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Book Author(s): Peter R. Grant and B. Rosemary Grant

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# Variation and Introgression

Introgression may be said to have taken over the role of excessive mutation in providing multitudes of new genetic combinations involving total blocks of cooperating genes.

(Svårdson 1970, p. 57)

To evaluate the evolutionary significance of hybridization requires a full accounting of the selective forces that act on the production of hybrids, and on their reproduction and survival. This includes consideration of the consequences of gene flow both within and between species.

(Schemske and Morgan 1990, p. 2151)

## Introduction

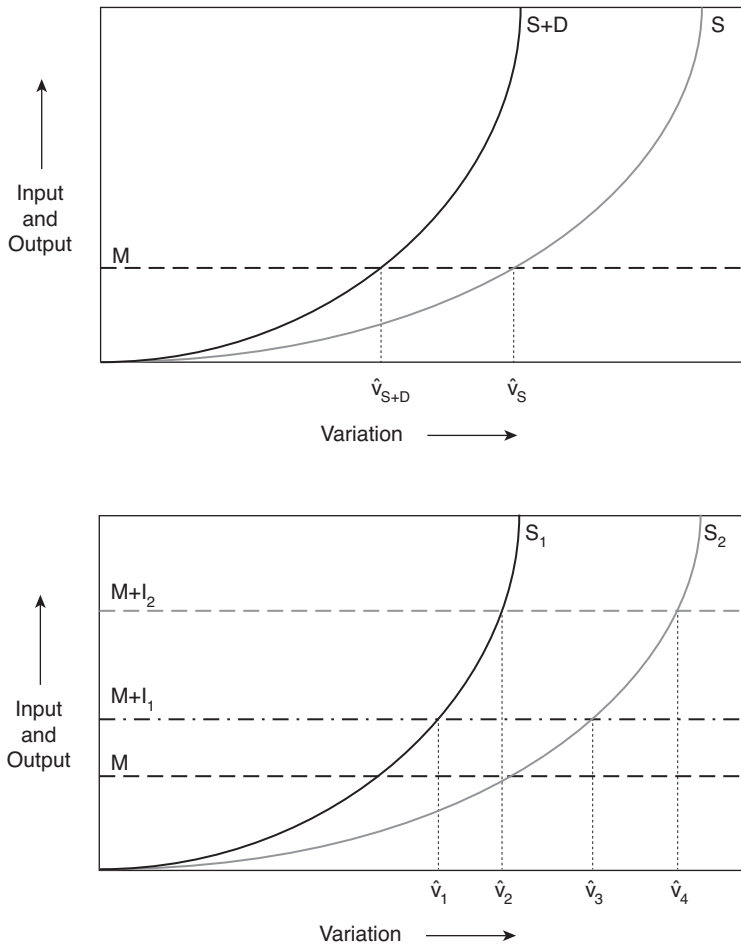
**M**ANY BIRD SPECIES, approximately one-tenth of roughly ten thousand species, are known to hybridize (Panov 1989, Grant and Grant 1992b, McCarthy 2006). Hybridization and subsequent introgression through backcrossing can be a powerful source of significant genetic variation because it can generate new combinations of genes in either or both parental populations, allowing a rapid evolutionary response to new or changing environments. In the Galápagos Archipelago closely related species occur in sympatry in various combinations (Grant 1986). This provides many opportunities for occasional hybridization with introgression to contribute to the high varia-

tion observed in morphological traits. As we mentioned in the previous chapter, our study of breeding yielded evidence of hybridization right at the beginning, in 1976 (Grant and Price 1981, Boag and Grant 1984b). This made us realize we could not afford to ignore it as a possible source of genetic input into a population and one of several factors affecting the observed levels of morphological variation in *fortis* and *scandens*.

When we began, studies of genetic variation in laboratory populations and in animal breeding considered standing variation at any one time to be the product of agents that increased or decreased variation: mutation and recombination elevate genetic variation, and stabilizing selection and random drift deplete it. The presence of abundant polygenic variation was nevertheless seen theoretically as a problem to be explained in the face of strong stabilizing selection that should rapidly eliminate it (Barton 1990). The primary evidence for strong stabilizing selection was the low fitness of extreme individuals in some studies (Endler 1986, Barton 1990). The problem disappears if mutation rates are high, for then losses due to selection are balanced at equilibrium by gains from mutation (Lande 1976).

