Unpredictable Evolution in a 30-Year Study of Darwin's Finches

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Evolution can be predicted in the short term from a knowledge of selection and inheritance. However, in the long term evolution is unpredictable because environments, which determine the directions and magnitudes of selection coefficients, fluctuate unpredictably. These two features of evolution, the predictable and unpredictable, are demonstrated in a study of two populations of Darwin's finches on the Galápagos island of Daphne Major. From 1972 to 2001, *Geospiza fortis* (medium ground finch) and *Geospiza scandens* (cactus finch) changed several times in body size and two beak traits. Natural selection occurred frequently in both species and varied from unidirectional to oscillating, episodic to gradual. Hybridization occurred repeatedly though rarely, resulting in elevated phenotypic variances in *G. scandens* and a change in beak shape. The phenotypic states of both species at the end of the 30-year study could not have been predicted at the beginning. Continuous, long-term studies are needed to detect and interpret rare but important events and nonuniform evolutionary change.

The value of long-term studies in ecology has become widely recognized among scientists and the media (*1–3*). Less widely appreciated is the similar value to be gained from long-term studies of evolution in nature. A classic study, spanning 49 years, was carried out by H. D. Ford and E. B. Ford (*4*) on phenotypic variation in a European butterfly, *Melitaea aurinia* (Marsh Fritillary). The most important discovery was made after about 40 years of monitoring that began with the collecting of specimens by amateur naturalists in 1881. In the early 1920s an outburst of phenotypic and presumed genetic variation occurred in association with a rapid increase in butterfly numbers from an extremely low density caused by parasitoids. Variation then declined to a lower and stable level, with phenotypes in the late 1920s being recognizably different from those in the same population at the beginning of the study. The inferred genetic reorganization helped to frame ideas about evolution in contemporary time, inspired other long-term studies of butterflies (*5*) and moths (*6*), and contributed to the development of at least one model of speciation (*7*).

Long-term studies of evolution involving annual or more frequent sampling have many potential benefits. These include documentation and understanding of slow and cryptic directional evolutionary change, perhaps in association with gradual global warming, reversals in the direction of evolution, rare events with strong effects such as genetic bottlenecks caused by population crashes, phenomena recurring at long intervals, and processes with high interannual variability such as erratic and intermittent gene flow. These benefits are beginning to be realized (*8–15*), but few studies have persisted long enough for us to be able to generalize about the temporal pattern and predictability of basic evolutionary processes in unconstrained natural populations.

Here, we report the results of a 30-year study of evolution of size and shape traits in two populations of Darwin's finches based on annual sampling and measurement. Distinctive features of the study are its length, continuity, entirely natural environmental setting, the availability of pedigree information to construct and interpret evolutionary change, and the macroevolutionary context of an adaptive radiation. The study reveals the irregular occurrence, frequency, and consequences of two evolutionary processes that are more often inferred than directly studied: natural selection and introgressive hybridization.

Natural selection and evolution. Populations of *Geospiza fortis* (medium ground finch) and *G. scandens* (cactus finch) have been studied on the Galápagos island of Daphne Major every year since 1973; adults that year were born (hatched) no later than 1972. Survival of marked and measured individuals has been recorded every year, and reproduction of most individuals has been recorded in most years (*16*). Six measured traits on adults whose growth has ceased have been reduced by principal components analyses to three interpretable synthetic traits: body size, beak size, and beak shape (*17–20*). The null expectation is that, subject to sampling error, means of these traits have remained constant across the period of study.

This expectation of no change is clearly not supported by the data (Fig. 1). Lack of independence of samples in successive years precludes year-by-year significance testing of the total samples. Nevertheless, comparisons across years show nonoverlapping 95% confidence estimates of the means at different times. Mean body size and beak shape were markedly different at the end of the period (2001) than at the beginning (1973) in both species (*21*). Between these two times mean body and beak size of *G. fortis* initially decreased, then increased sharply, and decreased again more slowly (Fig. 1, A and B). Beak shape abruptly became more pointed in the mid-1980s and remained so for the next 15 years (Fig. 1C). *G. scandens*, a larger species, displayed more gradual and uniform trends toward smaller size and blunter beaks (Fig. 1, D to F), thereby converging toward *G. fortis* in morphology.

