



# Hybridization increases population variation during adaptive radiation

Peter R. Grant<sup>a,1</sup> and B. Rosemary Grant<sup>a</sup>

<sup>a</sup>Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544

Contributed by Peter R. Grant, September 27, 2019 (sent for review August 6, 2019; reviewed by Leonardo Campagna and J. Albert Uy)

**Adaptive radiations are prominent components of the world's biodiversity. They comprise many species derived from one or a small number of ancestral species in a geologically short time that have diversified into a variety of ecological niches. Several authors have proposed that introgressive hybridization has been important in the generation of new morphologies and even new species, but how that happens throughout evolutionary history is not known. Interspecific gene exchange is expected to have greatest impact on variation if it occurs after species have diverged genetically and phenotypically but before genetic incompatibilities arise. We use a dated phylogeny to infer that populations of Darwin's finches in the Galápagos became more variable in morphological traits through time, consistent with the hybridization hypothesis, and then declined in variation after reaching a peak. Some species vary substantially more than others. Phylogenetic inferences of hybridization are supported by field observations of contemporary hybridization. Morphological effects of hybridization have been investigated on the small island of Daphne Major by documenting changes in hybridizing populations of *Geospiza fortis* and *Geospiza scandens* over a 30-y period. *G. scandens* showed more evidence of admixture than *G. fortis*. Beaks of *G. scandens* became progressively blunter, and while variation in length increased, variation in depth decreased. These changes imply independent effects of introgression on 2, genetically correlated, beak dimensions. Our study shows how introgressive hybridization can alter ecologically important traits, increase morphological variation as a radiation proceeds, and enhance the potential for future evolution in changing environments.**

speciation | hybridization | introgression | beaks | Darwin's finches

**B**y hybridizing 2 species of Australian fruit flies in the laboratory and following them for several generations, Lewontin and Birch (1) showed that introgression could account for an observed geographical expansion of a fruit fly, *Dacus tryoni*, into a new, hotter, and physiologically more stressful environment. In a parallel study of coregonid fish in Swedish lakes, Svårdson (2) found that introgressive hybridization of species with different numbers of gill rakers led to the production of new forms with the potential of evolving into new species. These 2 studies laid the foundation for modern investigations into the role of introgressive hybridization in the evolution of animal species (3, 4), and specifically into the possibility that interspecific gene exchange is important in initiating new evolutionary trajectories (5–8), and even new animal taxa (9–13), especially in adaptive radiations (7, 14–16). Together with studies of ongoing hybridization in a variety of animals (3, 17–19), they complement a large body of research into the better-known and widespread hybridization of plants (20–24). However, the link between hybridization in the present and the past has not been well established for adaptive radiations. Here we show, with an example of Darwin's finches on the Galápagos Islands, how introgression affects morphological variances of hybridizing contemporary populations, and how variances change as a radiation proceeds.

Several field studies have documented hybridization of Darwin's finches (25–27) or inferred it from phenotypic (28) and genetic data (29–32). On the small island of Daphne Major (0.34 km<sup>2</sup>) *Geospiza fortis* (medium ground finch, ~17 g) interbreeds rarely

with *Geospiza scandens* (cactus finch, ~21 g), a common resident, and *Geospiza fuliginosa* (small ground finch, ~12 g), an occasional immigrant; *G. scandens* and *G. fuliginosa* do not interbreed on this island. Significantly, none of them hybridize with the much larger *Geospiza magnirostris* (large ground finch, ~30 g). We measured large samples of hybrid and nonhybrid finches annually from 1973 until 2012 (33). Within this period breeding was documented in detail from 1976 to 1998 (34). Hybridization was first observed in 1976 (35, 36), but it only led to breeding of the hybrids and backcrossing in 1983 after a climatically induced change in the vegetation that was favorable to hybrid survival. Observations on the dynamics of hybridization were supported by parentage determined by microsatellite DNA analysis (26). Fitness of hybrids was as high as, or possibly higher than, the fitness of parental species (33, 34). Species received new alleles more frequently by hybridizing than by breeding with conspecific immigrants (26). After this period (>1998) hybridization was inferred from microsatellite-determined admixtures (*Methods*) supplemented by observations of mixed pairs.

The principal finding from the first half of the study was an increase in phenotypic and quantitative genetic variation as a result of hybridization (5, 34, 37). This led to the following reasoning concerning hybridization in the past (38). Total effects of gene exchange are a function of both the frequency of interbreeding and the morphological effects at each interbreeding episode. When populations begin to diverge, an exchange of genes may be frequent but will have little effect on the variation of each. As morphological divergence proceeds further to a point at which the populations become biological species—they seldom interbreed but suffer little or no loss of fitness when they do—phenotypic and genetic effects of gene mixing are expected to be greater, and at

## Significance

**Adaptive radiations, comprising many species derived from one or a small number of ancestral species in a geologically short time, are prominent components of the world's biodiversity. Introgressive hybridization of divergent species has been important in increasing variation, leading to new morphologies and even new species, but how that happens throughout evolutionary history is not known. A long-term field study of Darwin's finches on Daphne Major island, Galápagos, shows that introgression enhances variation and increases the potential for future evolution. We use a dated phylogeny to infer that populations became more variable in morphological traits through time, consistent with this enhancement effect, and then declined in variation after reaching a maximum. Introgression may be especially important with future climate change.**

Author contributions: P.R.G. and B.R.G. designed research; P.R.G. and B.R.G. performed research; P.R.G. analyzed data; and P.R.G. and B.R.G. wrote the paper.

Reviewers: L.C., Cornell University; and J.A.U., University of Miami.

The authors declare no competing interest.

Published under the PNAS license.

<sup>1</sup>To whom correspondence may be addressed. Email: prgrant@princeton.edu.

This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1913534116/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1913534116/-DCSupplemental).

First published October 28, 2019.

some point reach a maximum. Thereafter, population variation declines, caused by strengthening of premating isolating mechanisms and hence increased rarity of interbreeding, and/or by the accumulation of incompatible alleles through mutations that reduce or prevent exchange.

Darwin's finch populations possess several features that make them suitable for testing this hypothesis. These include a large number of phylogenetically young species for quantitative analysis, ages of the species as estimated by the dates of nodes in a phylogenetic tree, and measurements of population variation in ecologically significant (functional) traits. Eighteen species radiated rapidly from a common ancestor in little more than a million years (39, 40), and they comprise several populations of the same species on different islands. Dates of the last common ancestor of each species and a sister lineage have been estimated from whole-genome studies (40). Interspecific gene exchange has been a feature of the group's history, beginning at some time after the first split, as inferred from the results of ABBA-BABA tests (40). Means and SDs of beak length and beak depth measurements of 99 populations have been published (ref. 41; see also ref. 42). Beaks are tools for dealing with food and are therefore ecologically significant traits (41, 43). They are also cues to species identity (44, 45) that, together with song (25, 46–49), are used in the choice of mates (49, 50) (*SI Appendix, section 1*). They are known to be subject to natural selection in this climatically varying environment (10, 34, 35, 51, 52). Selection episodically causes a large effect on means and small effects on variances (33, 35).