This four-factor scheme with mutation-selection balance at its center is a useful theoretical framework for empirical studies and is sufficient for closed populations. In nature more is needed because populations there are open to exchange of individuals, environments are not constant in time or space, and selection regimes other than stabilizing may operate (Grant and Grant 1989). Variation at any one time is set by a resolution of all these factors. The point of resolution for Darwin's finches is likely to vary from one island to another as a result of variation in the relative strengths of the factors, and to vary from time to time because environments are not constant (Grant and Grant 1989, 2000a; fig. 9.1). For these various reasons the question of why populations display different levels of variation is as much an ecological genetics problem, dependent on the environment, as it is a population genetics problem, dependent on demography (Grant and Price 1981). Both genes and environment play essential roles (fig. 4.1), and the critical and debated question is how they do (e.g., Bürger 1999, Zhang 2012).

The goal of this chapter is to consider how introgressive hybridization might affect the maintenance of variation in individual traits, as well as covariation in correlated traits. Our study is not comprehensive but narrowly focused on introgression. To understand variation in its absence would require knowledge of how it arises from genetic mechanisms—mutation, gene duplication and conversion, recombination, pleiotropy, and linkage (Stern 2000, Barton 2001, Hill 2010, Hill



**Fig. 9.1** A graphical model for the maintenance of variation in a continuously varying trait. Input from mutation ( $M$ ) and introgressive hybridization ( $I$ ) is balanced by output from selection ( $S$ ) and random genetic drift ( $D$ ). Selection is either stabilizing or directional. **Upper:** An equilibrial level of variation ( $V$ ) is determined by a constant rate of input of genetic novelty through mutation, counterbalanced by loss from selection alone or selection combined with drift. At constant or occasionally low population sizes, where drift is likely to be most effective, variation is relatively low ( $V_{S+D} < V_S$ ). **Lower:** Two levels of constant introgression ( $I_1, I_2$ ) and two levels of selection intensity ( $S_1, S_2$ ) are added to the scheme above to show their joint determination of equilibrial levels of variation. Drift, not shown, could be added equally or unequally to the selection functions. Conspecific gene flow, not shown, could be added to the  $I + M$  levels of variation, or could be considered as a component of introgression more broadly defined. From Grant and Grant 2000a.

and Kirkpatrick 2010)—that are beyond the scope of this field study. Their relative roles in the maintenance of variation are currently uncertain (Hill 2010).

## Morphology of Hybrids

Hybrids are generally morphologically distinct (fig. 8.13), especially in the  $F_1$  generation but also in the first two generations of backcrosses (Grant and Grant 1994). They are almost always intermediate, on average, between the means of the parental groups that produce them, as expected from the polygenic nature of trait inheritance in the parental species. The *scandens*  $\times$  *fortis* hybrids are the only  $F_1$  group departing significantly from expectation. Those identified from the pedigrees were not midway between the means of the parental species, on average, but relatively small (fig. 8.13), perhaps because of a negative interaction between the two sets of parental genes in the growth of the hybrid offspring, or because the parents happen to be below average in size, or because they do not function well as a pair and the feeding of the offspring suffers in consequence. High fitness of hybrids (fig. 8.12) makes the last explanation the least likely.

Heritable variation in the hybrids is substantial. To estimate it, we had to combine  $F_1$  hybrids with backcrosses because individual sample sizes were small. We found that variation in all six measured traits was heritable, with averages of 0.802 for *fuliginosa*  $\times$  *fortis* heritabilities and 0.865 for *scandens*  $\times$  *fortis* (Grant and Grant 1994). These values are comparable to the heritabilities of the parental species, *fortis* and *scandens* (chapter 3).

## Effects of Hybridization on Variation

Hybridization results in backcrossing in diverse and complex ways (fig. 9.2). For example, even though *fuliginosa* and *scandens* have never been known to interbreed, each has bred with *fortis*. *G. fortis* acts as a conduit (Grant 1993) or bridge (Clarke et al. 1998, Broyles 2002) for the passage of genes from one species to another. A similar situation has been described for three species of *Nothonotus* fish in a North American river, with the difference being that in this linear environment only two coexist at any one place (Keck and Near 2010).