Apart from random sampling effects, annual changes in morphological means are caused by selective losses, as a result of mortality and emigration, and selective gains, as a result of breeding and immigration (*22*). Previous work has demonstrated directional natural selection on beak and body size traits associated with survival, in *G. fortis* at three times and in *G. scandens* once, when a scarcity of rain caused a change in the composition of the seed supply that forms their dry-season diets (*23–25*). Evolutionary responses of *G. fortis* to the two strongest selection episodes occurred in the following generations (*26*), as expected from the high heritabilities of the morphological traits [$h^2 = 0.5$ to 0.9 after corrections for misidentified paternity arising from extrapair copulations (*18*, *27*)].

Figure 2 provides the long-term perspective of repeated natural selection in both species (*28*). There are four main features of the figure. First, body and beak size traits were subject to selection more often than was beak shape. Setting α at 0.01, to allow for the lack of complete independence of the traits (*29*), we find that body size was subject to selection about once every 3 years in both species (*30*), that is, once each generation of 4.5 years (*G. fortis*) or 5.5 years (*G. scandens*) (*31*) on average. Second, considering only the statistically significant selection differentials, the species differed in the directions of net selection on size traits. *G. fortis* experienced selection in both directions with equal frequency (Fig. 2, A to C), whereas *G. scandens* experienced selection that repeatedly favored large body size and in no instance favored small beak size (Fig. 2, D and E). Third, unidirectional selection occurred in successive years, up to a maximum of 3 years in

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both species (Fig. 2, A, D, and E). Fourth, selection events in the two species were usually not synchronous, except in the late 1970s, when large size was selectively favored in both species during a drought (*23*). The demonstration here of natural selection occurring repeatedly in the same populations over a long time complements the widespread detection of natural selection in many different species of plants and animals over much shorter times (*32*, *33*, *34*). As in these broad surveys, and in three studies of birds lasting for 11 to 18 years (*15*, *35*, *36*), the magnitude of selection on the finch populations was usually less than 0.15 SD and rarely more than 0.50 SD (*33*, *34*). Median values (0.03 to 0.06) are well within the normal range (0.00 to 0.30) of other studies (*34*).

Evolution followed as a consequence of selection in both species because all traits are highly heritable (*18*, *20*, *27*). We compared the mean of a trait before selection with the mean of the same trait in the next generation by one-tailed t tests ($P < 0.05$) (*26*). Significant evolutionary events occurred in *G. fortis* eight times (body size, four; beak size, three; beak shape, one) and in *G. scandens* seven times (body size, two; beak size, five). Evolution below the level of statistical detectability may have followed other instances of directional selection, may have been masked by annual variation in environmental effects on growth to final size (*37*), or may have been nullified by countervailing selection on correlated traits not included in the analyses (*32*). Magnitudes of evolution of the two independent beak traits (size and shape) are correlated with values predicted from the products of selection differentials and heritabilities (Fig. 3). Similar results were obtained in analyses of the direct effects of selection on the six measured traits of *G. fortis* at two times of intense selection, taking into account genetic correlations among them (*26*). Thus evolution, as an immediate response to selection, was predictable.

