ENVIRONMENTAL CONDITIONS AFFECT THE MAGNITUDE OF INBREEDING DEPRESSION IN SURVIVAL OF DARWIN'S FINCHES

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Abstract.—Understanding the fitness consequences of inbreeding (inbreeding depression) is of importance to evolutionary and conservation biology. There is ample evidence for inbreeding depression in captivity, and data from wild populations are accumulating. However, we still lack a good quantitative understanding of inbreeding depression and what influences its magnitude in natural populations. Specifically, the relationship between the magnitude of inbreeding depression and environmental severity is unclear. We quantified inbreeding depression in survival and reproduction in populations of cactus finches (*Geospiza scandens*) and medium ground finches (*Geospiza fortis*) living on Isla Daphne Major in the Galápagos Archipelago. Our analyses showed that inbreeding strongly reduced the recruitment probability (probability of breeding given that an adult is alive) in both species. Additionally, in *G. scandens*, first-year survival of an offspring with $f = 0.25$ was reduced by 21% and adults with $f = 0.25$ experienced a 45% reduction in their annual probability of survival. The magnitude of inbreeding depression in both adult and juvenile survival of this species was strongly modified by two environmental conditions, food availability and number of competitors. In juveniles, inbreeding depression was only present in years with low food availability, and in adults inbreeding depression was five times more severe in years with low food availability and large population sizes. The combination of relatively severe inbreeding depression in survival and the reduced recruitment probability led to the fact that very few inbred *G. scandens* ever succeeded in breeding. Other than recruitment probability, no other trait showed evidence of inbreeding depression in *G. fortis*, probably for two reasons: a relatively high rate of extrapair paternity (20%), which may lead to an underestimate of the apparent inbreeding depression, and low sample sizes of highly inbred *G. fortis*, which leads to low statistical power. Using data from juvenile survival, we estimated the number of lethal equivalents carried by *G. scandens*, *G. fortis*, and another congener, *G. magnirostris*. These results suggest that substantial inbreeding depression can exist in insular populations of birds, and that the magnitude of the inbreeding depression is a function of environmental conditions.

Key words.—Extrapair paternity, *Geospiza fortis*, *Geospiza magnirostris*, *Geospiza scandens*, inbreeding, island population, lethal equivalents, pedigree, purging.

Received June 26, 2001.   Accepted March 18, 2002.

The deleterious effects brought about by matings among relatives (inbreeding depression) are one of the oldest and enduring topics in evolutionary biology (e.g., Darwin 1876; Wright 1922; Haldane 1924; Fisher 1949; Mayr 1963). Inbreeding among normally outbreeding organisms generally leads to a decline in fitness (e.g., Wright 1977). Although studies of laboratory (e.g., Charlesworth and Charlesworth 1987, 1999), agricultural (e.g., Abplanalp 1990), and captive populations (Hedrick and Kalinowski 2000) found this to be a very general result, evidence for inbreeding depression in natural populations proved more elusive (e.g., Thornhill 1993). However, recent efforts to gather empirical data on the magnitude of inbreeding depression in natural populations have produced enough evidence to suggest that inbreeding depression can be substantial in wild populations of animals and plants (e.g., reviews in Crnokrak and Roff 1999; Keller and Waller 2002). In addition, evidence is accumulating that inbreeding can be severe enough to affect the viability of populations (e.g., Saccheri et al. 1998; Westemeier et al. 1998; Madsen et al. 1999; Hedrick 2001), the measure of ultimate interest to conservation biologists.

Still lacking is a good quantitative understanding of inbreeding depression and what influences its magnitude in natural populations. Specifically, the relationship between the magnitude of inbreeding depression and environmental severity is understood incompletely (Keller and Waller 2002). Inbred individuals are thought to be less stress tolerant (e.g., Wright 1922) and stressful environmental conditions sometimes do increase inbreeding depression (e.g., Bijlsma et al. 1999; Coltman et al. 1999; Cheptou et al. 2000a; Meagher et al. 2000; see Keller and Waller 2002 for additional references). However, this is not a general pattern. Inbreeding depression is not universally higher under more stressful conditions or in wild as compared to captive populations (e.g., Waller 1984; Mitchell-Olds and Waller 1985; Dahlgaard et al. 1995; Hauser and Loeschcke 1996; Dahlgaard and Loeschcke 1997; Armbruster et al. 2000; Cheptou et al. 2000b; Dahlgaard and Hoffmann 2000). More measures of inbreeding depression in the wild are needed to gain insights into which demographic, environmental, and genetic factors contribute to the variation in inbreeding depression within and among populations and species. Here we present data on the occurrence of inbreeding and the magnitude of inbreeding depression in populations of two species of Darwin's finches living on the same island in the Galápagos Archipelago. We demonstrate significant inbreeding depression in juvenile and adult survival in the cactus finch *Geospiza scandens*, and present evidence that environmental conditions modify the magnitude of inbreeding depression. We compare the results from *G. scandens* with those from the medium ground finch *Geospiza fortis*, whose paternity is less accurately determined by observation (Keller et al. 2001). Finally, using the in-

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breeding load or lethal equivalents framework (e.g., Morton et al. 1956), we compare the observed magnitude of inbreeding depression with that in another congener, *Geospiza magnirostris*, and with that reported in other studies.

METHODS

Study Populations and Field Methods

Populations of medium ground finches (*Geospiza fortis*) and cactus finches (*Geospiza scandens*) on Isla Daphne Major, Galápagos, Ecuador, have been studied intensively every year from 1976 to 1991, and in less detail up to the present. Earlier publications describe the species, their habitat, and the study methods in detail (e.g., Abbott et al. 1977; Boag and Grant 1984a,b; Grant 1999). Here we provide a brief summary. Isla Daphne Major is a small (0.34 km²) island in the center of the Galápagos Archipelago, approximately 7.5 km to the north of the main island of Santa Cruz. Harmonic mean population sizes on Daphne were 80 for *G. scandens* and 198 for *G. fortis* over the period 1976–1992 (Grant and Grant 1995a). In addition to *G. scandens* and *G. fortis*, smaller populations of *Geospiza magnirostris* and *Geospiza fuliginosa* reside on Daphne.