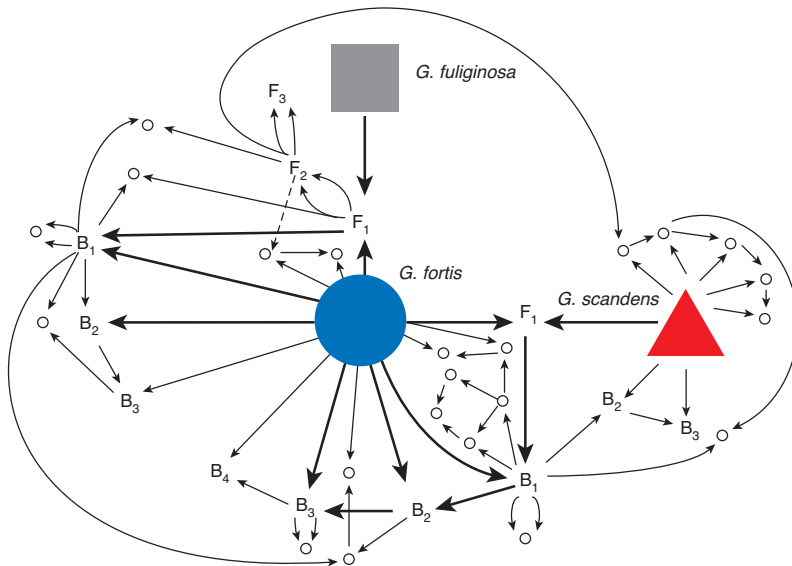


Fig. 9.2 Gene flow network reflecting known and quantified introgressive hybridization. All three species are connected by exchanging genes, but the relatively rare *fuliginosa* has not hybridized with *scandens*, and *fuliginosa*  $\times$  *fortis* hybrids have not backcrossed to *fuliginosa*. Thick lines indicate the primary pathways of genes from one population to another. From Grant and Grant 2010b.

Hybridization without loss of fitness should enhance variation (fig. 9.1). A sample of the parental species that contains a few hybrids should be more variable than one without them. The degree to which the expected enhancement is expressed and statistically detectable depends on the heritabilities of the traits, which are generally high, the mean differences between the species, which vary among the traits, and the frequencies and composition of the hybrids. We examined the expectations by first calculating variances for the samples of parental species that were known from pedigree data to lack hybrids, adding the hybrids and backcrosses to the samples, and then recalculating the variances. Additions were made to match the proportions in the population at large (Grant 1993). This entailed adding *fuliginosa*  $\times$  *fortis* and *scandens*  $\times$  *fortis* hybrids to *fortis*, but only *scandens*  $\times$  *fortis* hybrids to *scandens*.

As expected, introgressive hybridization increases phenotypic and genetic variation in all traits (table 9.1). Hybridization affects traits differently according to how much the hybridizing species differ from each other. Thus increases are small in beak depth and width in *scan-*

TABLE 9.1

Proportional effects of including  $F_1$  hybrids and backcrosses on estimates of heritabilities ( $h^2$ ) and coefficients of phenotypic ( $CV_P$ ), additive genetic ( $CV_A$ ), and residual ( $CV_R$ ) variation

	$h^2$	$CV_P$	$CV_A$	$CV_R$
<i>G. scandens</i>				
Mass	+33.9	+16.4	+32.2	+4.1
Wing length	+3.1	+15.0	+16.7	+43.0
Tarsus length	+3.3	+10.3	+10.2	+8.5
Beak length	+11.2	+22.4	+29.2	+2.7
Beak depth	-1.4	+3.6	+2.7	+4.8
Beak width	-9.0	+6.2	+1.0	+10.7
<i>G. fortis</i>				
Mass	+19.2	+37.9	+50.6	+18.9
Wing length	+40.0	+30.1	+53.9	+5.9
Tarsus length	+29.3	+15.4	+31.0	+4.8
Beak length	+7.8	+22.1	+26.8	+2.5
Beak depth	+11.0	+26.9	+33.8	+11.4
Beak width	+10.1	+28.4	+34.7	+12.0