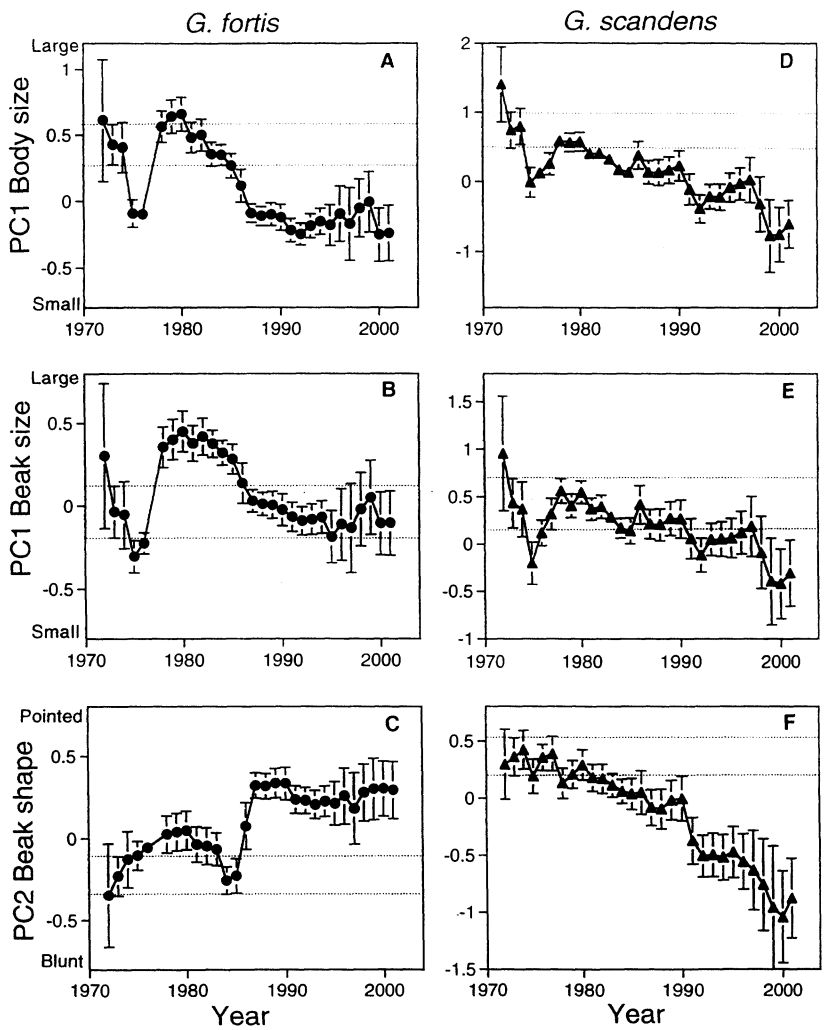

Fig. 1. Morphological trajectories of adult *Geospiza fortis* (**A** to **C**) and *G. scandens* (**D** to **F**). In the absence of change, mean trait values should have remained within the 95% confidence intervals (horizontal broken lines) of the estimates from the 1973 samples (body size: *G. fortis*, n = 115, *G. scandens*, n = 37; beak traits: *G. fortis*, n = 173, *G. scandens*, n = 62). Sample sizes varied from 45 (1997) to 976 (1991) for *G. fortis* and from 30 (1999) to 336 (1983) for *G. scandens*. The 1972 sample is composed of the adults (=1 year old) in 1973.

Introgressive hybridization. Annual changes in morphology (Fig. 1) are largely but not entirely accounted for by selective losses. The greatest discrepancy is in the 1990s when the single occurrence of natural selection on beak shape in *G. scandens* at the beginning (at $P < 0.05$; Fig. 2F) does not account for the continuing change in mean beak shape over the decade (Fig. 1F). Therefore, we next consider selective gains as a result of nonrandom recruitment to the adult population.

There are four potential contributors to nonrandom additions: conspecific and heterospecific residents and immigrants. Breeding immigrants are not known in *G. scandens* and are extremely rare in *G. fortis* (*16*). Biased conspecific breeding has minor effects on morphological trajectories. Prior analyses indicate some evidence for sexual selection on morphological traits (*38*), yet little influence of morphological variation on lifetime fitness as measured by the production of offspring that survive to breed (*39*). However, hybridization does occur rarely between resident *G. fortis* and *G. scandens*, and *G. fortis* also breeds with a rare immigrant species, *G. fuliginosa* (small ground finch) (*40*). In both cases there is generally little or no fitness loss (*41*, *42*). After the dry period of the late 1970s, and beginning in the extraordinarily prolonged wet season of 1983 (El Niño year), successful breeding of F_1 hybrids and backcrosses was documented (*40–43*). Effects of introgression on morphological means and variances have not been tested before, but are to be expected in view of the large additive genetic variation underlying the size and shape traits of both *G. fortis* and *G. scandens* (*18*, *20*, *27*).

A specific prediction of the introgression hypothesis is an increase in variance and skewness in the morphological distributions, beginning in 1983 in *G. fortis* and 1987 in *G. scandens* (*42*). Increases are expected to be greater in *G. scandens* than in *G. fortis*, despite bidirectional gene exchange, because F_1 hybrids and first-generation backcrosses made a proportionately greater numerical contribution to the *G. scandens* samples (Fig. 4, A and B) (*43*). The predicted increases in beak shape variance and skewness are observed in *G. scandens* (Fig. 5, E and F) and are scarcely noticeable in *G. fortis* (Fig. 5, B and C). Beak shape variance in *G. scandens* doubled from 0.430 in 1973 (95% confidence intervals 0.311, 0.636; n = 62) to 1.026 (0.674, 1.762; n = 35) in 2001, whereas the variance in *G. fortis* beak shape remained stationary: 0.627 (0.493, 0.824; n = 173) in 1973, and 0.887 (0.744, 1.316; n = 114) in

2001. Beak size and body size variances (not shown) are not as well estimated and show no significant variation across the study period (95% confidence intervals broadly overlap). Skewnesses in beak size and body size distributions vary in parallel with beak shape skewness: negatively in *G. scandens*, projecting toward *G. fortis*; and positively in *G. fortis*, projecting toward *G. scandens*.