We first present a morphological analysis of contemporary hybridizing species on Daphne Major island, thereby providing a foundation for making inferences of hybridization in the past, and then test predictions of the hypothesis of past hybridization. We conclude by integrating morphological effects of past and present hybridization in the context of adaptive radiations.

### Hybridization on Daphne Major Island

Backcrossing was determined by direct observation of breeding birds and microsatellite analysis of parentage from 1983 to 1998. Backcrossing occurred first in 1983 from *G. scandens* to *G. fortis* and then, beginning in 1987 and more extensively, from *G. fortis* to *G. scandens* (33). The incidence of hybridization can be inferred for the whole study period from the proportions of admixed individuals in populations of *G. scandens* and *G. fortis* (Fig. 1). The figure has 2 main features. First, proportions of hybrids (admixed individuals) were higher in the *G. scandens* samples than in the *G. fortis* samples throughout the 30-y period, except for 2 y. The contrast between species was evaluated statistically with newly produced admixed individuals ( $F_1$  and backcrosses) in 11 y of extensive breeding (*Methods*) because these are independent data. Frequencies of newly produced *scandens*  $\times$  *fortis* hybrids (SF, i.e., higher assignment to *G. scandens* than to *G. fortis*) in the *G. scandens* samples significantly exceeded the frequencies of *fortis*  $\times$  *scandens* hybrids (FS) in the *G. fortis* samples (paired  $t = 3.86$ , degrees of freedom = 10,  $P = 0.0032$ ). Second, the proportion of admixed individuals increased across years, as shown by linear regression: For FS hybrids  $F = 18.42$ ,  $P = 0.0020$ , and adjusted  $R^2 = 0.64$ , and for SF hybrids  $F = 7.25$ ,  $P = 0.0247$ , and adjusted  $R^2 = 0.38$ . Increases reflect greater gains (births) of hybrids than losses (deaths and emigration). In contrast to these increases, the proportions of *fortis*  $\times$  *fuliginosa* (Ff) hybrids in the *G. fortis* samples remained unchanged ( $F = 1.17$ ,  $P = 0.3074$ ) (*SI Appendix, section 2*) at an average frequency ( $0.08 \pm 0.014$  SE) no different from FS ( $0.06 \pm 0.008$  SE) and lower than SF ( $0.12 \pm 0.020$  SE) frequencies. These linear trends, and others below, are subject to the caveat that data for successive years are not independent samples, and the statistics are presented for their heuristic value.

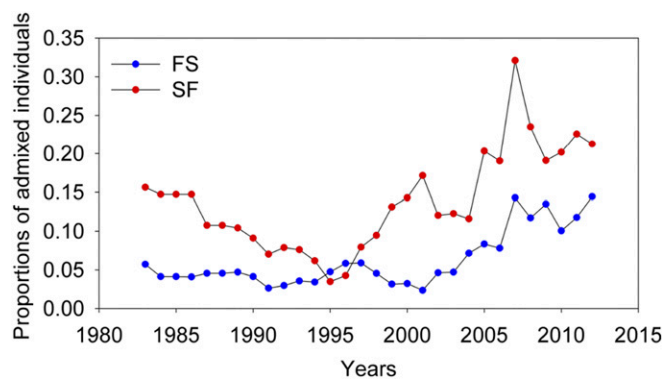


Fig. 1. Proportions of admixed individuals in samples of *G. fortis* (FS) and *G. scandens* (SF).

**Morphological Change.** Morphological effects of introgression are manifested as changes in mean values of beak length and beak depth, and in variation of both (34). Expected changes are based on the standard quantitative genetics model of additive genetic variation and covariation underlying continuously varying traits (5, 53). The changes are expected to be linear through time in view of high hybrid fitness (Introduction), except when affected by natural selection on the means (33–35). In some years there is no breeding (33–35, 54) and hence no gains (recruitment) of hybrids to the population. This results in small perturbations to linearity driven solely by losses (mortality and emigration).

**Means.** The average beak length of *G. scandens*, the larger species, should become smaller if introgressive hybridization of the 2 species is the sole cause of year-to-year changes. The predicted decline in average *G. scandens* beak length from 1987 onward is observed (Fig. 2). It is illustrated by a trend line fitted to the data by least squares regression (adjusted  $R^2 = 0.78$ ). In contrast to beak length, mean beak depth of *G. scandens* is not expected to decline regularly because it is much more similar to *G. fortis* than is beak length (Fig. 2). For example, overall averages with 95% confidence intervals for the 35 y are  $9.18 \pm 0.28$  mm for *G. fortis* and  $9.11 \pm 0.07$  mm for *G. scandens*. As expected, average beak depth of *G. scandens* fluctuates through random sampling without a net change (adjusted  $R^2 = 0.02$ ). The result of a systematic change in length but not in depth is a change in shape, in the direction of increasing bluntness.

For *G. fortis*, the all-else-equal proviso is not applicable as they, unlike *G. scandens*, hybridize with rare immigrants of the small ground finch *G. fuliginosa*. Mean beak dimensions of *G. fortis* gradually declined until 2004, possibly more influenced by breeding with *G. fuliginosa* than with *G. scandens* (*SI Appendix, Fig. S1*). In 2004 the means decreased further and sharply (Fig. 2) when large birds (including FS hybrids) were at a selective disadvantage during a prolonged drought (54). After 2004 there was no further change. Stability could reflect the absence of hybridization, but the high proportion of admixed samples at this time (Fig. 1) when breeding was not studied in detail suggests instead that morphological effects of new hybrids (births) approximately balanced the effects of those that died or emigrated. Morphological effects of adding the relatively large FS hybrids may have been counterbalanced to some extent by addition of the relatively small Ff individuals (*SI Appendix, Fig. S1*).