Note: Two *fortis* × *scandens*  $F_1$  families, five first generation backcross families and two second-generation backcross families, were added to the total of 149 *scandens* families lacking known hybrids and backcrosses; hybrids and backcrosses constituted about 5% of the total, which approximates their incidence in the population. The sample of *fortis* without hybrids was 167 families for which all four grandparents were known. To this were added 12  $F_1$  families, 13 families involving first-generation backcrosses to *fortis*, and another 15 second-generation backcross and other families that involved descendants of interbreeding (see fig. 9.2). Among the “other” category were two families in which one of the grandparents was a *fortis* × *scandens*  $F_1$  hybrid and the other was a *fortis* × *fuliginosa*  $F_1$  hybrid. Hybrid and backcross individuals constituted about 20% of the total, which approximated their frequency in the population in 1992. From Grant and Grant (2000a).

*dens* because the average size of these traits is almost identical in *fortis* and *scandens*. The increase in *scandens* beak length variation is much larger, and this corresponds to the large difference in averages between the species. Introgressive hybridization affects *fortis* variation more uniformly than *scandens* because it occurs mostly with *fuliginosa*, and *fuliginosa* and *fortis* differ to approximately the same extent in all six traits.

#### COMPARISON OF *G. FORTIS* AND *G. SCANDENS*

Additive genetic variances of the two species were approximately at equilibrium from 1976 to 1991 (fig. 3.4), with at most minor perturba-

tions that could be attributed to intermittent selection. The level of variation is consistently higher in *fortis* than in *scandens*, and the same is true for alleles at microsatellite loci (fig. 9.3).

There are three reasons why *fortis* varies more than *scandens*. First, introgressive hybridization has a larger influence on variation in *fortis* than in *scandens*, partly because the frequency of hybridization was higher in *fortis* in the early part of the study (Grant 1993) and partly because *fortis* receives genes from two different species, whereas *scandens* receives genes from only one. Second, *scandens* breeding populations are consistently smaller than *fortis* populations, their genetically effective population sizes are smaller (<100 ignoring gene flow: Grant and Grant 1992a), and as a result they are likely to lose variation through drift at a higher rate than *fortis* (Houle 1989, Caballero and Keightley 1994).

The third reason is ecological. Even though there is little spatial heterogeneity on the island, the food supply is diverse and allows a partitioning of the niche of the generalist granivore species, *fortis*, by phenotypes that are partially specialized on seeds of different sizes (chapter 2). This individual specialization of a generalist species is depicted as model 1b in figure 8.1. The between-phenotype component of the total niche width of the population has been estimated to be 11% (Price 1987). In contrast to *fortis*, *scandens* feeds in a uniform manner on seeds and cactus products (figs. 2.17–2.20), and all members of the population are specialized in similar ways: model 2 in figure 8.1.

Differences in variation between the species are not fully accounted for by our estimates of introgression. For example, the coefficient of additive genetic variation of *fortis* beak depth in the absence of hybrids and backcrosses (6.39) is almost twice as large as the comparable coefficient of *scandens* even with their hybrids included (3.43). Thus hybridization has a short-term effect of increasing variation, but differential introgression in the short term is not large enough to explain the actual differences in variation between the species. The most likely explanation for the discrepancy is a greater cumulative (storage) effect of past hybridization in *fortis* than in *scandens*. *G. fortis* hybridized with *fuliginosa* more frequently than with *scandens* before the El Niño event of 1982–83 (chapter 8), and perhaps for a long time before the study began. Other possibilities, such as higher mutation rates in *fortis* than *scandens*, seem less likely.

