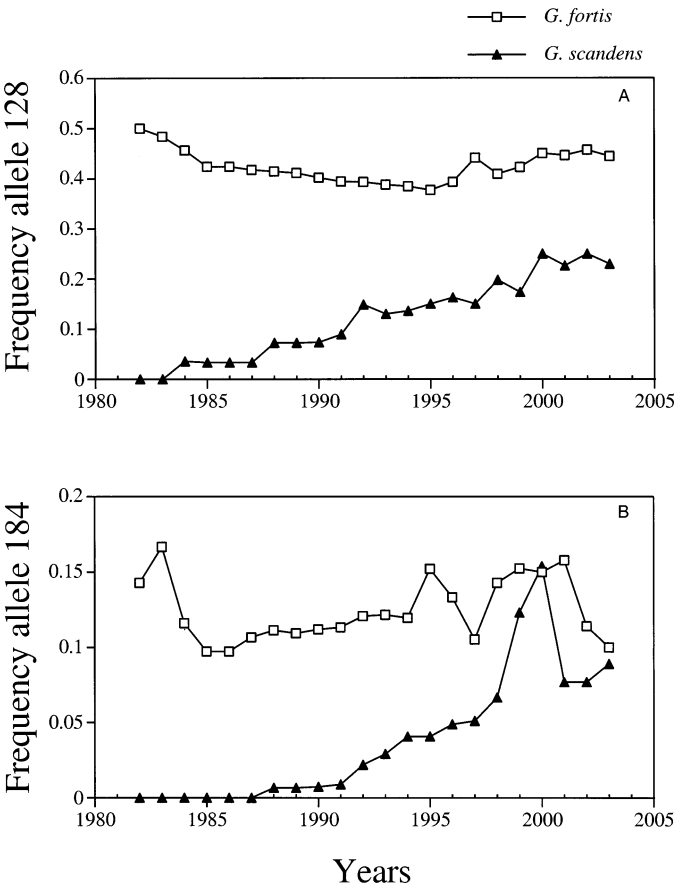

FIG. 3. Candidate introgressed alleles, from *Geospiza fortis* to *G. scandens*. The upper figure shows an allele (128 at locus Gf-16) that may have introgressed into the *G. scandens* population because it was present in *G. fortis* in 1982 and before but not present in *G. scandens* until 1984. Allele 184 at locus Gf-15 (lower figure) is a more likely candidate for introgression and is recognized as such in this study, because it did not appear in the *G. scandens* population until 1988. By 2000 it was as frequent as in the *G. fortis* donor population.

(7.7%, $n = 1023$). The two-fold difference is highly significant ($\chi^2_1 = 17.499$, $P < 0.0001$). Individuals identified from pedigrees as F_1 hybrids or backcrosses were often classified ambiguously and twice as frequently (31.9%) as the pure species ($\chi^2_1 = 62.941$, $P < 0.0001$). The frequency of ambiguously classified birds was three times higher in the *G. scandens* sample of 120 F_1 hybrids and backcrosses (42%) than in the *G. fortis* sample of 62 F_1 hybrids and backcrosses (13%). Nevertheless, some F_1 hybrids and backcrosses in generations 1–3 were indistinguishable genetically from either *G. fortis* (87%) or *G. scandens* (62%). Changing the probability criterion for recognizing F_1 hybrids and backcrosses from 90% to 85% or 95% changes the numbers but not the results of the statistical tests. Henceforth, we refer to ambiguously classified birds as ''suspected F_1 hybrids and backcrosses.''

The Temporal Pattern of Introgression

The frequencies of suspected F_1 hybrids and backcrosses identified by assignment tests were analyzed in six independent samples; birds hatched in 1976–1984, 1987, 1991, 1992–1997, 1998, and 2000–2003 (Fig. 4). There was significant temporal heterogeneity in the proportion of F_1 hybrids and backcrosses in the two species combined ($\chi^2_4 = 35.432$, $P < 0.0001$), as well as in the *G. fortis* ($\chi^2_4 = 16.756$, $P = 0.0022$) and *G. scandens* ($\chi^2_4 = 15.675$, $P = 0.0035$) populations analyzed separately.