**Variation.** Variances are predicted to increase as a result of introgression, more in beak length than in beak depth because the species differ more in beak length (34). Since means decreased in both species, with few exceptions, coefficients of variation (CVs) should increase. The expected increases are observed (Fig. 3)





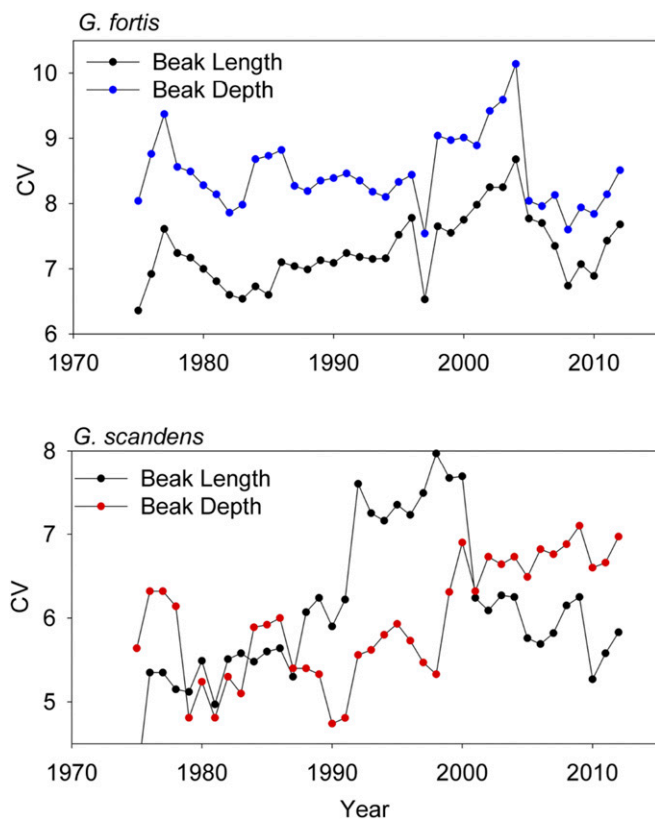


Fig. 3. Annual fluctuations in CVs.

length and depth are genetically correlated, more strongly in *G. fortis* than in *G. scandens* (5). Phenotypic, and possibly genetic, correlations are weakened in  $F_1$  hybrids of species differing in beak proportions such as *G. fortis* and *G. scandens* (5). We interpret the contrast between strong trait covariation in *G. fortis* and weaker covariation in *G. scandens* as a reflection of greater introgression into *G. scandens* than into *G. fortis* (Fig. 1).

Two other factors might have contributed to increases in variation: conspecific gene flow and diversifying selection. In the nonbreeding season Daphne Major island receives immigrants, mainly *G. fortis* and *G. fuliginosa*, from Santa Cruz and Santiago, but they rarely stay to breed (26, 35). The frequency of *G. fortis* immigrants breeding with residents during the period of intense study of breeding was extremely low, and much lower than the frequency of hybridization (26). Immigrant *G. fortis* individuals were larger than average but not extreme and consequently had little effect upon the CVs. However, immigration might have been higher in the last 14 y when breeding was not studied in detail. CVs of *G. scandens* beak length declined at this time, even though admixed individuals were present (Fig. 1), which is contrary to expectation from the hybridization hypothesis (next section). There is no evidence of diversifying selection on this ecologically simple island (33).

#### Ad Hoc Explanations of Beak Length Variation in *G. scandens*

Differential gains of morphologically unusual hybrids could explain the unexpected increase in CV from 1990 to 1991, and differential losses could explain the decrease in CV from 2000 to 2001. The first explanation is tested by a comparison of beak lengths of hybrids present in 1990 ( $n = 7$ ) with those newly recruited in 1991 ( $n = 5$ ). In agreement with expectation, the mean beak length ( $\pm$ SE) of the 1991 recruits ( $11.79 \pm 0.33$  mm)

was substantially smaller ( $t_{11} = 2.43$ ,  $P = 0.0381$ ) than the mean of the 1990 group ( $13.46 \pm 0.60$  mm).

The year 1999 was dry with little breeding and high mortality (33). The change in CV from the following year to the next was due to losses, 2 only, and not to gains. Further, the mean declined to a small extent (Fig. 3), and therefore the change in CV is not explained by the loss of (relatively small) hybrids. A change of variance without a change in mean is consistent with stabilizing selection. Hybridization appears not to have been relevant.

#### Hybridization as a Cause of Increases in Variance and Skewness.

Variation of a sample of measurements increases when hybrids are combined with nonhybrids. When a sample is split into hybrid and nonhybrids, these 2 components are predicted to differ in mean beak dimensions, more in length than in depth, and in a direction determined by the difference in means between species. We tested the prediction with samples of individuals (Table 1) produced in the 5 y of extensive breeding and largest numbers of admixed hybrids identified by microsatellites (2002, 2005, 2008, 2009, and 2010). Ff hybrids were excluded. Results agree with expectations (Table 1). For beak length, in all 5 y FS admixed individuals were larger on average than *G. fortis*, and SF admixed individuals were smaller on average than *G. scandens* (data combined, one-tailed binomial test,  $P = 0.001$ ). Despite small samples of admixed individuals, 5 of the 10 individual comparisons were significantly different. For beak depth, in all 5 y FS admixed individuals were larger on average than *G. fortis* ( $P = 0.031$ ), but SF admixed individuals were larger on average than *G. scandens* in only 3 comparisons ( $P = 0.812$ ). The same procedure resulted in predictable changes in skewness. Frequency distributions are predicted by the hybridization hypothesis to be right-skewed, positively, in *G. fortis* toward the larger species, and left-skewed, negatively, in *G. scandens* (SI Appendix, section 4). Removal of hybrids should reduce, if not eliminate, skewness in frequency distributions of beak measurements (34). Reduction of skewness was observed for *G. scandens* beak length in all 5 y ( $P = 0.031$ ) and in 4 y for *G. fortis* beak length. With data from the 2 species combined the one-tailed binomial probability is 0.011. Neither expectation was realized for beak depth ( $P = 0.188$ ).

#### Hybridization on Other Islands

**Patterns of Variation in Time.** Variation is expected to increase and then to decrease in phylogenetic time according to the argument in the Introduction. Differences in average population variation of the 18 species (SI Appendix, section 5) fit the expected temporal pattern (Fig. 4). A maximum in average beak length and depth variation is reached after about 200,000 y. Roughly 100,000 y after that, variability has decreased to an apparent long-term average. Thus, there is a window of unknown width during which gene exchange has the greatest effect on phenotypic variation. This interpretation assumes that the pattern reflects a process: that each species has the potential to increase and then decrease in variation through time. Enhanced phenotypic variation probably reflects enhanced genetic variation because beak traits are highly heritable in the 3 species that have been studied in detail (25, 55, 56).