Returning to the model in figure 9.1, *fortis* receives more genetic input from introgression than *scandens*; therefore its variation is at position  $V_2$  or  $V_4$ , and *scandens* is at  $V_1$  or  $V_3$ . Which of the alternatives is correct depends upon the long-term selection functions (S). However, *fortis* variation remains much higher than *scandens* when



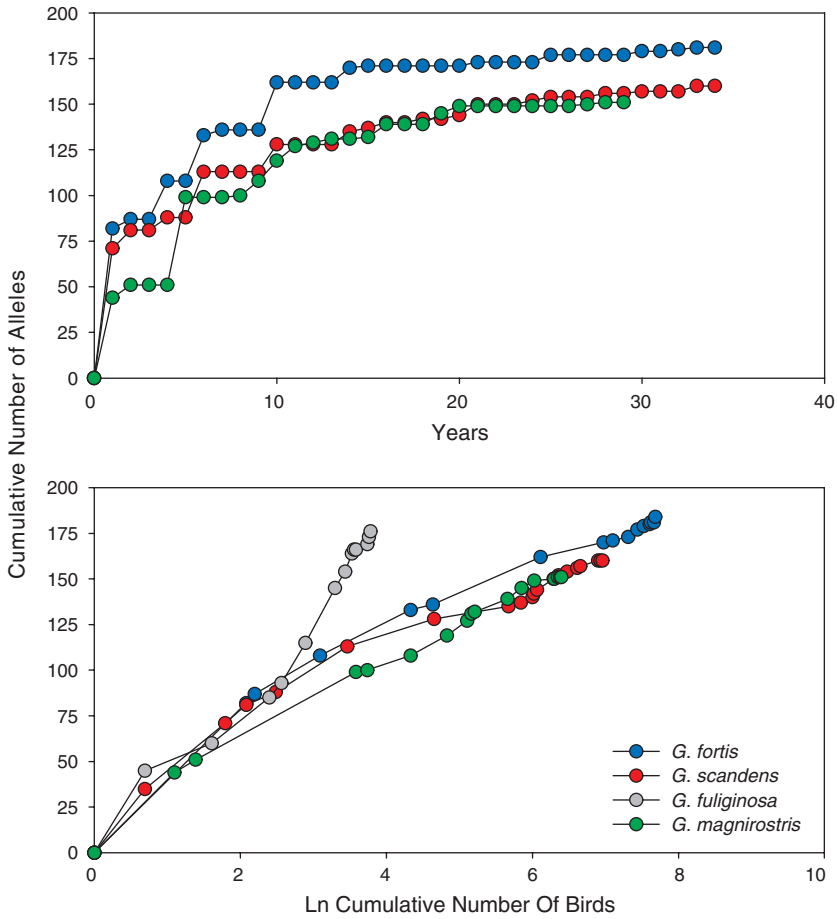


Fig. 9.3 Increase in allelic diversity at microsatellite loci with an increase in number of sampled individuals. **Upper:** Different asymptotes are approached by the different species. **Lower:** Addition of *fuliginosa* shows the effect of continuing immigration from a diverse source or sources. A sample of 24 *fuliginosa* from Santa Cruz Island alone has 168 alleles in total, almost the same as in the Daphne sample of 44 *fuliginosa* (176). Samples of the other three species are 2,169 *fortis*, 1,054 *scandens*, and 601 *magnirostris*.

hybrids are removed from the *fortis* sample but retained in the *scandens* sample. Therefore *fortis* cannot be at  $V_2$  and must be at  $V_4$ . By a similar argument *scandens* must be at  $V_1$ . The equilibril variation in figure 3.4 implies that *fortis* is subjected to stronger forces of stabilizing or directional selection than *scandens*, balancing the stronger

genetic input from introgression. Evidence in support of this expectation is given in chapter 11.

## Conspecific Gene Flow

Gene flow from conspecific populations on other islands does not alter the above comparisons. It is rare and contributes less to standing variation than does introgressive hybridization, but it may be sufficient to counteract losses from random genetic drift (Grant and Grant 2010b, Farrington and Petren 2011). Estimating it is not easy, because there are no diagnostic phenotypic traits possessed by *fortis* and *scandens* immigrants (fig. 9.4), nor do we have island-specific genetic markers. The best we can do is to use assignments tests with microsatellite DNA markers to identify probable immigrants among the *fortis* and *scandens* adults that lacked bands when first captured in the years 1976–98 (Grant and Grant 2010b; fig. 9.5). By this means we found that *fortis* and *scandens* receive genes more frequently by hybridizing than by breeding with conspecific immigrants. The net effect of hybridization upon morphological variation must be larger than implied by the difference in frequencies because species differ genetically from each other more than do populations of the same species.