Finches breed in response to the first heavy rain of the season, which tends to fall between November and February (Grant et al. 2000). In some years it never rains enough for finches to breed at all. During each breeding season from 1976 to 1991, we marked almost all nests on the island and identified the parents attending the nests. Nests were regularly checked to determine clutch size, hatching, and fledging success. All nestlings were banded with a unique combination of one metal and three color bands when they were approximately eight-days old. Regular censuses at the end of the nonbreeding (dry) season and during the breeding season established which birds were still alive. Banding efforts varied over time. Ninety percent of all breeders were banded by 1980, and 100% were banded by 1992. After 1992, less than 50% of nestlings were banded each year, and by 1998 the percentage of banded breeding adults had fallen to less than or about 20%. Censuses of birds alive were performed in all years, however, and we observed more than 98.8% of all birds actually alive, because few birds were not seen in one year but observed in the following one.

Determination of Parentage and Estimates of Extrapair Paternity Rates

Parentage for each brood was determined from behavioral observations at the nest. We identified the putative parents by observing them build the nest, incubate the eggs, and feed the nestlings. High levels of extrapair paternity (EPP) can render pedigree analyses meaningless (e.g., Brooker et al. 1990). Furthermore, if females mated to related males seek EPPs more often than females mated to unrelated males, analyses of inbreeding depression based on pedigrees may give biased results. Therefore, we used microsatellite markers to examine the occurrence of EPPs and to determine if they were associated most frequently with inbred matings. Overall estimates of EPP rates have previously been published for *G. scandens* by Petren et al. (1999) and for *G. fortis* by Keller

et al. (2001). Here, we report the results for *G. scandens* and *G. fortis* using a different set of individuals for which we also had inbreeding data (see below).

Blood samples were collected starting in 1988. A single drop of blood from the brachial vein was taken from nestlings during banding or from adults and immatures captured in mist nets (Petren at al. 1999). Details of the genotyping techniques as well as the primer sequences are given in Petren (1998). We used the following loci for our analyses here: GF1, GF2, GF3, GF5 (*G. scandens* only), GF7, GF8, GF9 (*G. fortis* only), GF11, and GF16. All young matched their mothers, which implies that intraspecific brood parasitism is absent or very low in this population. Samples from offspring and fathers that mismatched only at a few loci were genotyped repeatedly to exclude the possibility of genotyping error leading to a false paternity exclusion. All but two extrapair young mismatched their putative fathers at several loci (≥ 2). It is possible that the single locus mismatch of these two young was caused by a mutation event. However, because extrapair paternities are much more likely than mutations, we considered these two birds to be extrapair young.

Pedigree Construction and Inbreeding Estimates

Behavioral information on parentage allowed the construction of a pedigree for both species, spanning six generations for *G. scandens* and eight generations for *G. fortis*. We did not remove the extrapair young we discovered with the molecular markers from the pedigree because we only had information on EPPs from a few years of the study. Removing the error introduced by EPPs in some years but not in others could have biased our analyses. We used Wright's (1969) coefficient of inbreeding, *f*, to quantify the degree of inbreeding based on the pedigree. Inbreeding coefficients were calculated using PEDSYS (Southwest Foundation for Biomedical Research, San Antonio, TX, www.sfbr.org) with the Stevens-Boyce algorithm option (Boyce 1983). All measures of inbreeding are relative. Here, inbreeding coefficients were relative to the baseline population in 1976. Accordingly, the depth of the pedigree varied considerably over time. Owing to the irregularity of rainfall patterns in the Galápagos, finches do not breed every year. The 1980 breeding season was the first in which we could have detected matings among relatives, but only matings among first-degree relatives ($f = 0.25$). By 1992, the last year with complete data, we could have detected matings among relatives as remote as $f = 0.000031$ (assuming a common ancestor in 1976). Thus, early in the study pairings may have been assumed noninbred because their ancestry was unknown and not because the mates were not closely related. Although the inbreeding coefficients are accurate even early in the study (because they are all relative to the 1976 baseline population), they are highly dependent on the exact composition of the baseline population because most common ancestors were members of it. Thereafter, the influence of these individuals diminished. To allow for the effects of varying pedigree depths, we only used data from individuals with at least all four grandparents known. No individuals with known grandparents existed until 1980.

Survival Analyses

Estimates of survival are local, because individuals that disappeared from the island could have died or dispersed. We have very limited data on interisland dispersal: three juvenile cactus finches that were banded on Daphne Major were observed on Daphne Minor during a single visit to that neighboring island (Grant et al. 1980). However, many birds were found dead on Daphne over the years (Grant 1999), suggesting that mortality is the major cause of disappearance from the island. Thus, it is unlikely that our results were strongly biased by emigration. Data on adult survival of inbred birds was available from all years between 1983 and 1999. Because breeding is usually conditional on sufficient rainfall, data on juvenile survival was only available for nine years (*G. scandens*) and seven years (*G. fortis*), respectively.

Annual resighting probabilities (given that a bird was alive) were high: 99.8% in *G. scandens* and 98.8% in *G. fortis*. Survival data of finches on Daphne Major were grouped (or interval censored), because we only assessed survival once every year and not continuously over time. Therefore, we analyzed survival after banding using a proportional hazards model developed by Heisey (1992) for grouped survival data. Proportional hazards models analyze the influence of covariates such as inbreeding on time until death, assuming that the covariates act multiplicatively on a baseline hazard function. Heisey's (1992) model relates the covariates of interest to the survival probability of an individual k surviving year i as:

$$S_{ik} = e^{-e^{(\alpha_i + Z_k\beta)}} \tag{1}$$

where α_i is the baseline hazard for year i, Z_k is a vector of covariates and β is a vector of regression coefficients. Because survival of Darwin's finches is known to be strongly affected by environmental variables such as rainfall and population density (see below), we modeled the baseline hazard itself as a function of these two variables. This model accommodates left-truncated data. Because we chose the day of banding as the starting point for the survival analyses, any effects of inbreeding on survival from egg-laying to banding will have resulted in a biased, left-truncated sample. It is therefore important to adjust the likelihood functions accordingly (Heisey 1992).

