

## INBREEDING AND INTERBREEDING IN DARWIN'S FINCHES

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**Abstract.**—Studies of inbreeding and interspecific hybridization are generally pursued separately with different metrics. There is a need to integrate them because they have the common goal of seeking an understanding of the genetic and ecological basis of fitness variation in populations. We use mean expected heterozygosity as an axis of variation on which to compare the fitness of inbreeding and hybridizing Darwin's finches (*Geospiza scandens* and *G. fortis*) relative to the fitness of matched outbred controls. We find that relative fitness of inbred finches is less than one in the 1991 cohorts of both species. Inbreeding depression is stronger in the species (*G. scandens*) with the lower genetically effective population size. Relative fitness of hybrids (backcrosses) in the same cohort of *G. scandens* is greater than one. Evidence of heterosis in *G. fortis* is mixed. Thus the two interbreeding species displayed somewhat different fitness patterns under the same set of environmental conditions. Hybridization may enhance fitness to different degrees by counteracting the effects of inbreeding depression, by other additive and nonadditive genetic effects, and by producing phenotypes well suited to exploit particular ecological conditions.

**Key words.**—Backcross, expected heterozygosity, fitness, Galápagos, hybridization, inbreeding.

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The occurrence of inbreeding and hybridization in the wild is not trivial. With fragmentation of the environment occurring through human interference, many populations are becoming increasingly small, isolated, inbred and more strongly at risk of losing genetic variation through drift (Frankham 1998; Hedrick and Kalinowski 2000). When such populations are sympatric with closely related congeners they are liable to hybridize. Inbreeding and introgressive hybridization are two forms of genomic stress (Fontdevila 1992), with opposite effects on genetic variation. How do those effects compare in magnitude and evolutionary importance?

Theoretically, inbreeding is expected to depress fitness, whereas effects of hybridization are less predictable (Fig. 1), and the genetic causes may be different (Lynch and Walsh 1998; Whitlock et al. 2000). Empirically, the question of relative magnitude is not easy to answer because genetic effects of inbreeding and hybridization are typically studied separately. Part of the reason for this is the lack of a common scale for comparing the fitnesses of inbred and hybrid individuals. There is a need to integrate their fitness effects to understand the evolutionary implications and consequences of inbreeding depression and heterosis in the same population under the same environmental conditions. Using mean expected heterozygosity for this purpose, we present evidence of both inbreeding depression and elevated fitness of interspecific hybrids from a long-term field study of Darwin's finches living in an undisturbed habitat on the small Galápagos island of Daphne Major (0.34 km<sup>2</sup>).

Previous work on Daphne Major has produced evidence of both inbreeding (Gibbs and Grant 1989; Keller et al. 2002)

and introgressive hybridization (Grant and Grant 1992a, 2002; Grant and Grant 1998). The magnitudes of their effects are not strictly comparable because they were determined in different years and under different environmental conditions. *Geospiza scandens*, the cactus finch, breeds rarely with the smaller but more abundant *G. fortis*, the medium ground finch (Grant and Grant 2002). From 1976 to 1982 three interspecific pairs produced six offspring. In addition, one hybrid was produced in 1978 by an extra-pair fertilization (EPF) of a *G. fortis* female. With the exception of this EPF hybrid, none of the offspring survived to breed, but many offspring of the parental species also failed to breed at this time of relatively little rainfall. During an exceptional El Niño event in 1982–83 two mixed-species pairs fledged 12 offspring. Under the altered environmental conditions that have persisted since then, some of the F<sub>1</sub> hybrids and others produced subsequently survived to breed, backcrossing to both parental species. Similarly, the magnitude of inbreeding depression has been shown to vary annually and to a differing extent in the two species in relation to different environmental variables (Keller et al. 2002).