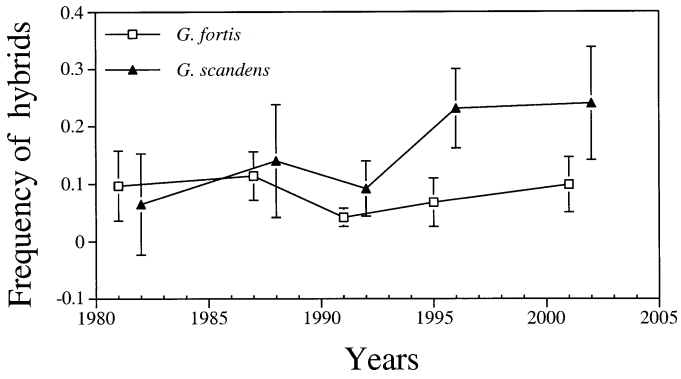

FIG. 4. Temporal heterogeneity in the proportions of known and suspected F_1 hybrids and backcrosses (hybrids) in six independent sample periods. Ninety-five percent confidence intervals are shown by vertical bars. Proportions for the two species in each time period are slightly offset for clarity.

According to the introgression hypothesis, based on observed hybridization F_1 hybrids and backcrosses should be proportionally most common in the *G. fortis* population early in the study and most common in the *G. scandens* population in the second half of the study. In agreement with expectations, F_1 hybrids and backcrosses were relatively most frequent in the *G. fortis* population in the 1980s ($\chi^2_1 = 4.234$, $P = 0.0376$) and in the *G. scandens* population in the last 10 years of the study (Fig. 4).

Intraspecific Genetic Divergence

The introgression hypothesis predicts a greater change in the *G. scandens* population than in the *G. fortis* population. This is observed (Fig. 5). By 2003 the samples differed in terms of Nei's *D* from the respective starting populations in 1982 by 12% for *G. scandens* and 5% for *G. fortis*. With data analyzed in six independent time units, the change in genotypes is linearly related to time in *G. scandens* ($b = 0.005 \pm 0.001$, $P = 0.0301$) but not in *G. fortis* ($b = 0.005 \pm 0.002$, $P = 0.0989$). The results are not dependent on a particular starting year, because the same patterns were evident when the starting year was changed to 1988 (the first year of blood sampling).

Interspecific Genetic Convergence

The introgression hypothesis makes two predictions about interspecific similarity. Genetically the two species should converge, and *G. scandens* should make a greater contribution to the convergence than *G. fortis*. Both predictions are supported. After 1982 the two species became more similar to each other (Fig. 6A), largely because of changes in *G. scandens* (Fig. 6B). The greater contribution of *G. scandens* to the convergence is shown by a comparison of the starting

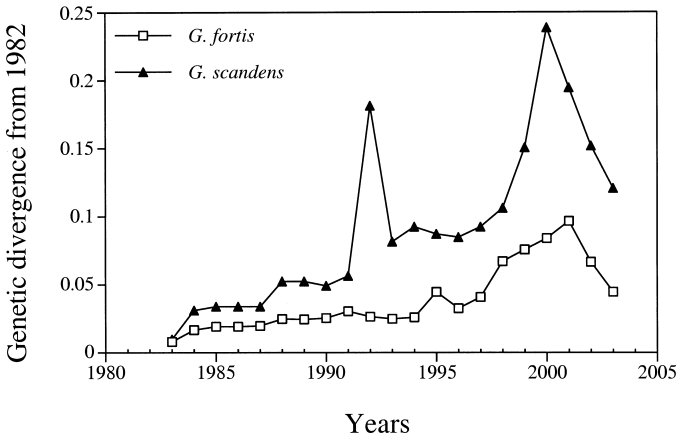

FIG. 5. The two *Geospiza* species became increasingly different in genetic composition from their respective populations in 1982, as measured by Nei's genetic distance (*D*) but changed at different rates. The sharp increase in 2000 may be an artifact of a partly biased sampling regime (see Materials and Methods) and small sample sizes (Fig. 1). There is no known bias causing the 1992 spike.