A second prediction is that variance and skewness will decrease if F_1 hybrids and first-generation backcrosses are deleted from the total samples. Deletions of these two classes of birds do indeed effectively eliminate the increases in skewness and variance in *G. scandens* beak shape, and reduce the degree to which the mean changed (Fig. 5, D to F), because on average F_1 hybrids and first-generation backcrosses are smaller in body size and beak size and have less pointed beaks than the parental *G. scandens* species (*44*). We conclude that introgressive hybridization caused a change in means and other moments of the frequency distributions of *G. scandens* measurements. Selection may have contributed as well but to a minor extent (Fig. 2F). Deletions had no obvious effect on the distributions of *G. fortis* traits (Fig. 5, A to C) (*44*, *45*).

The proportionally greater gene flow from *G. fortis* to *G. scandens* than vice versa has an ecological explanation. Adult sex ratios of *G. scandens* became male biased after 1983 (Fig. 4C) as a result of heavy mortality of the socially subordinate females. High mortality was caused by the decline of their principal dry-season food, *Opuntia* cactus seeds and flowers; rampantly growing vines smothered the bushes (*16*). *G. fortis*, more dependent on small seeds of several other plant species, retained a sex ratio close to 1:1 (Fig. 4C). Thus, when breeding resumed in 1987 after 2 years of drought, competition among females for mates was greater in *G. fortis* than in *G. scandens*. All 23 *G. scandens* females paired with *G. scandens* males, but two of 115 *G. fortis* females paired interspecifically. All their F_1 offspring later bred with *G. scandens* (*43*) because choice of mates is largely determined by a sexual imprinting-like process on paternal song (*42*).

Conclusion. The long-term study of Darwin's finch populations illustrates evolutionary unpredictability on a scale of decades. Mean body size and beak shape of both species at the end of the study could not have been predicted at the beginning. Moreover, sampling at only the beginning and at the end would have missed beak size changes in *G. fortis* in the middle. The temporal pattern of change shows that reversals in the direction of selection do not necessarily return a population to its earlier phenotypic state. Evolution of a population is contingent upon environmental change, which may be highly