Darwin's finches cannot be sexed reliably by phenotype until they are adults. Therefore, we performed two separate survival analyses; one from banding to one year of age (juvenile survival) without sex effects, and the other from one year of age onwards including sex effects (adult survival). Average annual survival rates fluctuate strongly among Darwin's finches on Daphne Major. Abundance of the major food sources (seeds, flowers, and arthropods) and population density are important factors contributing to variation in juvenile and adult survival rates (Price and Grant 1984; Gibbs and Grant 1987a; Grant and Grant 1996; Grant et al. 2000), and models of finch survival should therefore include these factors. Most juvenile mortality occurs in the transition period after they are no longer fed by their parents (Millington and Grant 1984). Pollen from flowers and arthropods are important food sources during the first few months of life (Boag and Grant 1984b; Millington and Grant 1984). We do not

have direct measures of the availability of the various types of food important to finches. However, annual rainfall provides a proxy measure of food availability since the numbers of insects and other arthropods increases with increasing annual rainfall (Grant et al. 2000). Similarly, the number of *Portulaca* flowers increases with increasing rainfall (Millington and Grant 1983). Therefore, we used the amount of annual rainfall as an index of food production. Accordingly, annual rainfall (in mm), population densities of *G. scandens* and *G. fortis* in a year (all variables are log transformed), and the interactions between these three variables were the environmental variables we considered in the survival models (except for juvenile *G. fortis* where we did not attempt to fit the three way interaction). Because different cohorts experience variation in environmental conditions at different ages, survival models can alternatively be stratified by cohort and age (e.g., Keller 1998). However, preliminary analyses (data not shown) revealed that a model including population densities of *G. scandens* and *G. fortis* and rainfall explained as much or more of the deviance explained by models stratified by cohort and age. Sex and all possible three-way interactions with it were also included in the proportional hazards models of adult survival because preliminary analyses showed that sexes differed in their annual survival probabilities. Data from birds still alive when the current study period ended in 1999 (three *G. scandens* and 13 *G. fortis*) were right censored. Thus, they contributed to the information about the survivor function but not to the information about age at death. Our model building strategy followed Hosmer and Lemeshow (2000) in first constructing a preliminary main effects model and then assessing the significance of interactions. Significance of all covariates was assessed by comparing the model excluding that covariate to a full model using likelihood ratio tests. Terms only remained in the models if they were significant at $P \leq 0.05$. Estimates for main effects were based on the final models including interactions. We graphically represent the main results of the proportional hazards models using cubic splines fitted to the raw data.

We considered the possibility that data points from juveniles born in the same nest were not independent data points. However, an analysis of juvenile survival based on each nest average as an independent data point gave virtually identical results to the proportional hazards model, suggesting that the causes of death were not correlated among siblings above and beyond the correlation due to shared covariates in the model. To allow straightforward comparison of the results between adult and juvenile survival, we present the data from the proportional hazards model of juvenile survival here.

Reproduction

We analyzed inbreeding depression in the following measures of reproductive success: the probability that an adult who was alive also attempted to breed in a given year (recruitment probability), the number of eggs, nestlings, and fledglings raised, hatching success, and fledging success. For all measures except recruitment probability, ANCOVAs were employed with maternal and paternal inbreeding coefficients and the pair's kinship coefficient as covariates and with year as a categorical variable (for details of this method, see Keller

1998). Inbreeding depression in recruitment probability was estimated using logistic regressions. Each individual contributed an observation (breeding or not) each year. This allowed the inclusion of year effects in the models, which was necessary given the large variations in average breeding probabilities between years (Grant 1999). However, individuals consequently were represented more than once in this analysis, possibly leading to nonindependence. We therefore used generalized estimating equations to fit population average models to the data (Hosmer and Lemeshow 2000). These models are an extension of the generalized linear model and are designed to handle binary data with repeated measures. They include random effects that account for the fact that repeated observations were taken from the same individual. The results we obtained were robust to different assumptions about the nature of the covariance matrix within individuals. The parameter estimates derived from these generalized estimating equations were used to calculate odds ratios (Hosmer and Lemeshow 2000) to aid interpretation.

Lethal Equivalents

One of the best ways to represent inbreeding depression in a standard way that allows for easy comparisons among studies and species is the framework of lethal equivalents (Morton et al. 1956). If the mutations at independent loci have independent effects on fitness, the logarithm of fitness (or a major fitness component like viability) is expected to decrease linearly with increased inbreeding. The negative slope (B) of this relationship is a standardized measure of inbreeding depression and equals the number of lethal equivalents in the haploid genome. A lethal equivalent is defined as the number of deleterious genes whose cumulative effects equal that of one lethal (Cavalli-Sforza and Bodmer 1971; Charlesworth and Charlesworth 1987).

The number of lethal equivalents (B) can be estimated from survival data using linear regressions (Morton et al. 1956). A common occurrence in many datasets, however, is zero survival at one or more inbreeding levels, leading to an undefined logarithm. This problem has been typically overcome by using a small sample correction. An alternative approach that does not require small sample corrections and that has been shown to be generally unbiased is the use of maximum-likelihood (Kalinowski and Hedrick 1998). We therefore used the maximum-likelihood approach given by Kalinowski and Hedrick (1998) to estimate lethal equivalents from survival data. We estimated the number of lethal equivalents for juvenile survival only, since adult survival does not have a natural cut-off time at which survival can be assessed and most other studies only have data on juvenile survival. We only calculated lethal estimates for those years for which we had sufficient data to get meaningful estimates (see Table 5).

All statistical analyses were performed using the SAS system (SAS Institute 1997).