Here we compare relative fitnesses of inbred birds and hybrids that hatched in 1991. All hybrids were backcrosses to one or the other of the two species and not F<sub>1</sub> hybrids. Our sample included all backcrosses produced in 1991. This was the last year of an intensive study of breeding with complete pedigree information (Grant and Grant 2002; Keller et al. 2002). Ninety-nine percent of finches on the island had been individually marked. It was also the first year with large samples of birds genotyped by microsatellite analyses (Keller et al. 2001). *Geospiza scandens* has a smaller genetically effective population size than *G. fortis* (Grant and Grant 1992b). At the start of the breeding season in 1991 there were

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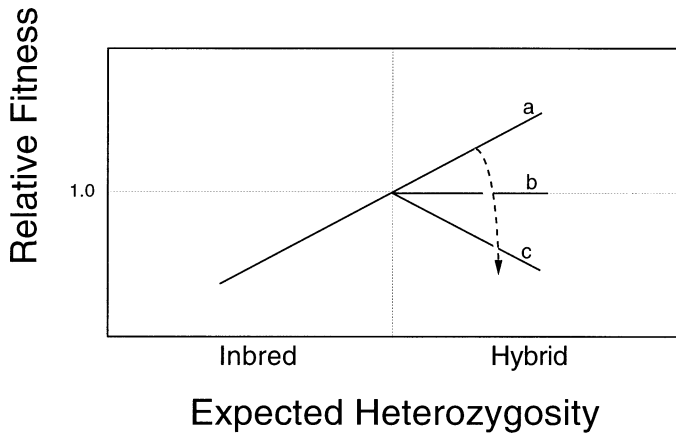


FIG. 1. Relative fitness in relation to expected heterozygosity for inbred birds and interspecific hybrids. Extreme heterozygosities are expected for close inbreeding ( $f = 0.25$ ) and  $F_1$  hybrids. Although a decline in fitness in relation to inbreeding coefficient is theoretically well justified and extensively supported empirically (Charlesworth and Charlesworth 1987; Keller and Waller 2002), relative fitness of hybrids and backcrosses is less predictable (Arnold 1997; Burke and Arnold 2001). Three alternatives are shown (a–c). A possible evolutionary sequence is indicated by the broken line with arrow, indicative of increasing genetic incompatibilities between species with time. Interbreeding of conspecific populations may produce similar effects on fitness in relation to time (Edmands 1999). Hybrids are assumed to be not inbred.

131 adult females and 143 adult males in the *G. fortis* population, but only 19 adult female and 51 adult male *G. scandens*.

#### METHODS

##### *Comparisons of Fitness*

Inbreeding is the breeding of relatives, in contrast to outbreeding, which refers to breeding of nonrelatives (Charlesworth and Charlesworth 1987; Keller and Waller 2002). Interspecific hybridization (Harrison 1990; Arnold 1997; Burke and Arnold 2001) is the most extreme form of outbreeding. Inbreeding is measured in terms of a coefficient of genetic relatedness due to common descent ( $f$ ), whereas hybridization is characterized differently in terms of offspring (F) or backcross (B) classes (Hartl and Jones 1998; Hedrick 2000). Comparing the relative fitness of inbreds and hybrids thus requires construction of a common axis of variation that spans the entire range from extreme inbreeding to hybridization. For this we use mean expected heterozygosity of each class of birds, estimated from allele frequency data (Petren et al. 1999).

Families with inbreeding coefficients of  $f > 0$ , as well as hybrids, were identified from pedigrees supported by analysis of parentage by microsatellite DNA (8–16 loci; Keller et al. 2001, 2002). Inbreeding families were compared to others (controls) with an inbreeding coefficient of less than 0.01. To minimize the large variation in fitness that can be attributed to substantial but unmeasured environmental variation (Grant and Grant 2000; Markert et al. 2004) we matched the inbred families with controls closest in space and time; they were often neighbors, and laid eggs within a few days of

each other (maximum 14). The same procedure was used for the hybrids to obtain an independent set of matched control families. Control pairs were not matched with more than one inbred or hybrid pair. Total nest failures and extra-pair young were excluded from all analyses.

On the basis of previous work (Grant and Grant 2000; Markert et al. 2004), absolute fitness was scored as number of years an individual survived (longevity) and whether it bred (recruitment). Absolute fitness was expressed as mean longevity of the brood or proportion that became recruits. Relative fitness was calculated as the difference in mean longevity (or proportion of recruits) between inbred or hybrid offspring and their matched controls. Relative fitnesses of inbred and hybrid birds were assessed in relation to their mean expected heterozygosities.