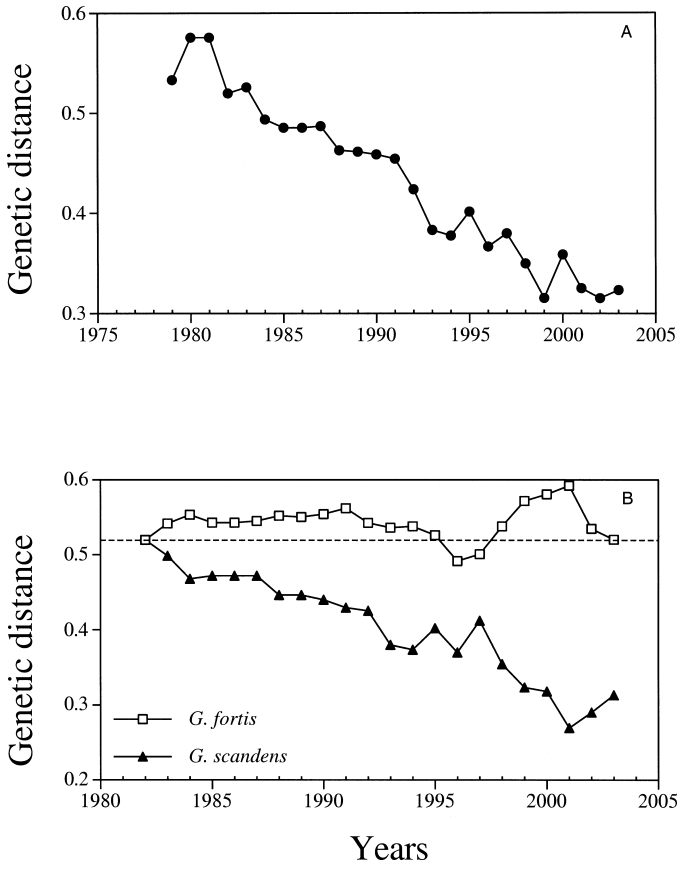

FIG. 6. Genetic convergence. (A) Genetic distances between *Geospiza fortis* and *G. scandens* calculated for each pair of annual samples of allele frequencies. (B) *Geospiza scandens* became increasingly similar in genetic composition to the initial (1982) *G. fortis* sample, whereas *G. fortis* retained its initial genetic distinctness and did not change.

genetic composition of each species with the genetic composition of the other species in succeeding time periods. The linear trend of *G. scandens* becoming increasingly similar to *G. fortis* is significant ($n = 6$, $b = -0.008 \pm 0.001$, $P = 0.0004$), whereas *G. fortis* neither converged nor diverged from the genetic composition of the initial *G. scandens* population ($b = 0.000 \pm 0.002$, $P = 0.9017$).

Deleting the known and suspected F_1 hybrids and backcrosses from the *G. scandens* samples has two effects upon these patterns of change in genotypes across time. First, it increases Nei's *D* between the species calculated for the combined samples from 0.35 to 0.44. In other words, inclusion of F_1 hybrids and backcrosses reduced the interspecific difference by 20.5%. Second, it reduces the slope of the relationship with time, although it does not eliminate it ($b = -0.005 \pm 0.001$, $P = 0.0151$, $n = 6$). In addition, deletion reduces expected heterozygosity values without altering the increase in *G. scandens* values over time (see Fig. 2).

Fractionally small samples of a population increase in similarity with increasing sample size, just like the similarity in species composition of communities (Plotkin and Muller-Landau 2002). Sample size effects may have contributed to genetic convergence of the species in the first few years when samples were very small, but cannot account for subsequent convergence when sample sizes were large proportions of total population sizes (Fig. 1), nor for the fact that only one, not both, species converged (Fig. 6B).