The low values of average coefficients of variation for species older than 400,000 y does not mean those species do not hybridize; it means only that introgression is not on a sufficient scale to affect morphological variation. Introgressive hybridization may persist for much longer. Price and Bouvier (57), surveying data from a large number of passerine bird species, estimated that infertility of hybrids arises on average 7 My after separation of the parental species from a common ancestor.

**Unusually Variable Populations.** Coefficients of variation of individual populations are informative about hybridization even in the absence of age estimates (Fig. 5). A previous study of North American and South American emberizids, and Hawaiian cardueline

**Table 1. Beak dimensions of species compared with admixed individuals (FS or SF)**

Year	Group	n	Length			Depth		
			Mean	SE	P	Mean	SE	P
2002	<i>G. fortis</i>	69	10.85	0.094	<b>0.0078</b>	9.10	0.099	0.2505*
	FS	7	11.75	0.371		9.70	0.469	
	<i>G. scandens</i>	67	13.48	0.099	0.0995*	9.06	0.075	0.6363
	SF	7	12.31	0.597		8.94	0.196	
2005	<i>G. fortis</i>	93	10.31	0.080	<b>&lt;0.0001</b>	8.62	0.062	0.1379*
	FS	10	12.11	0.257		9.88	0.343	
	<i>G. scandens</i>	29	13.17	0.124	0.1181*	9.05	0.124	0.5354
	SF	10	12.65	0.286		9.20	0.182	
2008	<i>G. fortis</i>	79	10.23	0.082	0.1186*	8.47	0.074	0.1514*
	FS	5	11.34	0.563		9.22	0.422	
	<i>G. scandens</i>	97	13.41	0.077	<b>0.0110</b>	8.99	0.058	0.2540
	SF	19	12.65	0.261		9.16	0.172	
2009	<i>G. fortis</i>	51	10.28	0.109	0.1406*	8.37	0.106	0.3342
	FS	5	11.50	0.666		8.72	0.320	
	<i>G. scandens</i>	40	13.46	0.142	0.2250*	9.10	0.103	0.4676
	SF	5	12.42	0.726		9.32	0.213	
2010	<i>G. fortis</i>	74	10.40	0.086	<b>0.0262*</b>	8.46	0.072	0.2171
	FS	10	11.36	0.359		8.73	0.268	
	<i>G. scandens</i>	59	13.28	0.125	<b>&lt;0.0001</b>	8.92	0.085	0.3132
	SF	15	11.91	0.317		8.73	0.177	

P values are associated with one-tailed t tests that assume unequal variances or, when variances were equal, by F tests as indicated by an asterisk (\*). Significance is indicated by boldface for emphasis. Original measurements were in millimeters.

finches, found that coefficients greater than 6.0 are rare and present in only 3 of 46 samples of beak length variation in 19 species and 2 of 44 samples of beak depth variation in 17 species (5, 25, 42). Coefficients exceeding 6.0 are much more common among Darwin's finch populations. In the total dataset (SI Appendix, Table S1), 27 of 186 coefficients (14.5%) are >6.0. They are more frequent in beak depth than in beak length (Table 2) and are heterogeneous in 3 respects. First, all but 2 of them are in the ground finch genus *Geospiza* (Table 2). Second, most of them are from populations of one species, *G. fortis*. Third, populations of the same species differ strikingly in levels of variation. *G. fortis* is the best example. Most (8) of the 13 populations of this species are unusually variable in one or both beak dimensions. Coefficients of beak depth variation range from 3.4 ( $\pm 0.08$  SD) on Marchena to 10.3 ( $\pm 1.43$ ) on Santa Cruz. Thus, variation itself varies among species and islands.

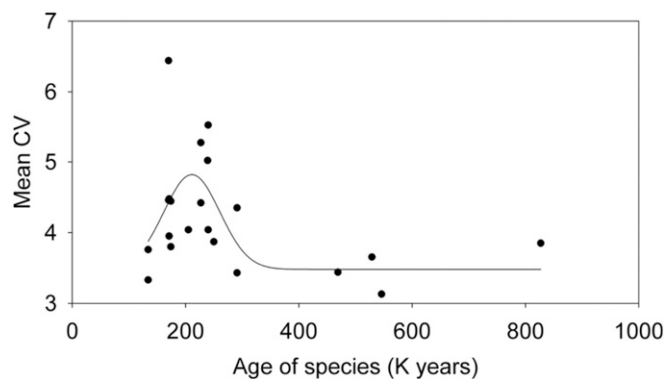
**Comparative Tests.** When 2 hybridizing species differ more in one beak dimension than in the other, the hybridization hypothesis predicts the dimension that differs most will be the more variable. Field observations allow 2 tests.

***G. fuliginosa* and *G. scandens* on San Cristóbal.** Interbreeding was observed in the Bosque de Cactus in April 1997 and February 2018 (personal observation). The 2 species differ more in mean beak length (51.3%) than in mean beak depth (20.7%) and therefore are predicted to be unusually variable in beak length. This is observed in both species (Table 2).

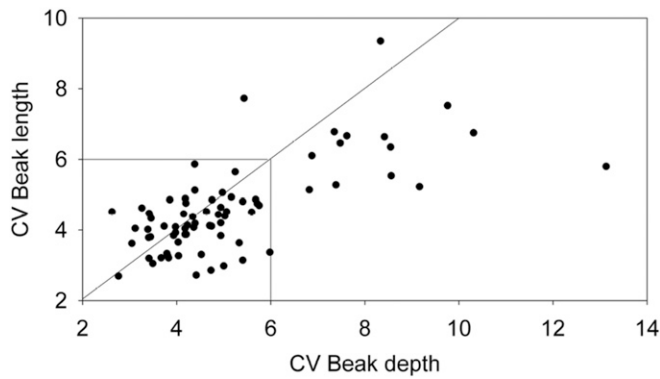
***Geospiza propinqua* and *G. magnirostris* on Genovesa.** Hybridization was studied in the years 1978 to 1988 (25). The species differ more in average mean depth (63.4%) than in mean beak length (15.1%). They are predicted to be unusually variable in beak depth but not in length. This is observed in *G. propinqua* but not in *G. magnirostris*. A possible reason for the difference is unidirectional gene flow through differential backcrossing into *G. propinqua*. The direction of backcrossing would be influenced not only by paternal song but also by the size difference between the sexes. Males are generally larger than females (25, 42) and *G. magnirostris* are larger than *G. propinqua*, so *G. propinqua*

males  $\times$  *G. magnirostris* females are more size-compatible than *G. magnirostris* males  $\times$  *G. propinqua* females. *G. propinqua* males  $\times$  *G. magnirostris* pairs produce offspring that imprint on paternal song, and these backcross to *G. propinqua* (25, 49).