Three points on the heterozygosity axis were estimated for inbreds at  $f = 0.25$ , outbreds at  $f < 0.01$ , and hybrids at  $F_1$ . For the outbreds, mean expected heterozygosity was calculated as  $1 - \sum$  squared allele frequencies at each of 14 microsatellite loci, averaged across loci. The loci are autosomal and unlinked, presumed to be selectively neutral, and show no significant sex differences in allele frequencies (Petren et al. 1999). The outbred sample was the total number of genotyped adults of each species alive in or before 1991: 75 *G. scandens* and 81 *G. fortis*. For the inbreds, the calculation was performed by randomly pairing noninbred full sibs as if they were breeders ( $n = 67$  *G. scandens* pairs and  $n = 70$  *G. fortis* pairs), and determining mean expected heterozygosity of the putative offspring as the mean of the fraction of 14 loci that were heterozygous in each offspring. For  $F_1$  hybrids the mean expected heterozygosity was calculated as  $1 -$  expected homozygosity by multiplying the *G. fortis* frequency of each allele at each locus by the corresponding *G. scandens* frequency, summing the products at each locus, then averaging the products at all loci and subtracting the value from 1.0. Other points on the scale were determined by linear interpolation according to the pedigree information for inbred ( $f = 0.01 - 0.25$ ) and backcross individuals. For example, individuals with an inbreeding coefficient of 0.125 had an expected mean heterozygosity midway between the values for individuals with  $f = 0.25$  and  $f < 0.01$ .

Regardless of whether they bred or not,  $F_1$  hybrids were assigned to either *G. fortis* or *G. scandens* on the basis of the song of their fathers because both males and females choose mates according to paternal species song (Grant and Grant 1997a). Like  $F_1$  hybrids, backcrosses mate according to paternal song (Grant and Grant 1997b). Therefore, hybrid individuals that were compared separately to *G. fortis* and *G. scandens* controls were mutually exclusive sets of birds.

##### *Statistical Analyses*

Inbred and controls were compared by one-tailed  $t$ -tests, based on the theoretically justified expectation of inbreeding depression (Keller and Waller 2002). Hybrids and their controls were compared by two-tailed  $t$ -tests because hybrids can be more or less fit than nonhybrids (Arnold 1997; Fig. 1). Variances of compared samples did not differ by a factor of more than two, and distributions were not markedly skewed. Different nests of the same inbreeding or hybridizing pair

TABLE 1. Survival and breeding of inbred and hybrid *Geospiza scandens* and *G. fortis* compared with outbred matched controls. All *G. scandens* hybrid pairs included one F<sub>1</sub> parent, and they produced first generation backcrosses. *Geospiza fortis* hybrid pairs produced first (one), second (eight), or third (four) generation backcrosses. For each species the two sets of outbred pairs do not differ significantly in either longevity or recruitment of their offspring (analyses of variance, all  $P > 0.05$ ). Outbred here means not inbred, and does not imply an avoidance of breeding with relatives.

	Pairs	Offspring	Nests	First year survival	Bred	Longevity in years	
						Maximum	Median
<i>G. scandens</i>							
Inbred	3	11	5	6	0	3	0.5
Outbred	3	12	5	10	8	7	3
Hybrid	4	17	7	13	8	8	3
Outbred	4	24	7	13	5	7	1
<i>G. fortis</i>							
Inbred	17	65	23	52	19	10	1.5
Outbred	20	72	23	55	32	8	2.5
Hybrid	3	13	5	12	6	4	2.5
Outbred	3	15	5	11	8	6	3

were treated as independent units because they were always produced at different times and were sometimes matched to different control pairs. Proportional hazard models of survival applied to a much larger dataset showed that causes of death among siblings were not correlated beyond the fact that siblings share the same inbreeding coefficient (Keller et al. 2002).