Interspecific Morphological Convergence

The two species differ in several morphological traits. *Geospiza scandens* is larger in most measures and has a longer but shallower beak (Table 2). The species converged morphologically between 1988, the first year of genotype sampling, and 2003, at the end (Fig. 7). None of the 1988 birds survived to 2003; therefore, the samples are independent. The samples for *G. fortis* are 314 in 1988 and 121 in 2003, and

TABLE 2. Morphological trait means and standard deviations (SD) of adult *Geospiza scandens* ($n = 252$) and *Geospiza fortis* ($n = 752$). Two-tailed probability values (*P*) are shown for *t*-tests of mean differences between species.

	Geospiza scandens		*Geospiza fortis*			
	Mean	SD	Mean	SD	*t*	*P*
Mass	19.14	2.286	15.95	1.945	21.476	<0.0001
Wing length	71.11	2.921	67.32	2.643	19.174	<0.0001
Tarsus length	20.39	0.810	19.02	0.816	26.364	<0.0001
Beak length	13.72	0.984	10.81	0.819	46.206	<0.0001
Beak depth	9.02	0.540	9.17	0.799	2.910	0.0037
Beak width	8.58	0.492	8.66	0.625	1.708	0.0879

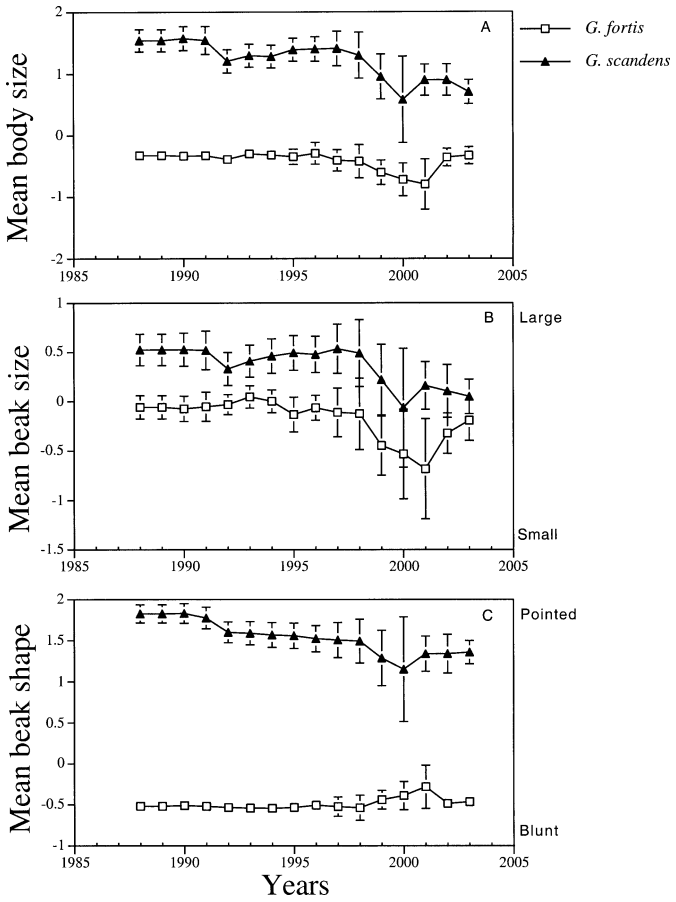

FIG. 7. Morphological convergence. *Geospiza scandens* became increasingly similar to *G. fortis* in three morphological traits, with different results: the two species became scarcely distinguishable in body size while retaining distinctiveness in beak shape. Body size is the first principal component (PC) in an analysis of mass, wing length, and tarsus length. Loadings were 0.909 for mass, 0.894 for wing, and 0.893 for tarsus. Total variance explained was 0.807. Beak size is the first component and beak shape is the second component of a PC analysis of beak length, depth, and width. Loadings of bill length, depth, and width were 0.577, 0.933, and 0.939 on PC1 and 0.817, −0.264, and −0.240 on PC2, respectively. For the beak analysis PC1 explained 0.695 of the variance and PC2 explained an additional 0.265. High positive mean values on the vertical axes signify large body and beak size and pointed beaks. Ninety-five percent confidence intervals are shown by vertical bars.