Results

Observed Levels of Inbreeding

All four grandparents were known for 120 unique *G. scandens* and 364 unique *G. fortis* matings. Of those, 16.7% of
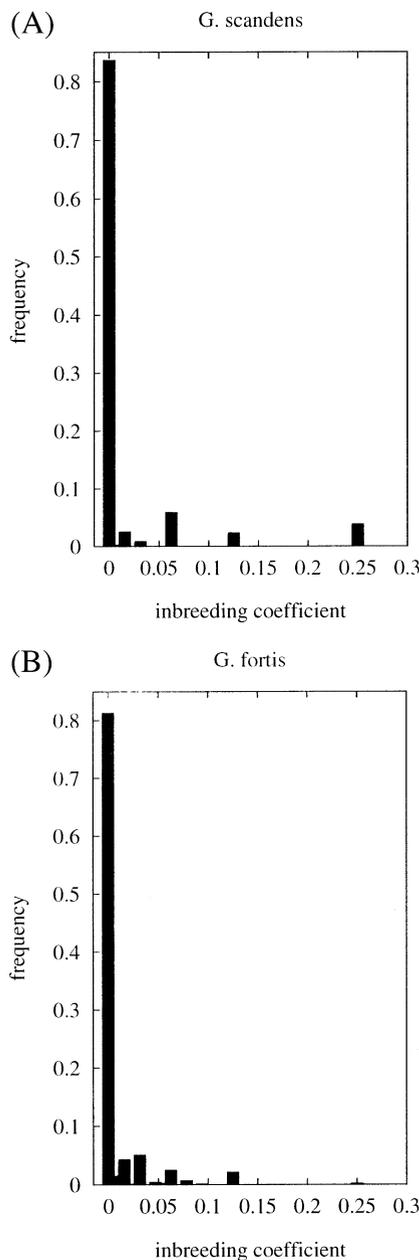

FIG. 1. Frequency distribution of inbreeding coefficients observed among (A) *Geospiza scandens* and (B) *G. fortis* on Daphne Major.

the *G. scandens* and 19.8% of the *G. fortis* were detectably inbred (Fig. 1) and 3.3% and 0.8%, respectively, were the product of matings among first-degree relatives ($f = 0.25$). Thus, the two species did not differ significantly in the proportion of birds with $f \geq 0.25$ (likelihood-ratio test, $G^2 = 3.36$, $P = 0.07$), but they did differ in the frequency of matings among first-degree relatives expressed as a proportion of all inbred matings. One fifth of all inbred matings among *G. scandens* led to $f \geq 0.25$, while only 4.2% of all inbred matings among *G. fortis* led to this high level of inbreeding (likelihood-ratio test, $G^2 = 4.56$, $P = 0.03$). Consequently, the mean f of all inbred matings among *G. scandens* was $f = 0.094$ (SE = 0.020, $n = 20$) while it was $f = 0.053$ (SE

TABLE 1. EPP rates are 2.6 times higher among *Geospiza fortis* than among *G. scandens*. The results are based on *G. fortis* and *G. scandens* with molecular data for both parents and known inbreeding coefficients.

	G. fortis	*G. scandens*
No. of offspring	225	116
No. of families	84	25
No. of EPPs	46	9
% EPP	20.4	7.8
No. of families with ≥1 EPP	32	7
% families with ≥1 EPP	38.1	28.0

$= 0.007$, $n = 72$) in *G. fortis*. Reflecting these differences and different average population sizes, the average kinship coefficients (f) differed slightly but not significantly. For *G. scandens* f averaged 0.016 ($n = 120$), while f averaged 0.011 ($n = 364$) in *G. fortis*. These average levels of inbreeding among pairs with known grandparents are lower than several others reported for island populations of birds (see table 2 in Keller 1998), presumably largely owing to the fact that *G. scandens* and *G. fortis* have larger average population sizes than the other island populations.

Occurrence of Extrapair Paternities and Their Effects on Inbreeding Estimates

The EPP rate was significantly higher among *G. fortis* than *G. scandens*: 20.4% of young *G. fortis* were not sired by their putative fathers, whereas the same applied to only 7.8% of *G. scandens* young (likelihood-ratio test, $G^2 = 10.1$, $P = 0.001$; Table 1). The EPP rate among the sample of *G. scandens* was 7.8% (Table 1). The combined exclusion probability (Weir 1990, p. 187) of the eight microsatellite loci was greater than 99.9%. It is unlikely, therefore, that we failed to exclude a father if in fact he did not sire an offspring. Maternity was apparently correctly identified by observation of behavior in both species: all offspring matched their mothers at all loci, indicating that intraspecific brood parasitism is either absent or very rare in both species on Daphne Major.

Inbreeding did not affect the occurrence of extrapair paternities in *G. fortis* or *G. scandens*, although the analysis for the latter species was based on a small sample (nine EPPs). There was no relationship between the likelihood of a young being an EPP and the degree of relatedness among its parents (logistic regression: *G. scandens*: $\chi^2 = 0.30$, $P = 0.58$, $n = 116$; *G. fortis*: $\chi^2 = 1.05$, $P = 0.30$, $n = 225$) suggesting that while EPPs introduced error into our estimates of inbreeding they did not bias them. This view is further supported by an analysis of the 16 cases of EPP among *G. fortis* for which we were able to identify the genetic fathers unequivocally. Albeit small, this dataset allows us to test directly the hypothesis that females prefer extrapair males that are unrelated. In eight of the 16 cases of EPP the presumed father and the genetic father were both unrelated to the female. In three of the remaining cases the genetic father was more closely related to the female than was the presumed father (in one case the extrapair male was the female's uncle). In five cases the opposite was true. The average difference between the coefficient of relatedness of the presumed pair and the actual pair should be positive if females prefer ex-

TABLE 2. Effects of inbreeding (f) on survival of juveniles from banding to one year of age in *Geospiza scandens* (A) and *G. fortis* (B). Estimates of all significant parameters in a discrete-time proportional hazards model and that for the effects of inbreeding are given. Numscan is the population size of *G. scandens*, and numfort is the population size of *G. fortis*. Positive parameter estimates indicate a negative influence of the variable upon juvenile survival. The values in parentheses represent 95% confidence intervals. Deviance values given are residual deviance.