#### RESULTS

Inbred *G. scandens* from five nests were unfit relative to outbred control birds of the same population (Table 1). The control birds were from five nests matched with nests of inbred birds closest in time and place of breeding. Five of the 11 inbred offspring died in their first year, the remainder died in the following year except for a single three-year survivor, and none bred. In striking contrast only two of the 12 outbred control birds failed to survive to the year after hatching, seven survived for three or more years (maximum seven), and eight individuals from four families bred. The difference in mean longevity of offspring from the two sets of families is significant by one-tailed paired  $t$ -tests ( $t_4 = 2.430$ ,  $P = 0.0093$ ). The two groups also differed significantly in proportions of offspring that survived to breed (recruits; paired  $t_4 = 3.091$ ,  $P = 0.0182$ ).

In contrast to these findings with inbred birds, *G. scandens* hybrids experienced higher fitness than the matched controls. All these hybrids were first generation backcrosses to *G. scandens*. They lived longer on average than nonhybrids when analyzed by two-tailed paired  $t$ -tests ( $t_6 = 3.341$ ,  $P = 0.0156$ ). Differences in the proportion of recruits are also significant ( $t_6 = 4.514$ ,  $P = 0.0040$ ). Tests applied to pairs instead of nests gave statistically similar results in all cases, but with higher  $P$ -values.

Considering the total *G. scandens* dataset, relative fitness increases in relation to mean expected heterozygosity across the full spectrum from inbred to hybrid (backcross) individuals (Fig. 2). Regressions are significant for longevity ( $F_{1,10} = 7.664$ ,  $P = 0.0198$ , adjusted  $r^2 = 0.377$ ) and recruits ( $F_{1,10} = 6.038$ ,  $P = 0.0338$ , adjusted  $r^2 = 0.314$ ). Unexpectedly, relative fitness (longevity) of just the inbred birds alone ap-

pears to decrease with increasing expected heterozygosity (Fig. 2). We consider this to be most likely an artifact of the small sample size ( $n = 5$ ). Thus across the full spectrum of expected heterozygosity, variation in relative fitness conforms most closely to alternative (a) in Figure 1.

Paired comparisons involving *G. fortis* provide support for alternative (b), and no support for alternative (c) (hybrid inferiority in Fig. 1). Despite larger sample sizes and greater statistical power than *G. scandens*, inbred *G. fortis* showed significant effects only in recruitment. They produced significantly fewer recruits than the matched controls (paired  $t_{19} = 2.535$ , one-tailed  $P = 0.0101$ ) but did not differ from them in longevity, nor were there any fitness differences between hybrids and their matched controls ( $P > 0.14$ ). For the total dataset, longevity increases with mean expected heterozygosity ( $F_{1,31} = 3.823$ ,  $P = 0.0596$ , adjusted  $r^2 = 0.081$ ; Fig. 2), as does production of recruits ( $F_{1,31} = 2.890$ ,  $P = 0.0991$ , adjusted  $r^2 = 0.056$ ), albeit not significantly.

Noteworthy is the fact that relative fitness increased with mean expected heterozygosity at the same rate in the two species, as shown by the results of analyses of covariance (ANCOVAs), with mean expected heterozygosity as a covariate and species as a factor. The relationships between fitness variables and mean expected heterozygosity were significant (longevity  $P = 0.0017$ ; recruits  $P = 0.0039$ ), the species did not differ (longevity  $P = 0.7218$ ; recruits  $P = 0.5512$ ) and interaction terms were not significant (longevity  $P = 0.7204$ ; recruits  $P = 0.3290$ ). These results imply that *G. fortis* experiences weak heterosis, despite the lack of statistical support from paired  $t$ -tests. Therefore this species conforms to alternative (a) in Fig. 1 on the basis of ANCOVA, or alternative (b) on the basis of  $t$ -tests, but certainly not to the hybrid inferiority alternative (c).