for *G. scandens* they are 83 and 75, respectively. The difference between the species decreased from 1988 to 2003 by 55.5% for body size, 24.5% for beak size, and 77.8% for beak shape. The convergence was almost entirely due to changes in *G. scandens*. The 15-year changes in this species were 1.02 standard deviations for body size ($F_{1,156}$ =39.163, $P < 0.0001$), 0.66 for beak size ($F_{1,156}$ = 16.169, $P < 0.0001$), and 0.92 for beak shape ($F_{1,156}$ = 27.478, $P < 0.0001$). The changes in *G. fortis* were trivial in comparison: 0.01 for body size ($F_{1,434}$ = 0.015, P = 0.9016), 0.13 for beak size ($F_{1,434}$ = 1.493, P = 0.2224), and 0.20 for beak shape ($F_{1,434}$ = 1.927, P = 0.1658).

Explanation of morphological convergence in terms of introgressive hybridization assumes that F_1 hybrid and backcross members of the *G. scandens* population differ morphologically from the nonhybrid (and nonbackcross) members in the direction of greater similarity to *G. fortis*. This assumption is supported by measurements of the 252 *G. scandens* with microsatellite genotypes. Known and suspected F_1 hybrids and backcrosses (n = 55) are smaller than non-hybrids (n = 197) in body size ($F_{1,248}$ = 18.930, $P < 0.0001$) and beak size ($F_{1,250}$ = 6.906, P = 0.0091) and have a less pointed beak shape ($F_{1,250}$ = 79.070, $P < 0.0001$). The largest mean differences are in body mass (9.0%) and beak length (9.2%).

Selection on Morphology

Natural selection contributed to the morphological trends in *G. scandens*. There were no deaths from 1988 to 1989 or from 2000 to 2001, therefore no selection occurred. During the 13 years following 1988, directional selection occurred on body size in four years, on beak depth in two years, and on beak shape in one year (Table 3). Large size was selectively favored in two years, 1991–1992 and 1998–1999 and disfavored in two others, 1992–1993 and 1994–1995.

Associating Morphology with Genotypes

Introgression should result in a transient, nonfunctional, association between neutral alleles and morphology-coding loci, although not necessarily in the form of physical linkage. We focus on the alleles in the *G. scandens* population (F_1 hybrids and backcrosses included) that changed in frequency by more than two standard deviations from the mean (by > 15%) to give us the best chance of detecting associations if present. From the beginning period (1984; n = 30 individuals) to the end (2003; n = 75 individuals) each of 11 alleles at nine loci changed by this amount. Individuals that possessed one of these allele differed significantly in at least one morphological trait from those that lacked the allele and were morphologically more like *G. fortis* in each case. The number of significant differences associated with the presence or ab-

TABLE 3. Selection on morphological traits of *Geospiza scandens*. Survivors and nonsurvivors were compared using one-way analyses of variance.

	Body size			Beak size			Beak shape		
Year	*n*	*F*	*P*	*n*	*F*	*P*	*n*	*F*	*P*
1990–1991							75	4.457	0.0382
1991–1992	102	8.261	0.0049	102	4.427	0.0379			
1992–1993	100	6.548	0.0120	100	8.295	0.0049			
1994–1995	101	4.644	0.0336						
1998–1999	43	5.681	0.0219						

TABLE 4. Morphological differences between *Geospiza scandens* with and without 11 alleles that contributed the most to the interspecific genetic convergence. Probabilities are shown without Bonferroni correction for multiple one-way analyses of variance (see text), but note that body size and beak size are not statistically independent (Materials and Methods).