## Hybridization versus Mutation

Mutation and introgression both contribute to the maintenance of variation (fig. 9.1), but how do they compare quantitatively? To address this question, we obtained an average value for mutational variance from several studies of laboratory organisms reported in the literature (Grant and Grant 1994): it is  $10^{-3} V_e$ , that is, expressed as a fraction of the environmental variance of a trait. Genetic variance resulting from introgressive hybridization is of approximately the same order of magnitude as the environmental variance ( $10^0$ ), and is three or more orders of magnitude greater than variation introduced by new mutations per generation ( $10^{-3}$ ). The reason for the great difference is that introgressive hybridization affects many more loci than mutation. This means that the effects of mutation on standing variation are dwarfed by effects of hybridization (fig. 9.1). Therefore it is reasonable to consider standing variation to be in equilibrium primarily because selection balances introgression.



Fig. 9.4 Immigrants and residents. **Upper:** Immigrant *fortis*, characterized by curvature of the beak, size, and genotype. **Middle:** Resident *fortis*. **Lower:** A pair of resident *magnirostris* derived from immigrants.

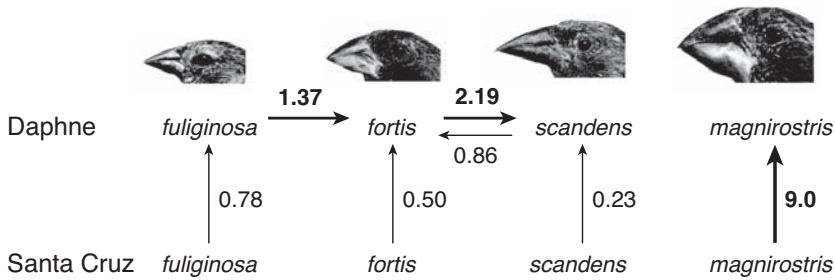


Fig. 9.5 Summary of gene flow through immigration and introgression on Daphne. Numbers refer to immigrants or hybridizing individuals per generation: those greater than 1.0 have been highlighted in boldface. Genes flow from *fortis* to *scandens* when the father of an  $F_1$  hybrid sings the *scandens* song, and vice versa for gene flow from *scandens* to *fortis*. The species hybridize on Santa Cruz Island to an unknown extent (Grant et al. 2005). *G. magnirostris* hybridizes with *fortis* on Santa Cruz (Huber et al. 2007) but not on Daphne. A rare *fortis* immigrant is illustrated in figure 9.4. From Grant and Grant 2010b.

## Correlations

Treating genetic variances of each trait alone as an indication of the potential to evolve ignores the fact that traits are correlated, so evolutionary change in one trait is affected by the correlation with other traits. Chapter 4 discussed this point in connection with natural selection. The six traits are intercorrelated in a similar way in all three species: strongly and positively. However, *scandens* differs from the other two species in beak proportions, and in the fact that beak length is relatively weakly correlated with beak depth and width. Correlations are important: when hybrids and backcrosses are formed, the correlations between pairs of traits depend upon the allometric relations of the hybridizing species (fig. 9.6).

Phenotypically, beak depth and width are strongly and positively correlated in all species and hybrid groups (Grant and Grant 1994). Introgressive hybridization has no effect on the beak correlations of *fortis* but does weaken the correlation between length and depth in *scandens*, by 11.7%. Genetically, all traits are positively correlated, strongly, in all species. Introgression strengthens the genetic correlations, more so in *fortis* than in *scandens*, with one difference between them being particularly striking. Addition of  $F_1$  hybrids and backcrosses increased the genetic correlation between depth and width by 50.7% in *fortis* but only by 4.5% in *scandens* (Grant and Grant 2000a).