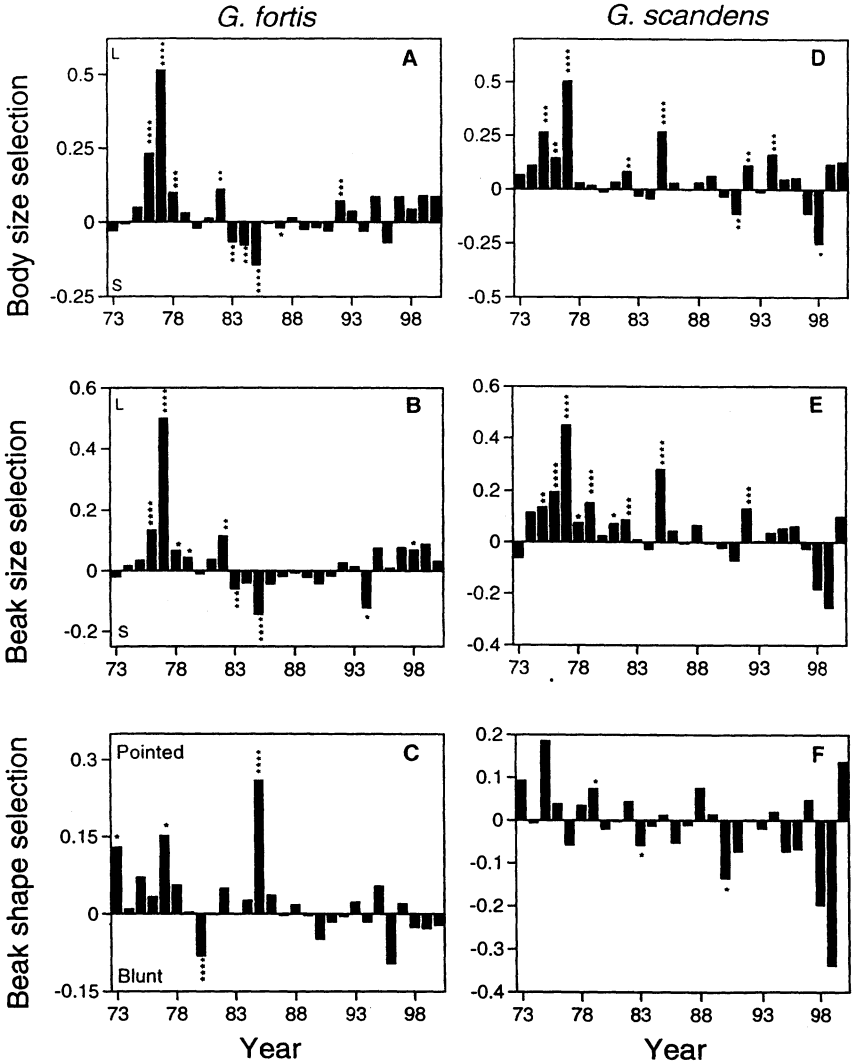

Fig. 2. (**A** to **F**) Standardized selection differentials, calculated for each sample surviving from year *x* to year *x*+1. Positive values indicate selection for large size or pointed beaks; L, large; S, small. Significance levels are shown without correction for multiple testing or lack of independence (*29*). Males and females were combined with adults of unknown sex because separate selection analyses of males and females give similar results (*23*). Differentials are temporally autocorrelated to varying degrees; autocorrelation coefficients are 0.416, 0.302, and 0.093 for *G. fortis* body size, beak size, and beak shape, respectively, and 0.171, 0.373, and 0.103 for the same traits in *G. scandens*. *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$; ****$P < 0.001$.

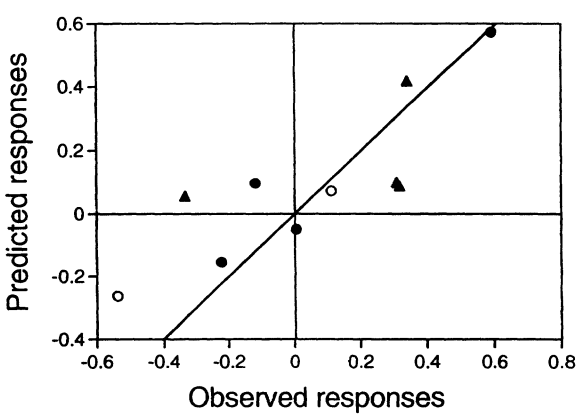

Fig. 3. Predicted and observed evolutionary responses to natural selection on beak size (●) and shape (○) in *G. fortis* and beak size in *G. scandens* (▲). Values are in standard deviation units. The line is drawn with a slope of 1.0 through the origin. Predictions are the products of standardized selection differentials (Fig. 2, $P < 0.01$) and heritabilities (*18*, *27*). Predicted and observed values are correlated ($r = 0.832$, $n = 10$, $P = 0.0028$). The signs of the beak shape variable are arbitrary with respect to the beak size axis, but this has little effect on the correlation when beak shape signs are reversed ($r = 0.781$, $n = 10$, $P = 0.0077$). Data for *G. scandens* after 1986 have not been included because of complications arising from introgressive hybridization (Figs. 4 and 5). Body size has not been included because it is not independent of beak size (*29*).