Term	df	Estimate (95% CI)	χ^2	P
(A)				
Intercept	1	−22.36 (−34.45, 10.16)	—	—
Numscan	1	9.60 (4.51, 14.68)	13.39	0.0003
Numscan²	1	−0.87 (−1.37, −0.38)	11.81	0.001
Numfort	1	−0.33 (−0.69, −0.01)	4.05	0.04
Rain	1	−0.25 (−0.42, −0.09)	8.58	0.003
f	1	18.16 (2.60, 34.36)	5.26	0.02
$f \times$ rain	1	−3.00 (−5.66, −0.56)	5.89	0.02
		$n = 502$, deviance = 606.7		
(B)				
Intercept	1	7.20 (−0.98, 15.09)	—	—
Numfort	1	−1.87 (−3.27, −0.41)	6.26	0.01
Numscan	1	0.64 (0.48, 0.81)	53.30	0.0001
Rain	1	−1.70 (−3.17, −0.41)	4.82	0.03
f	1	−1.08 (−3.93, 1.48)	0.64	0.42
		$n = 1994$, deviance = 2623.1		

trapair males that are unrelated. However, this difference was negative although not significantly so (mean difference = −0.013, paired t-test $P = 0.34$, $n = 16$). Thus, there is no evidence that females prefer extrapair males that are less related than their social mates.

Inbreeding Depression

Survival

Geospiza scandens.—Inbreeding significantly decreased annual survival of both juvenile and adult *G. scandens* after the model took account of environmental variables that affected survival (Tables 2 and 3). Number of finches alive on the island (both *G. scandens* and *G. fortis*) as well as rainfall all directly influenced annual survival of juvenile and adult *G. scandens*. Males and females did not differ significantly in the degree of inbreeding depression, but differences in survival between males and females approached significance ($P = 0.06$), with males surviving better than females for a given level of inbreeding.

Inbreeding depression varied as a function of environmental conditions (Fig. 2), as witnessed by the significant interaction between inbreeding and rainfall for juvenile survival (Table 2) and the significant three-way interaction between inbreeding, rainfall, and population density for adults (Table 3). An offspring of a full-sibling mating ($f = 0.25$) was on average 21% (95% CI: −21–44%) less likely to survive its first year than a noninbred bird. Note that although inbreeding negatively affects survival as a main effect, the confidence interval for the reduction in survival includes zero because of the interaction term between inbreeding and rainfall. Under conditions of low rainfall (less than 150 mm), the reduction in survival of a juvenile *G. scandens* with $f = 0.25$ was 65% compared to a noninbred juvenile. However,

TABLE 3. Effects of inbreeding (*f*) on annual survival of adult *Geospiza scandens* (A) and *G. fortis* (B). The notation is as in Table 2.

Term	df	Estimate (95% CI)	χ^2	P
(A)				
Intercept	1	−11.61 (−20.01, −3.21)	—	—
Numscan	1	2.62 (1.27, 3.97)	14.30	0.0002
Numfort	1	−0.50 (−0.94, −0.05)	5.14	0.02
Rain	1	2.31 (0.59, 4.04)	6.95	0.01
Numscan × rain	1	−0.44 (−0.77, −0.09)	6.33	0.01
f	1	12.09 (1.61, 22.31)	5.13	0.02
f × numscan × rain	1	−0.50 (−0.98, −0.01)	4.07	0.04
		n = 329, deviance = 370.4		
(B)				
Intercept	1	−2.91 (−3.63, −2.20)	—	—
Numfort	1	0.18 (0.07, 0.28)	10.55	0.001
Rain	1	0.92 (0.77, 1.07)	161.85	0.0001
Rain²	1	−0.16 (−0.19, −0.13)	167.61	0.0001
f	1	1.49 (−1.14, 4.11)	1.16	0.28
		n = 2730, deviance = 3108.4		

in years of medium or high rainfall (more than 150 mm), the survival of such an inbred individual was not significantly different from a noninbred juvenile (Fig. 2a). Thus, inbreeding depression was only evident in years with low rainfall. Results for adults were similar, but inbreeding depression was present under all conditions. Annual survival of an adult with *f* = 0.25 was on average reduced by 45% (95% CI: 10–80%). Adult survival of inbred (*f* = 0.25) birds was essentially zero in years with low rainfall (less than 10 mm) and high population density (more than 150 conspecifics), giving a reduction in survival of 99% compared to noninbred adults. In all other years combined, this reduction was only 19% on average (Fig. 2b).

Geospiza fortis.—Contrary to *G. scandens*, there was no evidence in *G. fortis* for inbreeding depression in either juvenile or adult survival. Inbreeding did not enter any of the survival models significantly, either as a main effect or as an interaction (Tables 2 and 3). However, the confidence limits around the parameter estimates for the effects of inbreeding were large and included values that represent high levels of inbreeding depression, thus indicating low statistical power of the analyses. The confidence limits indicate that our analyses could not have distinguished zero inbreeding depression from severe inbreeding depression, that is, a decline in annual survival of inbred *G. fortis* (*f* = 0.25) of 43%.

Reproduction

Geospiza scandens.—Inbreeding depression was also evident in reproduction of *G. scandens*. Not all adult *G. scandens* alive on Daphne were recruited into the breeding population. On average, only 23% of all adults alive were also breeding. The probability that an adult *G. scandens* would be breeding was significantly reduced by inbreeding (Table 4). Individuals with *f* = 0.25 were approximately one sixth as likely to breed than outbred individuals (odds ratio = 0.15). Recruitment probability differed between males (15%) and females (32%; Table 4), but the effects of inbreeding did not differ between the sexes (sex × inbreeding interaction: *P* ≥ 0.36).

Due to the substantial inbreeding depression in survival and recruitment sample sizes of inbred *G. scandens* that were

breeding were small. Only nine inbred *G. scandens* ever bred on Daphne. Based on these small samples, no inbreeding depression was evident in the production of either eggs, nestlings, or fledglings by inbred *G. scandens*. Similarly, inbred eggs and offspring did not experience reduced hatching or fledging success (ANCOVAs, all *P* ≥ 0.26). However, the statistical power of the analyses was very low.

Geospiza fortis.—On average 25% of all adult *G. fortis* alive on Daphne bred in a given year. As with *G. scandens*, the recruitment probability was negatively affected by inbreeding (Table 4): Individuals with *f* = 0.25 were one eighth as likely to breed than outbred individuals (odds ratio: 0.12). Recruitment probability did not differ between the sexes in *G. fortis* (*P* ≥ 0.95).

No other measures of reproductive success showed any evidence of inbreeding depression. Inbred mothers or fathers did not experience reduced reproductive success nor did inbred offspring experience lower hatching or fledging success (ANCOVAs, all *P* ≥ 0.18). These results and those for survival are in line with an earlier study on inbreeding depression in *G. fortis* based on a much smaller data set that also did not detect significant inbreeding depression in this species (Gibbs and Grant 1989).