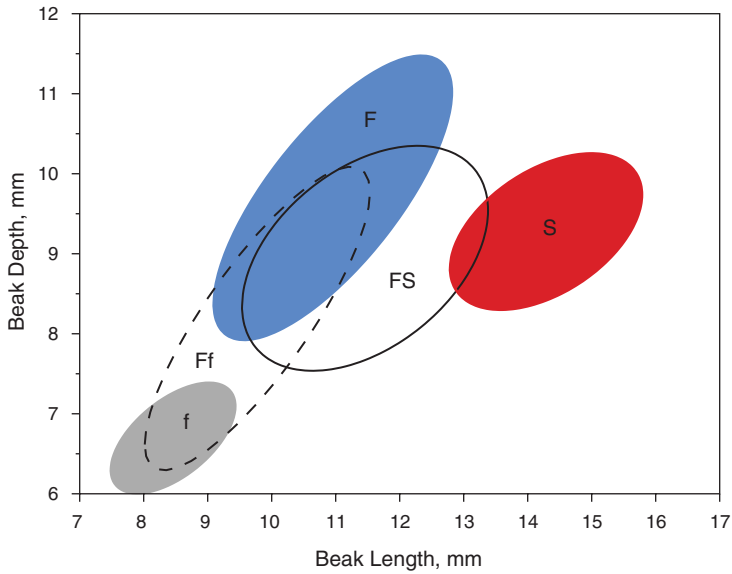


Fig. 9.6 Beak proportions of *fortis* (F), *scandens* (S), and *fuliginosa* (f), together with *fortis* × *scandens* hybrids (FS, ellipse with thick line) and *fortis* × *fuliginosa* hybrids (Ff, ellipse with broken line). Hybrids were identified from pedigrees as  $F_1$ , but some were probably backcrosses. Density ellipses approximate 99% of individuals. Based on Grant and Grant (1994).

## Evolutionary Potential

Enhanced variation facilitates directional evolutionary change, subject to constraints arising from genetic correlations between characters (chapter 4). These constraints become stronger when species with similar proportions hybridize, thereby rendering evolution in a new direction less, not more, likely. Only with species of transposed allometries, such as beak dimensions of *fortis* and *scandens*, are genetic correlations likely to be weakened or eliminated by hybridization. With enhanced genetic variation but reduced genetic constraints, a population has the potential to evolve in new directions more easily than in the absence of introgression. Thus the creation of individuals in novel genetic and morphological space could provide the starting point of a new evolutionary trajectory that is not easily reached by mutation alone (Miller 1956, Svårdson 1970, Lewontin and Birch 1966, Van Valen 1976, McDade 1990, Chiba 1993) or by directional selection acting on constrained genetic variation (Grant and Grant 1994, 2000a).

## Discussion

In the previous chapter we introduced the topic of morphological variation with an adaptive hypothesis to explain why some populations vary more than others in continuously varying traits such as beak or body size. The hypothesis proposes that high variation is maintained by diversifying natural selection in a heterogeneous environment. It is analogous to adaptive radiation theory. Both assume genetic variation. Both invoke natural selection from diverse ecological pressures in a heterogeneous environment. In both cases different units have different ecological niches as a result of those pressures; the units are species in an adaptive radiation and morphologically extreme individuals in adaptive population variation.

However, variation is not adaptive in the model of figure 9.1. Variation that is elevated by introgressive hybridization is not maintained by diversifying selection; on the contrary increases in variation are opposed by stabilizing or directional selection. In reality, too, selection is not diversifying but directional (appendix 9.1). Either small or large birds may be at a selective advantage but not at the same time. In fact the selective advantage of one extreme size class arises from the selective disadvantage of the other.

A second reason for not accepting the adaptive variation hypothesis is that extreme individuals of either species do not have unique diets. There is no component of the dry-season diets of either *fortis* or *scandens* that is exploited only by extreme individuals (fig. 9.7), and therefore nothing comparable to the unique ecological niches of species in an adaptive radiation.

Nonetheless the increase in population variation caused by introgressive hybridization is adaptive in a more restricted sense. The *fortis* population exploits the environment more fully as a result of being more variable. The evidence for this is the greater feeding efficiency of *fuliginosa*  $\times$  *fortis* hybrids and backcrosses (small phenotypes) when feeding on small and soft seeds compared with nonhybrid *fortis* (Grant and Grant 1996b): hybrids consumed seeds faster. The difference between phenotypes supports model 1 in preference to model 2 in figure 9.7 as a representation of feeding efficiency in relation to seed size and hardness. Moreover *scandens*  $\times$  *fortis* hybrids were more efficient than *fortis* when feeding on *Opuntia* seeds, although less efficient than *scandens*. The hybrids are generally at the small end of the body and beak size spectrum (fig. 9.6). At the opposite end of the size spectrum the largest individuals are the most efficient in extracting seeds from *Tribulus* fruits (figs. 2.15 and 4.7), although we are unable to say if they are large because of hybridization with *scandens*.