			Body size		Beak size		Beak shape	
Locus	Allele	*n*	*F*	*P*	*F*	*P*	*F*	*P*
Gf-1	163	248	6.413	0.0119	0.447	0.5043	5.632	0.0184
Gf-3	188	195	15.846	<0.0001	8.602	0.0038	19.482	<0.0001
Gf-6	181	192	13.319	0.0003	6.305	0.0129	20.820	<0.0001
Gf-8	101	245	7.146	0.0080	15.197	0.0001	0.299	0.5851
Gf-8	123	245	3.196	0.0778	3.944	0.0482	0.398	0.5258
Gf-9	165	250	16.668	<0.0001	22.660	<0.0001	0.536	0.4647
Gf-10	228	240	13.815	0.0003	10.266	0.0015	2.835	0.0936
Gf-11	225	230	1.786	0.1828	4.975	0.0267	1.515	0.2196
Gf-13	167	241	12.308	0.0005	7.938	0.0052	5.625	0.0185
Gf-16	128	236	11.845	0.0007	10.276	0.0015	32.910	<0.0001
Gf-16	144	236	0.056	0.8131	0.823	0.3654	3.956	0.0479

sence of alleles is eight for body size, nine for beak size, and six for beak shape (Table 4). It is not surprising that body size and beak size analyses give similar results because the two traits are correlated (Materials and Methods). With sequential Bonferroni correction for multiple tests, the number of significant differences is eight, six, and three, respectively. Deleting known and suspected F_1 hybrids and backcrosses does not reduce the number of significant associations with body size (eight), but does reduce the number for beak size from nine to five and for beak shape from five to four.

Genetic Consequences of Selection on Morphology

Given the morphology-allele associations, selection on morphological traits is expected to have correlated effects on allele frequencies at the microsatellite loci. In those years with significant selection on morphology, the frequencies of associated alleles were compared between survivors and nonsurvivors. The majority of frequency changes (29/42) occurred in the direction predicted from morphological change, which is significantly more than 50% under the simplest expectation (binomial test for large samples, $Z = 2.50$, one-tailed $P = 0.0062$). Furthermore, the magnitude of deviations from expected frequencies was significantly greater for those in the predicted direction than in the opposite direction (Mann-Whitney $U' = 262.5$, $Z' = 2.014$, one-tailed $P = 0.0220$).

DISCUSSION

Genetic Evidence of Introgression

Microsatellite markers are useful for detecting and measuring gene exchange between populations of the same or different species (McDonald and Potts 1997; Rannala and Mountain 1997; Hansson et al. 2003; Hutchinson et al. 2003). Using such markers we have provided genetic evidence of introgression of genes contributing to morphological convergence of populations of *G. scandens* and *G. fortis* over 15–20 years. Both morphological and genetic convergence were asymmetrical, with *G. scandens* becoming more like *G. fortis* in allele frequencies at 16 polymorphic microsatellite loci, and in body size, beak size, and beak shape. The average number of alleles per locus and expected heterozygosity increased in *G. scandens* but not in *G. fortis*. Consistent with observed hybridization, introgression was estimated to be three times greater into *G. scandens* than into *G. fortis*, and occurred later in *G. scandens*.

Part of the genetic evidence for introgression is a higher frequency of misassigned individuals in the *G. scandens* population than in the *G. fortis* population. Members of the *G. scandens* population were classified ambiguously by assignment tests twice as often as were members of the *G. fortis* population. Individuals identified from pedigrees as F_1 hybrids or backcrosses were classified ambiguously twice as frequently as the pure species (i.e., not hybrids or backcrosses) and three times more frequently in the *G. scandens* sample of F_1 hybrids and backcrosses than in the comparable *G. fortis* sample.