**Indirect Evidence of Hybridization.** Populations of *Geospiza conirostris* and *G. fuliginosa* coexist on Española and Gardner with no other congeneric species. If they hybridize, they are expected to be unusually variable in both beak dimensions because on Española they differ substantially in both beak length (76.9%) and beak depth (92.9%); data for *G. fuliginosa* on Gardner are lacking. The expectation is realized in *G. conirostris* in both beak dimensions and on both islands, but not in *G. fuliginosa*. (SI Appendix, Table S1).



**Fig. 4.** Average CVs of beak length for male specimens of each species of Darwin's finches in relation to their ages. Points fitted by nonlinear, polynomial regression: peak, Gaussian, and 4 parameters. The adjusted  $R^2$  value is 0.23. Included are 2 pairs of populations, *Certhidea fusca* from Española and San Cristóbal and *Geospiza septentrionalis* from Wolf and Darwin. These are the only pairs of conspecific populations with estimated ages of independence (40). Morphological data are from ref. 41. The curve for beak depth variation is almost identical.



**Fig. 5.** CVs for beak dimensions of adult males of 75 populations of Darwin's finches (41).

The difference between the species can be rationalized as the result of unidirectional gene exchange. This hypothesis should be tested. Breeding patterns on these islands are almost unknown (58).

***G. fortis* has several unusually variable populations.** The species is morphologically intermediate between a smaller (*G. fuliginosa*) and a larger (*G. magnirostris*) species with similar but not identical beak proportions. If *G. fortis* receives genes from one or both of the others it should be unusually variable in beak depth, because this dimension is more strongly correlated with size (5) than is beak length, or in both beak dimensions, but not in beak length alone. Expected differences are observed. Six populations are unusually variable in both beak length and beak depth, and 2 populations are unusually variable in only beak depth. Five populations are not unusually variable in either beak dimension (SI Appendix, Table S1). There are no modern studies of them that could serve as a basis for an explanation. Sample sizes are small ( $n = 10$  to 15) for 2 of them (Rábida and Fernandina). There are also no studies of the exceptionally variable population of *G. magnirostris* on Darwin whose variation is difficult to interpret (SI Appendix, section 6).

**Solitary Finches.** *Pinaroloxias inornata* occurs on the strongly isolated Cocos Island (43). In the absence of other finch species, it is neither expected nor observed to be unusually variable

(SI Appendix, Table S1). In the Galápagos, *G. fuliginosa* is the only species present on Los Hermanos, a cluster of 3 islets off the coast of Isabela. It is unusually variable in both beak depth and length. The variation could be the product of diversifying selection, but the islets are small and with little heterogeneity in vegetation, so the unusual variation is more likely to be the result of breeding with occasional *G. fortis* immigrants from nearby Isabela (59).

**Conclusions and Discussion**

Adaptive radiations provide rich material for understanding how and why natural and sexual selection cause populations to diverge, how barriers to interbreeding arise, why some taxa radiate and others do not (60), and how species accumulation through speciation is reduced by extinction (61–63). Introgressive hybridization in the early stages of a radiation may play a central role in both speciation (3, 4, 15, 64, 65) or its opposite, the collapse of the speciation process (38, 66–70). Those roles can be inferred as historical processes using genomic data (15, 16, 71) but can only be studied directly with currently hybridizing species, which is why there is a need to integrate the findings of hybridization in the past and the present in those radiating taxa where this is possible, such as cichlid fish (15, 16, 71, 72), estrildid finches (73), heliconiine butterflies (9, 74) and the Hawaiian silversword alliance (75–77). It is not possible in older radiations, such as *Anolis* lizards in the Caribbean that experience little or no contemporary hybridization (78), and Hawaiian honeycreeper finches that have lost many species through human-caused extinctions (79, 80).

Darwin's finches are a model group for the study of adaptive radiation, and the finches on Daphne Major island are a model subgroup for studying evolution in contemporary time. Here we have combined the study of evolution in the past with evolution in the present by 1) quantifying contemporary hybridization and 2) examining the implication of increased variation in phylogenetically young species. Exchange of genes through introgressive hybridization provides a working hypothesis and an organizing framework for understanding levels of continuous morphological variation that are elevated above a background determined by mutation, drift, and intraspecific gene flow (6).

We found that population variation in Darwin's finch beak traits increased with time since sharing a common ancestor with a sister species, reached a peak, and then fell. If the pattern of rise and fall of variation is due to a changing gene exchange, as

**Table 2. Populations with unusually variable beak length or depth by the criterion of CV >6.0 are shown in red**

Species	Island	Sample length	Mean length	CV length	Sample depth	Mean depth	CV depth
<i>G. magnirostris</i>	Darwin	17	14.99	5.831	16	16.53	13.134
<i>G. magnirostris</i>	Santa Cruz	13	15.28	5.229	14	19.11	9.173
<i>G. fortis</i>	Seymour	26	11.69	6.672	24	12.55	7.625
<i>G. fortis</i>	Santa Cruz	105	11.95	6.770	100	12.82	10.304
<i>G. fortis</i>	Pinzón	30	11.26	6.465	25	11.46	7.522
<i>G. fortis</i>	N. Isabela	65	11.49	6.319	64	12.41	8.501
<i>G. fortis</i>	S. Isabela	70	12.45	6.675	65	13.86	8.434
<i>G. fortis</i>	San Cristóbal	109	12.20	5.475	101	13.21	8.562
<i>G. fortis</i>	Floreana	181	11.65	7.579	173	12.50	9.776
<i>G. fortis</i>	Daphne	31	10.51	5.157	26	10.67	6.823
<i>G. fuliginosa</i>	San Cristóbal	125	8.79	7.702	112	8.08	5.446
<i>G. fuliginosa</i>	Los Hermanos	12	9.32	9.367	11	9.55	8.398
<i>G. scandens</i>	San Cristóbal	15	12.86	6.112	12	9.54	2.943
<i>G. propinqua</i>	Genovesa	43	14.35	5.310	40	12.98	7.381
<i>G. conirostris</i>	Española	87	15.43	6.079	80	15.97	6.913
<i>G. conirostris</i>	Gardner	72	14.64	6.742	67	15.05	7.395
<i>Camarhynchus psittacula</i>	Santiago	17	9.84	3.394	12	11.15	6.018
<i>Certhidea fusca</i>	Pinzón	21	7.56	6.151	—	—	—

CV =  $100 \times \text{SD}/\text{mean}$ . Original measurements were in millimeters.





of the assignments. Ninety-seven percent of 167 *G. fortis* families produced only *G. fortis* offspring ( $n = 590$ ), and 99% of 47 *G. scandens* families produced only *G. scandens* offspring ( $n = 175$ ). Statistical analyses were performed in JMP (SAS Institute). All tests were 2-tailed, unless indicated otherwise.