#### DISCUSSION

One species (*G. scandens*) experienced strong inbreeding depression and heterosis in the backcrosses. The other species (*G. fortis*) showed weaker inbreeding depression and heterosis. The difference between the species is not explicable in terms of different power of statistical tests resulting from

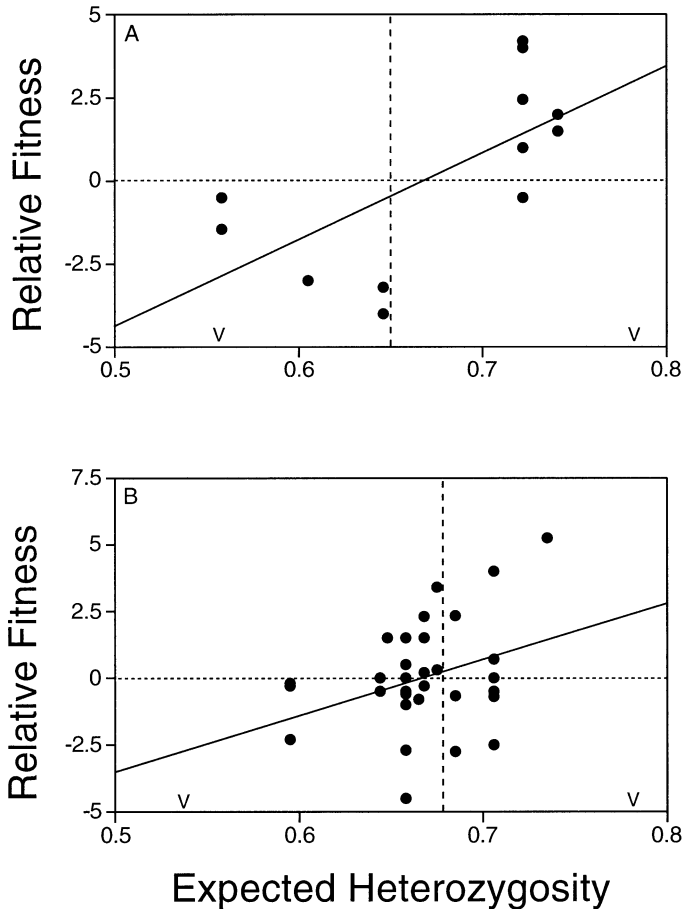


FIG. 2. Relative fitness of inbred birds and interspecific hybrids (backcrosses) in relation to mean expected heterozygosity. Relative fitness is expressed as the difference in mean longevity between inbred or hybrid offspring and their matched controls for *Geospiza scandens* (A) and *G. fortis* (B). None of the backcrosses are inbred. The mean expected heterozygosity of outbred birds is indicated by a broken vertical line. Positions of maximally inbred birds ( $f = 0.25$ ) on the left and  $F_1$  hybrids on the right are shown by arrowheads. The empirically determined position of the inbred birds is slightly to the right of the theoretically expected position, partly due to a small difference in allele frequencies between the sexes. Regression equations are (A)  $Y = 26.081X - 17.407$  ( $F_{1,10} = 7.664$ ,  $P = 0.0198$ , adjusted  $r^2 = 0.377$ ) for *G. scandens* and (B)  $Y = 21.014X - 14.020$  ( $F_{1,32} = 3.823$ ,  $P = 0.0596$ , adjusted  $r^2 = 0.081$ ) for *G. fortis*. The slopes of the two regression lines do not differ significantly (see Results); confidence intervals are 7.139 and 45.923 for the *G. scandens* slope and  $-0.482$  and 42.510 for the *G. fortis* slope.

different sample sizes. Thus two interbreeding congeneric species living in the same environment displayed somewhat different fitness patterns under the same set of environmental conditions.

The contrast between the species in the effects of hybridization was expected on the basis of their respective population sizes. Loss of heterozygosity due to inbreeding and drift should be greater in the smaller population of *G. scandens* than in *G. fortis*. Correspondingly, the fitness-enhancing effects of introgression are expected to be greater in *G. scandens*. The enhancement may arise from favorable combina-

tions of alleles with additive effects, from an increase in heterozygote frequency beyond that which counteracts the effects of inbreeding depression within populations caused by homozygous recessives, and from positive epistatic interactions among loci (Whitlock et al. 2000; Barton 2001; Burke and Arnold 2001). The advantage experienced by  $F_1$ s should be diluted in the backcrosses such as those analyzed here. Dilution is an additional and perhaps major reason why heterosis was weaker in *G. fortis* than in *G. scandens*. Most of the *G. fortis* hybrids were second and third generation backcrosses, whereas all *G. scandens* hybrids were first generation backcrosses.