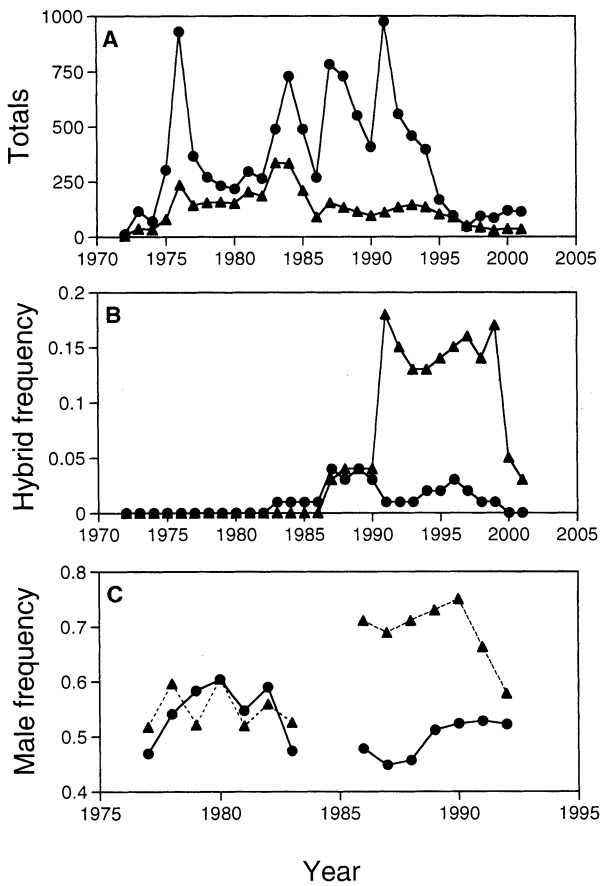

Fig. 4. Total samples of measured birds (**A**), proportional contributions of F_1 hybrids and first-generation backcrosses to the totals (**B**), and minimal frequencies of adult males (**C**) before and after habitat and demographic changes were caused by the El Niño event of 1983; *G. fortis* (●) and *G. scandens* (▲). In (B), the decline in proportion of hybrids in the *G. scandens* samples after 1999 reflects the addition of birds of unknown parents. They have been classified as *G. scandens*, but some were probably unidentified hybrids and backcrosses. In (C), birds of unknown sex in female-like plumage (0 to 10%) have been added to the female samples, and therefore proportions of males are minima. Sexes of all birds were known in some years (e.g., 1989). Data for 1984 and 1985 are not shown because of the large proportion of birds of unknown sex (>15%) produced in 1983 and 1984, comprising both males and females.

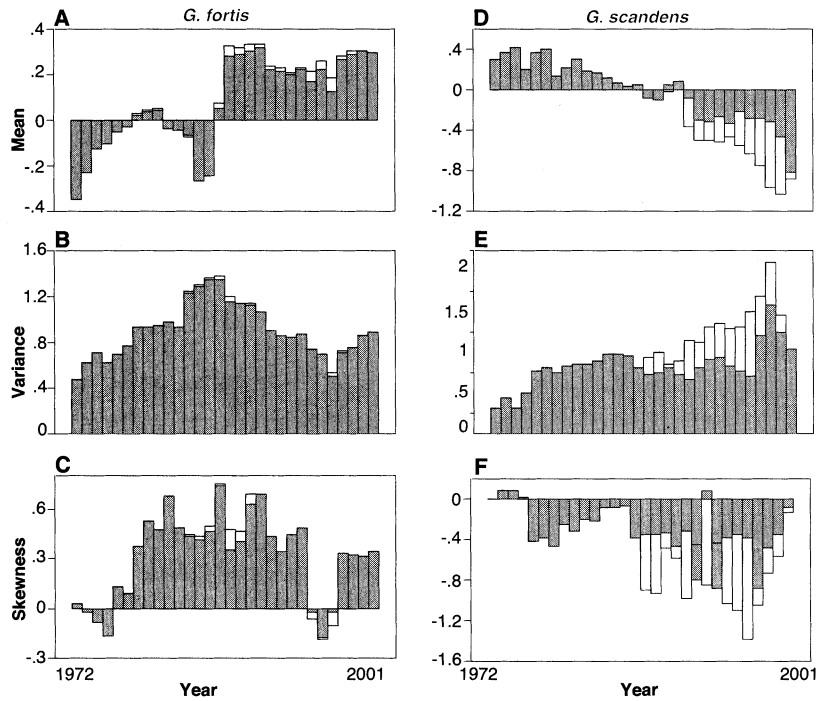

Fig. 5. Effects of hybrids and backcrosses on the mean, variance, and skewness of the beak shape frequency distributions of *G. fortis* (**A** to **C**) and *G. scandens* (**D** to **F**). Removal of F_1 hybrids and first-generation backcrosses (white bars) has a strong effect on *G. scandens* but a minor effect on *G. fortis* parameters (*45*). The proportion of known hybrids declined after 1991; not all hybrids and backcrosses could be identified with pedigree information, because some nestlings were not banded after 1991 (*16*), and therefore some may have been included as *G. fortis* and *G. scandens*. Negative skewness indicates a prolonged tail toward less pointed beaks. Additional removal of *G. fortis* × *G. fuliginosa* F_1 hybrids and backcrosses has scarcely detectable effects in these diagrams.

irregular, as well as on its demography and genetic architecture (*33*, *46*).