**Data Availability.** Data are available from the authors upon request.

- R. C. Lewontin, L. C. Birch, Hybridization as a source of variation for adaptation to new environments. *Evolution* **20**, 315–336 (1966).
- G. Svärdson, “Significance of introgression in coregonid evolution” in *Biology of Coregonid Fishes*, C. C. Lindsey, C. S. Woods, Eds. (University of Manitoba Press, Winnipeg, 1970), pp. 39–59.
- R. Abbott *et al.*, Hybridization and speciation. *J. Evol. Biol.* **26**, 229–246 (2013).
- S. A. Taylor, E. L. Larson, Insights from genomes into the evolutionary importance and prevalence of hybridization in nature. *Nat. Ecol. Evol.* **3**, 170–177 (2019).
- P. R. Grant, B. R. Grant, Phenotypic and genetic effects of hybridization in Darwin’s finches. *Evolution* **48**, 297–316 (1994).
- P. W. Hedrick, Adaptive introgression in animals: Examples and comparison to new mutation and standing variation as sources of adaptive variation. *Mol. Ecol.* **22**, 4606–4618 (2013).
- O. Seehausen, Conditions when hybridization might predispose populations for adaptive radiation. *J. Evol. Biol.* **26**, 279–281 (2013).
- R. J. Pereira, F. S. Barreto, R. S. Burton, Ecological novelty by hybridization: Experimental evidence for increased thermal tolerance by transgressive segregation in *Tigriopus californicus*. *Evolution* **68**, 204–215 (2014).
- J. Mavárez *et al.*, Speciation by hybridization in *Heliconius* butterflies. *Nature* **441**, 868–871 (2006).
- P. R. Grant, B. R. Grant, Synergism of natural selection and introgression in the origin of a new species. *Am. Nat.* **183**, 671–681 (2014).
- P. Lavretsky, A. Engilis, Jr, J. M. Eadie, J. L. Peters, Genetic admixture supports an ancient hybrid origin of the endangered Hawaiian duck. *J. Evol. Biol.* **28**, 1005–1015 (2015).
- T. O. Elgvin *et al.*, The genomic mosaicism of hybrid speciation. *Sci. Adv.* **3**, e1602996 (2017).
- S. Lamichhane *et al.*, Rapid hybrid speciation in Darwin’s finches. *Science* **359**, 224–228 (2018).
- O. Seehausen, Hybridization and adaptive radiation. *Trends Ecol. Evol.* **19**, 198–207 (2004).
- M. Malinsky *et al.*, Whole-genome sequences of Malawi cichlids reveal multiple radiations interconnected by gene flow. *Nat. Ecol. Evol.* **2**, 1940–1955 (2018).
- I. Irisarri, *et al.*, Phylogenomics uncovers early hybridization and adaptive loci shaping the radiation of Lake Tanganyika cichlid fishes. *Nat. Comm.* **9**, 3159 (2018).
- J. Kulmuni, P. Pamilo, Introgression in hybrid ants is favored in females but selected against in males. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 12805–12810 (2014).
- R. A. Duckworth, G. A. Semenov, Hybridization associated with cycles of ecological succession in a passerine bird. *Am. Nat.* **190**, E94–E105 (2017).
- D. Wheatcroft, A. Qvarnström, Reproductive character displacement of female, but not male song discrimination in an avian hybrid zone. *Evolution* **71**, 1776–1786 (2017).
- M. L. Arnold, *Natural Hybridization and Evolution* (Oxford University Press, Oxford, 1997).
- L. H. Rieseberg *et al.*, Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* **301**, 1211–1216 (2003).
- S. Stankowski, M. A. Streisfeld, Introgressive hybridization facilitates adaptive divergence in a recent radiation of monkeyflowers. *Proc. Biol. Sci.* **282**, 20151666 (2015).
- B. E. Goulet, F. Roda, R. Hopkins, Hybridization in plants: Old ideas and new techniques. *Plant Physiol.* **173**, 65–78 (2017).
- A. Suarez-Gonzalez, C. Lexer, Q. C. B. Cronk, Adaptive introgression: A plant perspective. *Biol. Lett.* **14**, 20170688 (2018).
- B. R. Grant, P. R. Grant, *Evolutionary Dynamics of a Natural Population* (University of Chicago Press, Chicago, 1989).
- P. R. Grant, B. R. Grant, Conspecific versus heterospecific gene exchange between populations of Darwin’s finches. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **365**, 1065–1076 (2010).
- K. J. Peters, S. A. Myers, R. Y. Dudaniec, J. A. O’Connor, S. Kleindorfer, Females drive asymmetrical introgression from rare to common species in Darwin’s tree finches. *J. Evol. Biol.* **30**, 1940–1952 (2017).
- R. I. Bowman, “The evolution of song in Darwin’s Finches” in *Patterns of Evolution in Galapagos Organisms*, R. I. Bowman, M. Berson, A. E. Leviton, Eds. (American Association for the Advancement of Science, Pacific Division, San Francisco, CA, 1983), pp. 237–537.
- P. R. Grant, B. R. Grant, K. Petren, Hybridization in the recent past. *Am. Nat.* **166**, 56–67 (2005).
- H. L. Farrington, L. P. Lawson, C. M. Clark, K. Petren, The evolutionary history of Darwin’s finches: Speciation, gene flow, and introgression in a fragmented landscape. *Evolution* **68**, 2932–2944 (2014).
- S. Kleindorfer *et al.*, Species collapse via hybridization in Darwin’s tree finches. *Am. Nat.* **183**, 325–341 (2014).
- L. P. Lawson *et al.*, Slow motion extinction: Inbreeding, introgression, and loss in the critically endangered mangrove finch (*Camarhynchus heliobates*). *Conserv. Genet.* **18**, 159–170 (2017).
- P. R. Grant, B. R. Grant, *40 Years of Evolution. Darwin’s Finches on Daphne Major Island* (Princeton University Press, Princeton, 2014).