We suspect that not all F_1 hybrids and backcrosses have been correctly identified by multilocus assignments. For example, the tests did no better than detect two-thirds of those identified from pedigrees as F_1 hybrids and half of those identified as first generation backcrosses. Changing the criteria for assigning individuals to F_1 hybrids and backcrosses yielded only a small improvement. The same has been reported in other studies. In horses, for example, precision in assigning individuals to cross-breeds (hybrids) is a function of number of loci, number of individuals, and the genetic difference between the breeds that have been crossed (Bjørnstad and Røed 2002). Based on these findings, those of others (Blott et al. 1999; Cornuet et al. 1999; Davies et al. 1999; Hansson et al. 2003; McDonald 2003) and a study of the related species *Geospiza magnirostris* (Grant et al. 2001), we are confident that our sample sizes of individuals, number of loci, and number of alleles per locus are adequate. The degree of genetic difference between *G. scandens* and *G. fortis* is probably a more important factor limiting the capacity of the tests to identify hybrids. An additional factor is the tendency for both species to show heterozygote deficits that cannot be attributed to null alleles (Petren 1998; Petren et al. 1999a), but may instead be due to spatially restricted breeding.

In 1979–1986 F_1 hybrids and backcrosses, identified by assignment tests, constituted less than 10% of the samples of each species. By 2001–2003 their frequency in the *G.*

scandens population had risen to 24% Given problems of detection, these estimates are too low. Nonetheless, taken at face value they are far higher than the overall estimate of immigrant frequency. The frequency of *G. fortis* immigrants is not known, but is likely to be low. In some years (1990–1992) there was no immigration. This is known because more than 99% of the birds were identifiable by their bands. In other years when banded birds were less frequent, three birds suspected of being immigrants from subtle features of plumage constituted less than 1% of the population. Immigrants in the *G. scandens* population were only detected by assignment tests. Up to and including 1998 their frequency was estimated to be 2.4%. Over the same time period the estimate of F_1 hybrids and backcrosses in the *G. scandens* population was 18.0%. Making allowance for additional F_1 hybrids and backcrosses identified from pedigrees but not identified as such by assignment tests, the true estimate is more likely to be in the range 25–30%. The three alleles introduced by immigrants contrast with 30 introduced by F_1 hybrids and backcrosses. We conclude that introgressive hybridization caused the species to converge and had increasing cumulative effects over 15–20 years.

Current hybridization is not the only cause of changes in allele frequencies. Deleting known and suspected F_1 hybrids and backcrosses reduced but did not eliminate the genetic convergence of *G. scandens* on *G. fortis*. The influence of F_1 hybrids and backcrosses on allele frequencies may have been underestimated in two ways: (1) our detection and deletion techniques missed several F_1 hybrids and backcrosses; and (2) hybridization prior to our study may have had persistent effects during the study. An important additional factor in genetic change is known to be directional natural selection on morphological traits (Grant and Grant 2002).

Allele frequencies changed as a correlated effect of selection on morphological traits in the *G. scandens* population. We found that many alleles at microsatellite loci changed in frequencies, and those that changed the most were associated with one or more morphological traits that changed. Deleting known and suspected F_1 hybrids and backcrosses reduced but did not eliminate the number of significant associations.

Field studies of nonhybridizing but heterogeneous populations of other species (Coulson et al. 1998), including Darwin's finches (Grant et al. 2001), have reported evidence of associations between microsatellite loci and morphology (but see Chan and Arcese 2003). In our study an association between a particular microsatellite allele and a morphological trait is likely to be a transient consequence of hybridization. It need not necessarily take the form of physical linkage between morphology-coding loci and microsatellite loci—or even presence on the same chromosome. The diploid number of chromosomes of Darwin's finches is about 80 (Jo 1983); thus, it is possible for a haploid set from a hybridizing parent to contain 16 microsatellite loci and 24 morphology-coding loci, all on separate chromosomes. On the other hand, there may indeed be physical linkage, in which case some of the microsatellite loci would be acting essentially as quantitative trait loci markers.