Magnitude of Inbreeding Depression Compared to Other Species

Estimates of inbreeding depression in juvenile survival as measured by the number of lethal equivalents (*B*) varied substantially from year to year among both juvenile *G. scandens* and *G. fortis* (Table 5). In both species, there was no inbreeding depression (*B* = 0) in two or more years but extremely high estimates of *B* in others (Table 5). The extremely high values in 1990 for *G. fortis* and in 1984 and 1992 for *G. scandens* were due to zero survival of all inbred young in two years with little rainfall and one year with moderate rainfall. Although zero survival of all inbred young clearly represents strong inbreeding depression, the resulting estimates of lethal equivalents in these years are unreliable because of the numerical problems that arise when survival is zero. Thus, these very large values should not be taken at
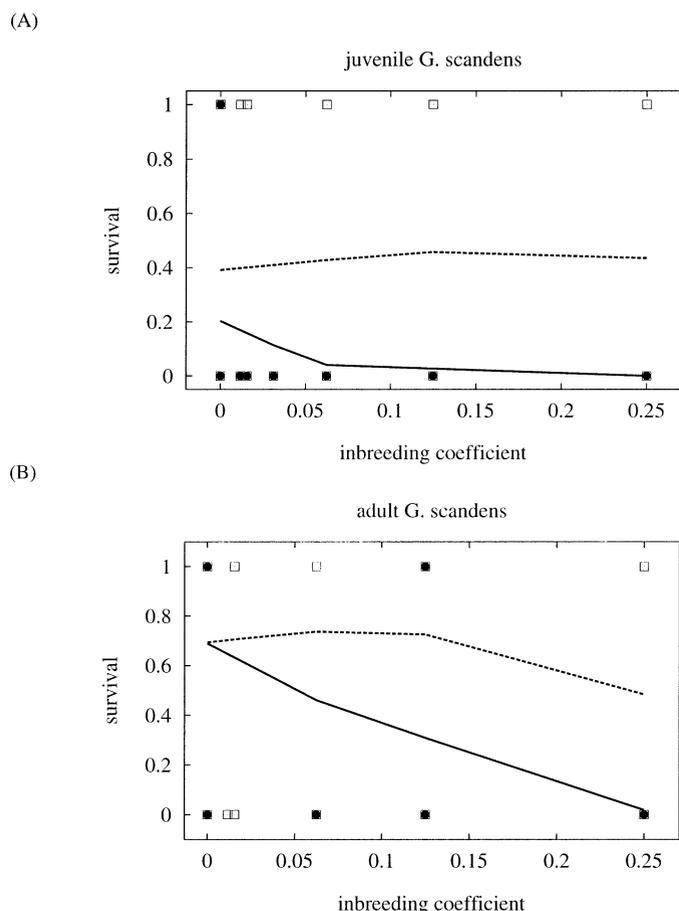
(A)

(B)

FIG. 2. Inbreeding depression in survival of (A) juvenile and (B) adult *Geospiza scandens* was a function of environmental conditions. The circles and squares represent the raw data and the lines are cubic splines. Note that many of the original data points are overlapping in the figure. (A) Inbreeding depression in juvenile *G. scandens* was only evident in years with low rainfall (less than 150 mm; filled circles and solid line) and not in all other years combined (open squares and broken line). (B) Inbreeding depression in adult survival was a function of rainfall (an index of food availability) and the population density of conspecifics (intraspecific competition). Inbreeding depression was extreme under very dry (less than 10 mm) and crowded (more than 150 conspecifics) conditions (filled circles and solid line), and less pronounced but still significant under all other conditions(open squares and broken line).

TABLE 4. Inbreeding)(f reduces the probability of breeding of (A) adult *Geospiza scandens* and (B) adult *G. fortis* in a given year, provided that they are at least one year of age. Parameter estimates from a GEE logistic regression are given with 95% confidence intervals in parentheses. The individual estimates for the year effects are omitted for simplicity.

Term	df	Estimate (95% CI)	χ^2	P
(A)				
Intercept	1	-0.15 (-2.50, 0.69)	1.29	0.26
Year	6		30.86	0.0001
Sex	1	-1.12 (-1.83, -0.41)	6.49	0.01
f	1	-7.64 (-14.92, -0.36)	4.71	0.03
		$n = 206$, deviance $= 186.0$		
(B)				
Intercept	1	0.18 (-0.52, 0.88)	0.18	0.67
Year	8		169.55	0.0001
f	1	-8.43 (-14.26, -2.60)	7.56	0.01
		$n = 1504$, deviance $= 1792.1$		

face value but rather as an indication that inbreeding depression was strong. Note that the survival analyses revealed significant inbreeding depression whereas the confidence limits for most estimates of *B* include zero. This reflects the larger sample sizes of the survival analyses achieved by combining all years and allowing for environmental covariates and their interaction with inbreeding.

Given the highly skewed distribution of estimates of lethal equivalents and the unreliability of the exact estimates for the years with zero survival, the median appears to be the most appropriate measure of average inbreeding depression. The median number of lethal equivalents was zero among juvenile *G. fortis* and 4.27 among juvenile *G. scandens*. For comparison, we used data given in Grant et al. (2001) to estimate *B* for a third species of Darwin's finches breeding on Daphne, *G. magnirostris*. Based on data from two cohorts ($n = 56$), we estimated *B* for juvenile survival to be 4.5 (95% CI: 0.8–9.1). Thus, *G. scandens* and *G. magnirostris* show substantial inbreeding depression, whereas juvenile survival of *G. fortis* apparently was not affected by inbreeding.

DISCUSSION

Occurrence of Extrapair Paternities and Their Effects on Inbreeding Estimates

Geospiza fortis were 2.6 times more likely to engage in EPPs than *G. scandens*. However, in both species, females mated to a relative were not more likely to engage in EPPs than females unrelated to their mates. Because so few inbred

TABLE 5. *B* refers to number of lethal equivalents per haploid genome for juvenile (first-year) survival in *Geospiza scandens* and *G. fortis*, with the 95% confidence interval in parentheses. *N* refers to the number of juveniles each year on which the calculations are based.