The situation is different when more genetically divergent species interbreed, for then both inbreeding and hybridization may lower fitness. An example is the collared flycatcher (*Ficedula albicollis*) on the Swedish island of Gotland, which hybridizes with the pied flycatcher (*F. hypoleuca*). It experiences both inbreeding depression (Kruuk et al. 2002) and depressed fitness of hybrids (Tegelström and Gelter 1990; Gelter et al. 1992), thereby conforming to pattern (c) in Figure 1. Female hybrids are sterile. *Geospiza scandens* and *G. fortis* are younger species, having originated in the last half million years to judge from their mtDNA difference (Sato et al. 2001). This is a much shorter time than the 2.5-million-year separation apparently required for postzygotic incompatibilities to develop between populations of passerine birds (Price and Bouvier 2002).

Outcomes of both inbreeding (Keller et al. 2002) and hybridization (Grant and Grant 1998) are to some extent dependent upon environmental conditions. This makes it desirable to extend the comparison to other cohorts experiencing different sets of environmental conditions to see how general our results are. Samples of birds of known pedigree and paternity for doing this are limited; nevertheless they indicate a consistent pattern. In the years prior to 1991, inbred *G. scandens* experienced relatively low survival, especially under poor environmental conditions. There was no detectable effect of inbreeding on the survival of *G. fortis* (Keller et al. 2002). This contrast is consistent with results reported here for the 1991 cohorts. In addition, the survival of  $F_1$  hybrids was higher than the survival of both parental species. In 1983, 12  $F_1$  hybrids were produced and they all backcrossed to *G. fortis*, and in 1987, seven  $F_1$  hybrids were produced and they backcrossed to *G. scandens*. Each of these two groups survived slightly better than their respective parental species for the first 10 years of life (Grant and Grant 1998).

Despite these consistencies there are likely to be subtle variations in relative fitnesses attributable to variation in the strength of ecological factors under different environmental conditions. As a consequence the slopes of the relationships depicted in Figure 1 should vary somewhat at different times. This can be inferred from the variation in relative fitness of inbred *G. scandens* in relation to environmental conditions (Keller et al. 2002). With regard to hybrids, their intermediate size (Grant and Grant 1994) confers a relative advantage when there is an abundance of seeds of intermediate size and hardness. This was demonstrated in a study of the diets and feeding efficiencies of *G. fortis*, *G. scandens*, and their  $F_1$  hybrids and backcrosses in the years following the El Niño

event in 1982–83 (Grant and Grant 1996, 1998). However, they experienced no feeding or fitness advantage prior to 1983 when the seed supply was biased towards large and hard seeds (Grant and Grant 1998).

In conclusion, two classes of factors, genetic and ecological, may work separately or together, and their effects on fitness are likely to vary with environmental conditions (Emms and Arnold 1996; Parris 2001), genetic differences between breeders (Edmands 1999; Rieseberg et al. 1999) and evolutionary history of the interbreeding species (Moore 1977). Occasional hybridization may have important evolutionary potential (Stebbins 1959; Lewontin and Birch 1966; Grant and Grant 1994) in complex ways that are still being explored (Arnold 1997; Barrier et al. 1999; Kim and Rieseberg 1999; Barton 2001; Burke and Arnold 2001; Martinson et al. 2001). Hybridization has the potential to alleviate the effects of inbreeding in small populations in fragmented habitats (Frankham 1998; Woodruff 2001) or in island settings such as the ones studied here. Habitat fragments and small islands are arenas where the long-term fates of populations depend critically upon the interplay of ecological factors, inbreeding, genetic drift, conspecific gene flow, hybridization, and selection. It is in such arenas that our framework of analysis should be most useful because it involves measurement of inbreeding, hybridization, and fitness. With the adoption of a relaxed definition of hybridization to include interdemic (conspecific) gene exchange, the framework could also be useful for addressing questions of metapopulation dynamics (e.g. see Edmands 1999; Saccheri and Brakefield 2002).

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