- P. R. Grant, B. R. Grant, Unpredictable evolution in a 30-year study of Darwin’s finches. *Science* **296**, 707–711 (2002).
- P. T. Boag, P. R. Grant, Darwin’s finches (*Geospiza*) on isla Daphne major, Galápagos: Breeding and feeding ecology in a climatically variable environment. *Ecol. Monogr.* **54**, 463–489 (1984).
- P. R. Grant, T. D. Price, Population variation in continuously varying traits as an ecological genetics problem. *Am. Zool.* **21**, 795–811 (1981).
- P. R. Grant, B. R. Grant, Hybridization of bird species. *Science* **256**, 193–197 (1992).
- P. R. Grant, B. R. Grant, J. A. Markert, L. F. Keller, K. Petren, Convergent evolution of Darwin’s finches caused by introgressive hybridization and selection. *Evolution* **58**, 1588–1599 (2004).
- K. J. Burns *et al.*, Phylogenetics and diversification of tanagers (Passeriformes: Thraupidae), the largest radiation of Neotropical songbirds. *Mol. Phylogenet. Evol.* **75**, 41–77 (2014).
- S. Lamichhane *et al.*, Evolution of Darwin’s finches and their beaks revealed by genome sequencing. *Nature* **518**, 371–375 (2015).
- D. Lack, The galapagos finches (geospizinae): A study in variation. *Occas. Pap. Calif. Acad. Sci.* **21**, 1–159 (1945).
- P. R. Grant, I. Abbott, D. Schluter, R. L. Curry, L. K. Abbott, Variation in the size and shape of Darwin’s finches. *Biol. J. Linn. Soc. Lond.* **25**, 1–39 (1985).
- P. R. Grant, *Ecology and Evolution of Darwin’s Finches* (Princeton University Press, Princeton, 1986).
- L. M. Ratcliffe, P. R. Grant, Species recognition in Darwin’s finches (*Geospiza*, gould). I. Discrimination by morphological cues. *Anim. Behav.* **31**, 1139–1153 (1983).
- L. M. Ratcliffe, P. R. Grant, Species recognition in Darwin’s Finches (*Geospiza*, Gould). II. Geographic variation in mate preference. *Anim. Behav.* **31**, 1154–1165 (1983).
- L. M. Ratcliffe, P. R. Grant, Species recognition in Darwin’s Finches (*Geospiza*, Gould). III. Male responses to playback of different song types, dialects and heterospecific songs. *Anim. Behav.* **33**, 290–307 (1985).
- P. R. Grant, B. R. Grant, Mating patterns of finch hybrids determined by song and morphology. *Biol. J. Linn. Soc. Lond.* **60**, 317–343 (1997).
- B. R. Grant, P. R. Grant, Simulating secondary contact in allopatric speciation: An empirical test of premating isolation. *Biol. J. Linn. Soc. Lond.* **60**, 317–343 (2002).
- B. R. Grant, P. R. Grant, Cultural inheritance of song and its role in the evolution of Darwin’s finches. *Evolution* **50**, 2471–2487 (1996).
- P. R. Grant, B. R. Grant, Role of sexual imprinting in assortative mating and premating isolation in Darwin’s finches. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E10879–E10887 (2018).
- P. T. Boag, P. R. Grant, Intense natural selection in a population of Darwin’s finches (geospizinae) in the Galápagos. *Science* **214**, 82–85 (1981).
- H. L. Gibb, P. R. Grant, Oscillating selection on Darwin’s finches. *Nature* **327**, 511–513 (1987).
- D. G. Falconer, T. F. C. Mackay, *Introduction to Quantitative Genetics* (Longman, Harlow, UK, ed. 4, 1996).
- P. R. Grant, B. R. Grant, Evolution of character displacement in Darwin’s finches. *Science* **313**, 224–226 (2006).
- P. R. Grant, B. R. Grant, “Quantitative genetic variation in populations of Darwin’s Finches” in *Adaptive Variation in the Wild*, T. A. Mousseau, B. Sinervo, J. Endler, Eds. (Oxford University Press, New York, 1999), pp. 3–41.
- L. F. Keller, P. R. Grant, B. R. Grant, K. Petren, Heritability of morphological traits in Darwin’s finches: Misidentified paternity and maternal effects. *Heredity* **87**, 325–336 (2001).
- T. D. Price, M. M. Bouvier, The evolution of F1 postzygotic incompatibilities in birds. *Evolution* **56**, 2083–2089 (2002).
- J. F. Downhower, Observations on the nesting of the small ground finch *Geospiza fuliginosa* and the large cactus finch *Geospiza conirostris* on Española, Galápagos. *Ibis* **120**, 340–346 (1978).
- D. Schluter, T. D. Price, P. R. Grant, Ecological character displacement in Darwin’s finches. *Science* **227**, 1056–1059 (1985).
- O. Seehausen, African cichlid fish: A model system in adaptive radiation research. *Proc. Biol. Sci.* **273**, 1987–1998 (2006).
- D. L. Rabosky, I. J. Lovette, Explosive evolutionary radiations: Decreasing speciation or increasing extinction through time? *Evolution* **62**, 1866–1875 (2008).
- D. L. Rabosky, R. E. Glor, Equilibrium speciation dynamics in a model adaptive radiation of island lizards. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 22178–22183 (2010).
- L. F. Henaó Diaz, L. J. Harmon, M. T. C. Sugawara, E. T. Miller, M. W. Pennell, Macroevolutionary diversification rates show time dependency. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 7403–7408 (2019).
- K. J. Parsons, Y. H. Son, R. C. Albertson, Hybridization promotes evolvability in african cichlids: Connections between transgressive segregation and phenotypic integration. *Evol. Biol.* **38**, 306–315 (2011).
- J. Mallet, N. Besansky, M. W. Hahn, How reticulated are species? *Bioessays* **38**, 140–149 (2016).