	1983	1984	1987	1990	1991	1992	1993	Median
Juvenile *G. scandens*								
B	0 (0, 2.2)	276 (0, 683)	7.38 (0, 81.8)	—	1.13 (0, 6.7)	545 (351, 740)	0 (0, 6.0)	4.27
N	161	107	90	—	86	12	18	6
Juvenile *G. fortis*								
B	0 (0, 2.8)	1.20 (0, 10.9)	0.36 (0, 3.4)	652.7 (0, 1375)	0 (0, 2.4)	0 (0, 24.7)	0 (0, 6.8)	0
N	387	243	688	21	485	44	74	7
Rain (mm)	1359	51	649	40	190	210	234	

G. scandens ever bred, we were unable to study mate choice through EPPs directly in this species. However, in the congener *G. fortis*, there is no evidence that females prefer extrapair males that are less related than their social mates. Therefore, EPPs will have introduced error variance into our estimates of inbreeding and considerably more so in *G. fortis* than *G. scandens*. But they are unlikely to have led to biased estimates because extrapair males were not any less related to the females than the presumed fathers. Unbiased errors in the inbreeding coefficients are likely to lead to an underestimate of inbreeding depression. In regressions, unbiased error in the explanatory variable leads to a downward bias of the resulting regression coefficient provided that the sum of the variance of the error in the explanatory variable and the covariance between the true value of the explanatory variable and its error are greater than zero (Draper and Smith 1981, p. 123). That is, if the variance of the error in the inbreeding coefficients (σ_δ^2) plus the covariance between f and its error ($\sigma_{f,\delta}$) exceeds zero, the estimates of inbreeding depression are underestimates. In *G. fortis* the errors in f appear to be unbiased and simple simulations suggest that under these conditions the sum $\sigma_\delta^2 + \sigma_{f,\delta}$ is positive under most circumstances. Thus, the magnitude of inbreeding depression in reality is likely to be higher than is indicated by the analyses presented above. More in-depth analyses are required to determine the degree to which the assumptions underlying Draper and Smith's (1981) treatment are violated in analyses of inbreeding depression and to quantify the magnitude of the underestimate caused by EPPs. The proportion of EPPs will not directly translate into the amount of error in estimates of inbreeding, because the identity of the actual father will also affect the degree of pedigree error. The sample of actual fathers identified in *G. fortis* is not large enough to allow for a rigorous analysis.

Inbreeding Depression

Recruitment probability displayed strong inbreeding depression in both species and juvenile and adult survival showed evidence of inbreeding depression in *G. scandens* but not *G. fortis*. Two factors likely explain the paucity of apparent inbreeding depression in *G. fortis*. First, the relatively high rate of EPPs (2.6 times higher than in *G. scandens*) increases the error in our estimates of inbreeding coefficients, thus reducing the likelihood of detecting statistically significant inbreeding depression when it exists. The errors in f also likely result in an underestimate of the actual inbreeding depression. Second, the low number of highly inbred ($f = 0.25$) *G. fortis* results in low statistical power of the survival analyses. The low statistical power is reflected in the large confidence intervals for the magnitude of inbreeding depression (Tables 2–5). Thus, although our results provide evidence for inbreeding depression in only one trait in *G. fortis*, they do not provide evidence that inbreeding depression was absent or even low in all other traits in *G. fortis*.

Survival

Environmental conditions strongly affected the magnitude of inbreeding depression among juvenile and adult *G. scandens*. We used two measures of environmental conditions:

rainfall as a proxy measure of food availability and the number of conspecifics of all ages as a measure of the magnitude of competition. Among juvenile *G. scandens*, inbreeding depression was only evident when food availability was low. Thus, inbreeding depression was not only most pronounced under stressful conditions, but for juvenile survival it was only detectable when conditions were poor. Coltman et al. (1999) found a comparable situation among Soay sheep on the island of Hirta. Both lamb and adult survival were only associated with heterozygosity (which correlates with inbreeding) in years with severe overwinter mortality.

Results for adults were similar, yet differed in interesting ways. As with juvenile survival, environmental conditions strongly affected the magnitude of inbreeding depression. Inbreeding depression was five times stronger under conditions of low food availability and high conspecific competition than under all other conditions. In fact, highly inbred ($f = 0.25$) adults had virtually zero survival in such dry and crowded years, representing very severe inbreeding depression. However, contrary to the results for juvenile survival, no similarly clear patterns of interactions between environmental quality, average survival, and magnitude of inbreeding depression emerged. Survival of adult *G. scandens* (irrespective of inbreeding) was lowest in dry and crowded years, but also in years with very high rainfall independent of density (Table 3). Based on mortality rates, years with high rainfall were therefore similarly as stressful as dry and crowded years. Inbreeding depression, however, was average in wet years and much less than in dry, crowded years.

Our data show that adults differed from juveniles in the way environmental conditions, mortality, and magnitude of inbreeding depression interacted. Two mechanisms may explain why adults did not show the same pattern as juveniles. First, juvenile and adult *G. scandens* differ in their use of food sources, and rainfall as an index of food abundance does not capture this variation. Although juvenile *G. scandens* largely feed on arthropods, pollen, and small seeds from various plants, adult *G. scandens* on Daphne Major are highly dependent on cactus (*Opuntia echios*) flowers and fruits as a food source (Millington and Grant 1983; Grant and Grant 1996). Wet El Niño conditions cause cacti to be overgrown by vines and, consequently, cacti produce few flowers or fruits in El Niño years (Gibbs and Grant 1987b). Cacti seeds and fruits are therefore rare in such years and this may contribute to the high mortality rates observed among adult *G. scandens* under such wet conditions. Therefore, the relationship between rainfall and adult mortality is not as straightforward as it is for juveniles.

Second, when little food was available (low annual rainfall), most inbred juvenile *G. scandens* died and therefore never reached adulthood. On Daphne Major, very wet years (caused by El Niño events; Grant et al. 2000) tended to be preceded by periods of dry and crowded conditions. For example, the 1987 El Niño was preceded by three dry years with high population density (Grant et al. 2000). Inbreeding depression among juveniles and adults was very high during these three years leading to very high mortality of inbred birds, and insufficient rain fell in two of the three years to allow breeding. Therefore, no inbred adults were alive in the *G. scandens* population on Daphne in 1987 when large

amounts of rain fell. The situation was similar in 1991, another El Niño year. Consequently, sample sizes of inbred adults in very wet years were very small and the lack of an increased magnitude of inbreeding depression in those wet years with high general mortality may be due to a lack of adequate sample sizes.