Implications of Introgression

Hybridization is of current research interest in three interrelated contexts. First, hybridization reveals how genetic incompatibilities evolve during speciation, leading to complete reproductive isolation (Barton 2001; Ortíz-Barrientos et al. 2002; Navarro and Barton 2003; Rieseberg et al. 2003a,b; Servedio and Sætre 2003; Sætre et al. 2001, 2003). A prime source of information is hybrid zones, where gene flow can be selective for genetic reasons, behavioral reasons, or both (Kim and Rieseberg 1999; Sattler and Braun 2000; Martinsen et al. 2001; Rowher et al. 2001; Bensch et al. 2002; Thulin and Tegelström 2002; Bronson et al. 2003; Gee 2003). Second, introgressive hybridization results in novel gene combinations being formed (Stebbins 1959; Svärdson 1970; Mecham 1975), and these create the potential for niche, habitat, or range expansion if environmental circumstances are favorable (Lewontin and Birch 1966; Chiba 1993; Pierotti and Annett 1993; Grant and Grant 1994; Arnold 1997; Rieseberg et al. 2003a,b; Smith et al. 2003). This is a possible route to speciation (Svärdson 1970; Salzburger et al. 2002; Rieseberg et al. 2003b). Third, hybridization at one trophic level has ecological effects at one, sometimes two, other trophic levels (Preszler and Boecklen 1994; Whitham et al. 1994, 1999; Dungey et al. 2000; Brown et al. 2001). A fourth reason for paying attention to hybridization and estimating its effects is its potential to obscure evolutionary history (Avise 1989; McDade 1990; Smith 1992; Clarke et al. 1998).

Our study is most relevant to speciation and the potential for new directions of evolution (Grant and Grant 1994). For example, *G. fortis* and *G. scandens* have different allometries, and when introgressive hybridization occurs the allometries are genetically altered through a reduction in strength of genetic correlations between traits. With enhanced genetic variation but reduced genetic constraints, the two populations have the potential to evolve in new directions more easily than in the absence of introgression (Grant and Grant 1994).

Our study reveals nothing about the final stages of speciation. Instead it shows that premating isolation evolves before postmating isolation (Grant and Grant 1992b, 1997c; see also Price and Bouvier 2002). This has been known or suspected for a long time in birds (Mayr 1963) as well as other taxa (Kaneshiro and Val 1977; Powell 1989; Sperling 1990). Much less well known is the fact that the divergent process of speciation can be reversed at this stage when the environment changes. Ecological conditions in the terrestrial Galápagos environment depend on climatic conditions that in turn depend on oceanographic conditions (Grant et al. 2000). Sea-surface temperatures fluctuate at approximately 25-year intervals (Chavez et al. 2003). Our study began at the end of a relatively cool period, and the Galápagos are now experiencing the next cool period. Thus, the two species on Daphne may approximately follow the 25-year periodicity in alternately converging through hybridization and selection and diverging through selection alone.

Our findings can be generalized. Closely related populations in sympatry may undergo merge-and-diverge oscillations when the incidence of interbreeding or the fitness of F_1 hybrids and backcrosses is dependent on particular conditions in a strongly fluctuating environment. If, when merging, the populations evolve in the direction of their ancestral population, or one becomes genetically assimilated into the other (Carney et al. 2000), they may be said to be despeciating. Recently diverged populations are likely to do that (Cade

1983). On the other hand, if truly novel genotypes and phenotypes are created by introgressive hybridization between older populations (McDade 1990), then each population in the next divergence phase could evolve along a new evolutionary trajectory, in situ or having expanded its range and colonized new habitats (Lewontin and Birch 1966; Chiba 1993; Wang and Szmidt 1995) or islands (Grant 1998).

The main implication of these considerations is that there is a time during speciation when introgression has the largest evolutionary effect. It is neither very early nor very late, but perhaps occurs after some genetic incompatibilities have arisen (Kim and Rieseberg 1999) yet before the point is reached at which interbreeding inevitably incurs a fitness cost.

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