The study also illustrates how the value of long-term studies increases with time. Not only is regular monitoring at short intervals desirable, but sampling for many years is to be recommended, especially for long-lived organisms like vertebrates and perennial plants. Yet evolutionary studies are rarely pursued in the field for as many as 10 years (*33*). If we had stopped sampling after 10 years, our conclusions would have been different because at that time the only difference from the starting point was in beak size of *G. fortis*. By persisting beyond then, we witnessed a natural-selection event that affected beak shape in *G. fortis*, documented interbreeding and morphological effects of introgression on *G. scandens*, and gained a better quantitative estimate of the frequency of evolutionary events.

Unlike the Marsh Fritillary study (*4*), we did not witness a release of genetic variation following a population crash. The evolutionary dynamics were different. Natural selection occurred frequently in our study, occasionally strongly, unidirectionally in one species and oscillating in direction in the other as a result of their dependence on different food supplies (*16*). Introgressive hybridization, a phenomenon whose importance has been relatively underappreciated until recently, except in plants (*47*), had different effects on the two species for demographic reasons. Hybridization and selection are often connected through the selective disadvantage experienced by hybrids and backcrosses (*13*, *40*, *47*, *48*). In the present study they appear to have been connected synergistically in the sense that interbreeding may have been facilitated in part by selection for more pointed, *G. scandens*–like, beaks in the *G. fortis* population in the mid-1980s. Choice of mates is partly determined by imprinting on parental beak morphology, as well as on paternal song (*41*). The principal causes of selection have been identified as changes in food supply (*23*–*26*) mediated in large part by droughts. The ultimate cause of repeated natural selection and introgressive hybridization may have been a change in the seasonal movement of water masses in the eastern subtropical and tropical Pacific (*49*, *50*), triggering altered climatic patterns, including the intensification of El Niño and La Niña cycles.

Regardless of the precise chain of causality, field studies such as ours, in conjunction with multigenerational studies of microorganisms in the laboratory (*51*, *52*) and experimental studies of selection in the field (*53*–*55*), provide an improved basis for extrapolating from microevolution to patterns of macroevolution; in the present

case, from evolutionary dynamics of populations on the scale of decades to speciation and further adaptive radiation on the scale of hundreds of thousands of years (*56*). In conclusion, the long-term unpredictability of evolutionary change that arises from unpredictable ecological change, together with the need to strengthen generalizations about the frequency and importance of selection and hybridization, are reasons for encouraging additional, continuous, long-term studies of evolution in nature.