66. T. Dobzhansky, *Genetics and the Origin of Species* (Columbia University Press, New York, ed. 2, 1941).
67. J. M. Rhymer, D. Simberloff, Extinction by hybridization and introgression. *Annu. Rev. Ecol. Syst.* **27**, 83–109 (1996).
68. E. B. Taylor *et al.*, Speciation in reverse: Morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Mol. Ecol.* **15**, 343–355 (2006).
69. W. C. Webb, J. M. Marzluff, K. E. Omland, Random interbreeding between cryptic lineages of the common raven: Evidence for speciation in reverse. *Mol. Ecol.* **20**, 2390–2402 (2011).
70. P. Vonlanthen *et al.*, Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature* **482**, 357–362 (2012).
71. W. Salzburger, Understanding explosive diversification through cichlid fish genomics. *Nat. Rev. Genet.* **19**, 705–717 (2018).
72. J. I. Meier *et al.*, Ancient hybridization fuels rapid cichlid adaptive radiations. *Nat. Comm.* **8**, 14363 (2017).
73. K. F. Stryjewski, M. D. Sorenson, Mosaic genome evolution in a recent and rapid avian radiation. *Nat. Ecol. Evol.* **1**, 1912–1922 (2017).
74. J. Enciso-Romero *et al.*, Evolution of novel mimicry rings facilitated by adaptive introgression in tropical butterflies. *Mol. Ecol.* **26**, 5160–5172 (2017).
75. M. Barrier, R. H. Robichaux, M. D. Purugganan, Accelerated regulatory gene evolution in an adaptive radiation. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 10208–10213 (2001).
76. M. D. Purugganan, R. H. Robichaux, Adaptive radiation and regulatory gene evolution in the Hawaiian silversword alliance (Asteraceae). *Ann. Mo. Bot. Gard.* **92**, 28–35 (2005).
77. A. Lawton-Rauh, R. H. Robichaux, M. D. Purugganan, Diversity and divergence patterns in regulatory genes suggest differential gene flow in recently derived species of the Hawaiian silversword alliance adaptive radiation (Asteraceae). *Mol. Ecol.* **16**, 3995–4013 (2007).
78. J. B. Losos, *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles* (University of California Press, Berkeley, 2009).
79. P. R. Grant, Population variation and hybridization: Comparison of finches from two archipelagos. *Evol. Ecol.* **8**, 598–617 (1994).
80. H. R. Lerner, M. Meyer, H. F. James, M. Hoffreiter, R. C. Fleischer, Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Curr. Biol.* **21**, 1838–1844 (2011).
81. O. M. Selz, K. Lucek, K. A. Young, O. Seehausen, Relaxed trait covariance in interspecific cichlid hybrids predicts morphological diversity in adaptive radiations. *J. Evol. Biol.* **27**, 11–24 (2014).
82. R. Stelkens, O. Seehausen, Genetic distance between species predicts novel trait expression in their hybrids. *Evolution* **63**, 884–897 (2009).
83. W. Moore, J. A. Robertson, Explosive adaptive radiation and extreme phenotypic diversity within ant-nest beetles. *Curr. Biol.* **24**, 2435–2439 (2014).
84. C. Hughes, R. Eastwood, Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the andes. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 10334–10339 (2006).
85. E. M. Prager, A. C. Wilson, “Phylogenetic relationships and rates of evolution of birds” in *Proceedings of the 16th International Ornithological Congress* (Verlag der Deutsche Ornithologen-Gesellschaft, Berlin, 1995), pp. 1209–1214.
86. T. L. Parchman *et al.*, The genomic consequences of adaptive divergence and reproductive isolation between species of manakins. *Mol. Ecol.* **22**, 3304–3317 (2013).
87. J. L. Rifkin, A. S. Castillo, I. T. Liao, M. D. Rausher, Gene flow, divergent selection and resistance to introgression in two species of morning glories (*Ipomoea*). *Mol. Ecol.* **28**, 1709–1729 (2019).
88. Y. Song *et al.*, Adaptive introgression of anticoagulant rodent poison resistance by hybridization between old world mice. *Curr. Biol.* **21**, 1296–1301 (2011).
89. L. C. Norris *et al.*, Adaptive introgression in an African malaria mosquito coincident with the increased usage of insecticide-treated bed nets. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 815–820 (2015).
90. D. Wen, Y. Yu, M. W. Hahn, L. Nakhleh, Reticulate evolutionary history and extensive introgression in mosquito species revealed by phylogenetic network analysis. *Mol. Ecol.* **25**, 2361–2372 (2016).
91. J. Bechsgaard, T. H. Jorgensen, M. H. Schierup, Evidence for adaptive introgression of disease resistance genes among closely related *Arabidopsis* species. *G3 (Bethesda)* **7**, 2677–2683 (2017).
92. D. M. Hooper, S. C. Griffith, T. D. Price, Sex chromosome inversions enforce reproductive isolation across an avian hybrid zone. *Mol. Ecol.* **28**, 1246–1262 (2019).
93. J. M. Sardell, J. A. C. Uy, Hybridization following recent secondary contact results in asymmetric genotypic and phenotypic introgression between island species of *Myzomela* honeyeaters. *Evolution* **70**, 257–269 (2016).
94. S. E. Kingston, T. L. Parchman, Z. Gompert, C. A. Buerkle, M. J. Braun, Heterogeneity and concordance in locus-specific differentiation and introgression between species of towhees. *J. Evol. Biol.* **30**, 474–485 (2017).
95. A. Runemark *et al.*, Variation and constraints in hybrid genome formation. *Nat. Ecol. Evol.* **2**, 549–556 (2018).
96. D. A. Marques, J. I. Meier, O. Seehausen, A combinatorial view on speciation and adaptation. *Trends Ecol. Evol.* **34**, 531–544 (2019).
97. S. Lamichhaney *et al.*, A beak size locus in Darwin’s finches facilitated character displacement during a drought. *Science* **352**, 470–474 (2016).
98. J. A. Chaves *et al.*, Genomic variation at the tips of the adaptive radiation of Darwin’s finches. *Mol. Ecol.* **25**, 5282–5295 (2016).
99. A. Abzhanov *et al.*, The calmodulin pathway and evolution of elongated beak morphology in Darwin’s finches. *Nature* **442**, 563–567 (2006).
100. A. Abzhanov, M. Protas, B. R. Grant, P. R. Grant, C. J. Tabin, *Bmp4* and morphological variation of beaks in Darwin’s finches. *Science* **305**, 1462–1465 (2004).
101. S. A. Brugmann *et al.*, Comparative gene expression analysis of avian embryonic facial structures reveals new candidates for human craniofacial disorders. *Hum. Mol. Genet.* **19**, 920–930 (2010).
102. P. R. Grant, B. R. Grant, *How and Why Species Multiply* (Princeton University Press, Princeton, 2008).
103. D. Schluter, P. R. Grant, Determinants of morphological patterns in communities of Darwin’s finches. *Am. Nat.* **123**, 175–196 (1984).
104. A. P. Hendry *et al.*, Possible human impacts on adaptive radiation: Beak size bimodality in Darwin’s finches. *Proc. Biol. Sci.* **273**, 1887–1894 (2006).
105. S. K. Huber, L. F. De León, A. P. Hendry, E. Bermingham, J. Podos, Reproductive isolation of sympatric morphs in a population of Darwin’s finches. *Proc. Biol. Sci.* **274**, 1709–1714 (2007).
106. G. Bell, Evolutionary rescue and the limits of adaptation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **368**, 20120080 (2013).
107. O. Razzgour *et al.*, Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 10418–10423 (2019).
108. V. Radchuk *et al.*, Adaptive responses of animals to climate change are most likely insufficient. *Nat. Commun.* **10**, 3109 (2019).
109. P. R. Grant *et al.*, Evolution caused by extreme events. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **372**, 20160146 (2017).