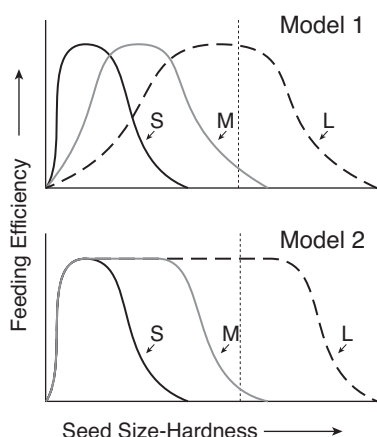


Fig. 9.7 Different ways in which feeding efficiency varies among small (S), medium (M), and large (L) phenotypes in relation to the size and hardness of seeds in the diet. The key difference between the models is in how small and soft seeds are exploited by the different phenotypes. *G. fortis* phenotypes and seeds on Daphne span the range to the left of the broken line. On other islands seeds (Abbott et al. 1977) and beak sizes (fig. 2.1) are larger. For example, on Santa Cruz only the largest *fortis* are able to crack the seeds of *Cordia lutea* ( $DH^{1/2} > 13.0$ ).

Consideration of diets leads to the question of whether temporal variation in environmental conditions is a factor favoring a high level of morphological variation, depending on the exact nature of the variation, as has been suggested sometimes for other systems (e.g., Sasakai and Ellner 1997, Zhang 2012). Annual variation in food supply affects morphological variation in *fortis* more than *scandens*. Extreme excursions from long-term average morphology in *fortis*, caused by selection in either direction (figs. 7.3 and 7.6), are rare, and this gives the impression of long-term relaxation of selection or environmental tolerance of extreme individuals. However, this does nothing to promote, let alone generate, morphological variation, because new food items are not added to the diet when selection does or does not occur. The same range of resources is exploited at all times.

If not temporal variation, then how about spatial variation? Strong spatial variation in habitat features can promote diversifying selection, as has been shown with freshwater fish (e.g., Robinson and Schluter 2000, Nosil and Reimchen 2005), but such selection is not possible on Daphne, because habitat heterogeneity there is far from comparable to the vertical stratification of habitats in lakes in degree or extent.

In conclusion, introgressive hybridization increases the level of morphological variation in the *fortis* population, and enhanced feeding efficiency of the morphologically extreme individuals maintains it.

## Summary

To understand population variation in nature, two questions must be addressed: what is the origin of the variation, and what maintains it?

The first is a question of genetics, and the second is a question of ecology. In this chapter we concentrate on the role of one genetic factor, introgressive hybridization, and one ecological factor, food. *G. fortis* hybridizes with two species, immigrant *fuliginosa* and resident *scandens*, whereas *scandens* hybridizes only with *fortis*. We explore the morphological consequences of hybridization by comparing the features of a combined sample of parental species and hybrids with those of the parental species alone. For this we use observations of breeding, pedigrees, morphological measurements, and microsatellite DNA measures of genetic effects. Hybrids and backcrosses by themselves were found to be intermediate in mean morphology between the respective parental species, and their variation is highly heritable. Introgressive hybridization results in an increase in phenotypic and genetic variances, and a moderate increase in heritabilities. Further increases in variation are opposed by selection. *G. fortis* varies more than *scandens* in all body and beak traits, largely as a result of receiving a more diverse genetic input from hybridization, and varies more ecologically. Enhanced variation facilitates directional evolutionary change, subject to constraints arising from genetic correlations between characters. These constraints become stronger when species with similar proportions hybridize, such as *fortis* and *fuliginosa*, thereby rendering evolution in a new direction less likely. *G. fortis* and *scandens* have different beak allometries—they are transposed and have different slopes—and interbreeding of these two species tends to weaken the genetic correlations, thereby increasing their evolutionary scope.