References and Notes

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17. For each species, two principal-components analyses were performed on the correlation matrix with untransformed data from all birds [males, females, and birds of unknown sex of both species; see (*18*)]. Mass (weight), wing length, and tarsus length were used in the first analysis, and beak length, depth, and width (*19*) were used in the second analysis. For *G. fortis* (n = 3204), PC1 in analysis 1 is interpreted as a body size factor (69.2% variance explained), because factor loadings were high and of the same sign: 0.856 (mass), 0.839 (wing), and 0.800 (tarsus). PC1 in analysis 2 is interpreted as a beak size factor (85.8% variance explained); factor loadings are 0.890 (length), 0.951 (depth), and 0.937 (width). PC2 is a beak shape factor (10.1% additional variance explained) with factor loadings of different sign: 0.455 (length), -0.167 (depth), and -0.262 (width). Factor loadings and their interpretation were similar in analyses of *G. scandens* ($n \approx$ 1037). Percentage variance was 67.5 for PC1 in the first analysis, 69.8 for PC1 in the second analysis, and 23.2 for PC2 in the second analysis. Separate analyses of males and females gave similar results.
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19. The six measured traits (*17*) have high repeatabilities (*20*). Nine specimens of *G. fortis* in the Charles Darwin Research Station museum, Galápagos, were measured by P.R.G. in 1975, 1976, and again in 2001 to check for possible changes in methods of linear measurement. No heterogeneity among years was found for any of the five traits [analyses of variance (ANOVAs), all $P > 0.67$], and no difference between pairs of years was found in any trait (Fisher's protected least significant difference post hoc tests, all $P > 0.38$).
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21. The first samples in 1973 (221 *G. fortis* and 72 *G. scandens*) were compared by ANOVAs with the last samples in 2001 (114 *G. fortis* and 35 *G. scandens*). The data were trimmed to 2.5 SD on either side of the mean by removing one to three individuals from the samples of each species. This corrected for skewness and unequal variances. Sex was included in two-factor ANOVAs because males are generally larger than females. Mean body size was significantly smaller in *G. fortis* ($F_{1,132}$ = 7.773, P = 0.0061) and in *G. scandens* ($F_{1,50}$ = 11.272, $P < 0.0001$) in 2001 than in 1973. There was a significant effect of sex in each species ($P < 0.002$) but no sex-by-year interaction ($P > 0.1$). Mean beak size did not differ between years in either *G. fortis* ($F_{1,166}$ = 0.004, P = 0.9480) or *G. scandens* ($F_{1,50}$ = 3.108, P = 0.0840); sex effects were significant in both species ($P < 0.007$), but there were no sex-by-year interactions ($P > 0.1$). Beak shape differed between years in both species. For *G. scandens* there was a strong year effect ($F_{1,72}$ = 17.168, $P < 0.0001$), a weak sex effect ($F_{1,72}$ = 5.943, P = 0.0172), and no interaction. The *G. fortis* sexes do not differ in beak shape (P = 0.9715), and therefore a one-factor ANOVA was performed with adult males, females, and birds of unknown sex. It demonstrated a strong difference between years ($F_{1,287}$ = 30.246, $P < 0.0001$).
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28. Directional selection differentials were calculated as the difference in trait means before and after selection, then standardized in each case by dividing the difference by the standard deviation of the sample before selection (*32*). After checking for normality, differences between survivors and nonsurvivors were tested by two-tailed t tests. Significant skewness (*57*) was eliminated by deleting outliers (more than 2.5 SD beyond the mean), with minor effects on probability values. Equality of variances was tested with F tests. Only beak traits displayed unequal variances. Correcting for skewness reduced the number of significant variance inequalities from three to two in *G. fortis* and from nine to four in *G. scandens*. Means in the remaining six cases were tested with a median test (*57*); five were nonsignificant ($P > 0.1$). Selection was diversifying in three of the five cases. Selection differentials measure the combined direct effects of selection on a trait and indirect effects of selection on correlated traits. Direct effects on the individual measured traits have been assessed by partial regression (selection gradient analysis) in some cases (*24–26*), yielding similar interpretations; body size, beak size, and beak shape are independently selected traits.
29. Body size and beak size are strongly correlated traits in *G. fortis* (r^2 = 0.464 to 0.650; median 0.540) and *G. scandens* (r^2 = 0.393 to 0.694; median 0.553) in all 30 years at $P < 0.0001$. Beak shape is occasionally and weakly correlated with body size in *G. fortis* (r^2 = 0.001 to 0.082; median 0.015) and *G. scandens* (r^2 = 0.001 to 0.179; median 0.026).
30. *G. scandens* body size was selected in 8 out of 22 years in which a minimum sample size of 75 was met, and beak size was selected in 7 of those years. Frequency of selection in *G. fortis* was 8 out of 25 years for body size, 5 out of 26 years for beak size, and 2 out of 26 years for beak shape. These are minimal frequencies; selection below the level of statistical detectability may have occurred in other years.
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43. Eight F_1 hybrids (both sexes), all from families with a *G. scandens* father that sang a *G. scandens* song, backcrossed to *G. scandens* and produced 16 measured offspring. Six F_1 hybrids (both sexes), all from families with a *G. scandens* father that sang a *G. fortis* song, backcrossed to *G. fortis* and produced 26 measured offspring. Other F_1 hybrids and backcrosses were produced but not captured and measured.
44. The 1991 sample of *G. scandens* (n = 112), the last with complete pedigree information, included 20 hybrids (seven F_1 hybrids and 13 first-generation backcrosses). The hybrids were smaller in body size (ANOVA, $F_{1,109}$ = 38.522, $P < 0.0001$) and beak size ($F_{1,109}$ = 34.583, $P < 0.0001$) and had less pointed beaks ($F_{1,109}$ = 55.116, $P < 0.0001$) than the rest. In the same year, one F_1 hybrid and nine first-generation backcrosses in the sample of 510 *G. fortis* were larger in body size than the rest ($F_{1,507}$ = 21.817, $P < 0.0001$) and had more pointed beaks ($F_{1,507}$ = 8.323, P = 0.0041) but did not differ in beak size ($F_{1,507}$ = 1.098, P = 0.2953).
45. The fractional contribution to beak shape variance made by hybrids was on average 0.265 ± 0.140 SD for *G. scandens* (maximum 0.528; n = 15 years) and 0.024 ± 0.018 for *G. fortis* (maximum 0.064; n = 17 years).
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