It is likely that the nature and shape of the relationship between inbreeding depression, environmental variables, and average mortality depends on the actual mechanisms that lead to reduced fitness of inbred individuals and the nature of the environmental stress applied. Environmental factors such as heat or drought stress and exposure to stressful chemicals (Miller 1994; Bijlsma et al. 1999) are likely to lead to different patterns of interactions between environmental conditions, mortality, and inbreeding depression than factors mediated by population density such as parasite prevalence (Coltman et al. 1999), intrasexual competition (Meagher et al. 2000), or food limitation (this study). This fact was borne out clearly in recent experiments with the outcrossing plant, *Crepis sancta* (Cheptou et al. 2000a,b). Although field experiments revealed that interspecific competition greatly increased inbreeding depression, no such general pattern was found when the same species was subjected to drought stress. More data on the effects of different types of environmental factors on the magnitude of inbreeding depression are necessary to elucidate this issue.

Magnitude compared to other species

Geospiza scandens and *G. magnirostris* show substantial inbreeding depression in comparison to other studies (Table 6). The magnitude of inbreeding depression observed among juvenile *G. scandens* and *G. magnirostris* suggests that purging has had little effects on the genetic load in these populations. In small, isolated populations, selection against deleterious recessives made homozygous due to inbreeding is expected to reduce the genetic load over time, at least the load due to genes of large effects (e.g., Hedrick 1994). Many factors determine the efficiency of purging, and theoretical and empirical studies suggest that purging is unlikely to act consistently in natural populations (Byers and Waller 1999; Bijlsma et al. 1999; Kirkpatrick and Jarne 2000). Apart from possible genetic reasons for the lack of efficient purging (e.g., Keller and Waller 2002), there is an ecological mechanism that may prevent purging in *G. scandens*, *G. fortis*, and *G. magnirostris*: the populations are probably not genetically isolated enough to prevent reintroduction of deleterious recessive alleles that may have been purged on Daphne. The *G. magnirostris* population receives a constant flux of immigrants (Grant and Grant 1995b; Grant et al. 2001) and a few immigrant *G. fortis* have also been identified (Grant and Grant 2000). No immigrant *G. scandens* has been observed to breed on Daphne during the study period (Grant and Grant 2000), but on average 0.8% of breeding *G. scandens* individuals hybridized with *G. fortis* during the study period. F_1 offspring have had high fitness since 1983 and several of them backcrossed to *G. scandens* after 1990 (Grant and Grant 1998). Thus, hybridization may lead to sufficient gene flow to prevent substantial purging among *G. scandens*. Levels of variation at 16 microsatellite loci are similar in *G. fortis*, *G.*

TABLE 6. Comparison of the magnitude of inbreeding depression in several species. Estimates of lethal equivalents per haploid genome (*B*) are given for other studies of wild vertebrate populations. Note that the estimate in Koenig et al. 1999 is based on simulations rather than direct estimation. For comparison, some estimates of *B* from laboratory and human studies are given in the bottom part of the table.

Species	Trait	B	Reference
Medium ground finch	*Geospiza fortis*	banding to first year	0	this study
Large ground finch	*Geospiza magnirostris*	banding to first year	4.47	this study, based on data in Grant et al. 2001
Cactus finch	*Geospiza scandens*	banding to first year	4.27	this study
Song sparrows	*Melospiza melodia*	egg to first breeding	2.69	Keller 1998
Great tits	*Parus major*	hatch rate	0.77	van Noordwijk and Scharloo 1981
Great tits	*Parus major*	egg to fledging	0.72–1.16	Bulmer 1973
White-footed mice	*Peromyscus leucopus*	three-week survival	6.3	Jiménez et al. 1994
Acorn woodpeckers	*Melanerpes formicivorus*	R_O	0.6–0.9	Koenig et al. 1999
Humans	*Homo sapiens*	neonatal and juvenile survival	0.58–2.57	Lee et al. 1996
Squinting bush brown	*Bicyclus anynana*	juvenile survival	1.20	van Oosterhout et al. 2000
Speke's gazelle	*Gazella spekei*	survival to 30 days	0.57–6.56	Kalinowski et al. 2000

magnirostris, and *G. scandens* on Daphne Major (Petren et al. unpubl. data), supporting the view that none of the populations has been isolated for a long time.

CONCLUSION

The main results of this study are, first, severe inbreeding depression was evident in an island population of *G. scandens* and, to a lesser extent, in *G. fortis*. Together inbreeding depression in survival and recruitment probability was so severe that no inbred *G. scandens* ever produced any grandoffspring at all! The comparison of lethal equivalents suggests that inbreeding in natural populations can easily be as high as in artificial systems such as laboratories, farms, or zoos. Our results and those by Keller (1998) also make clear that inbreeding depression may be pronounced even in island populations.

Second, the degree of inbreeding depression was strongly affected by the environmental conditions to which the birds were exposed. For one trait (juvenile survival), inbreeding depression was only detectable when environmental conditions were poor. For adult survival, inbreeding depression was five times more severe under poor environmental conditions. Nevertheless, although evidence is accumulating that the magnitude of inbreeding depression in natural animal populations can be substantially modified by environmental conditions, it is also becoming apparent that inbreeding depression is not necessarily always most pronounced when environmental conditions are poorest (Cheptou et al. 2000b, Keller and Waller 2002).

Third, a 20% extrapair fertilization rate may have contributed to the fact that we were unable to detect statistically significant inbreeding depression in all but one trait in *G. fortis*. Studies with relatively high EPP rates are therefore unlikely to detect significant levels of inbreeding depression unless these are extremely high. Similarly, the absence of detectable inbreeding depression should not be interpreted as evidence for zero inbreeding depression if paternity is uncertain and/or if the sample size of highly inbred individuals is small.

ACKNOWLEDGMENTS

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