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A population founded by a single pair of individuals: establishment, expansion, and evolution

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Abstract

Events occurring at the founding of a population, and in the next few generations, are potentially of great importance for the future evolution of the population. This study reports demographic, genetic, and morphological changes that took place during and after the colonization of the small Galápagos island of Daphne Major by three male and two female large ground finches, *Geospiza magnirostris*, at the end of 1982. Using assignment tests with microsatellite DNA data we demonstrate heterogeneity among the immigrants. Their sources included both a near island (Santa Cruz) and a far island (Marchena). However, almost all immigrants that stayed to breed were from an intermediate island (Santiago) and its satellites. Song may have been responsible for this selectivity. Mean heterozygosity stayed roughly constant over the next 15 years while allelic diversity almost doubled, after an initial decline, as the breeding population increased to a maximum of 30 pairs. Although close inbreeding occurred, with a reduction in heterozygosity, an expected net decline in heterozygosity did not occur, for two reasons: it was counteracted by continuing gene flow from immigrants at a low rate, and inbred birds (in one cohort) were at a selective disadvantage. An abrupt step-function shift in beak shape occurred after 9 years. Thus the study provides evidence of drift and selection causing morphological and genetic divergence in the establishment of a new population and in the first few generations.

The founding of a new population

When a new population is established by a few individuals colonizing an unoccupied area, genetic changes are likely to occur solely as a result of random processes. Rare alleles are likely to be lost in the founding event, and heterozygosities will be reduced in subsequent generations at a rate that is dependent upon population growth (Nei, 1987; Hedrick, 1998). As a consequence of these changes the new population diverges genetically from the parent population. Much theoretical attention has been given to the nature of those changes because they have a bearing on several issues in population genetics and evolution. These include the question of what maintains genetic variation, whether gene flow is sufficient to prevent local adaptation, and how speciation occurs. The founding of a new population by a small number of breeders may lead to a change in genetic architecture (Wright, 1931; Mayr, 1954; Bryant, McCommas & Coombs, 1986; Whitlock & Wade, 1995; Armbruster, Bradshaw & Holzapfel, 1998), and specifically to an increase in additive genetic variance (Bryant & Meffert, 1995, 1996; Cheverud & Routman, 1996; Cheverud et al., 1999) that could facilitate rapid evolution. These are not inevitable outcomes however. Although the theory of founder effect speciation lays great stress on the genetic changes occurring in the early stages of population establishment (Mayr, 1992; Gavrilets & Hastings, 1996; Slatkin, 1996; Templeton, 1996; Gavrilets & Boake, 1998), the evidence for profound changes sufficient for reproductive

isolation to arise at this time has been repeatedly questioned (Coyne, 1994; Futuyma, 1994; Barton, 1996; Grant & Grant, 1996a; Charlesworth, 1997), hence the magnitude, frequency of occurrence and evolutionary significance of founder effects remain in doubt.

These issues can be partly resolved in the laboratory or greenhouse, but their final resolution requires field research. Needed, but conspicuously missing, is a body of empirical work that follows the genetic and phenotypic fate of populations, and individuals within those populations, from their natural establishment onwards. Typically, instead, a new population is discovered some time after it has become established. Changes occurring during and immediately after establishment are then inferred from what can be learned at that later time (e.g., Baker & Moeed, 1987; Conant, 1988; Fleischer, Conant & Morin,1991; Degnan, 1993; Merilä, Björklund & Baker, 1996a, 1996b; Mundy et al., 1997; Tarr, Conant & Fleischer, 1998; Losos, Warheit & Schoener, 2001; Reznick & Ghalambor, 2001).

Here we report the results of a study of a population of Darwin's finches in the 18 years following its establishment on the small and undisturbed Galápagos island of Daphne Major (Grant & Grant, 1995a). Morphologically and demographically the population was well characterized throughout this period. Genetic variation was assayed with microsatellite DNA markers in the last 12 years. The goals of the study were to investigate the genetic and morphological changes that might be expected to occur in the founding of the population and in the next few generations. Specifically we attempted to determine the source of the immigrants with genetic and song markers, whether the immigrants that stayed to breed were a random or non-random sample of those that arrived, whether fitness was a function of degree of relatedness, heterozygosity or morphology, and whether the population changed over time in both genetic and morphological constitution as it increased in size. The answers to these questions provide a unique insight into what must have happened numerous times during the adaptive radiation of the finches.

Early history of the Daphne population

An earlier report described the main events up to 1993 (Grant & Grant, 1995a). They are summarized as follows. Immigrant large ground finches were present on Daphne in the dry seasons of every year from 1973 to 1982, but never stayed to breed. They had temporarily invaded from another island, tentatively identified as the nearest large island of Santa Cruz (Figure 1) on the basis of geographical position as well as the song types of males. In breeding seasons they were scarce or absent, and none bred. In December 1982, a breeding population became established by three males and two females that bred in three pairs several times in the next 6 months and produced a total of 17 fledglings. Only one of the pairs produced offspring that survived to breed, therefore the genetically effective size of the founder population size was two. The original colonizers appeared to be a non-random sample with respect to morphological variation, but the small sample of measured colonists (two) precluded a more definite statement. By uniquely marking nestlings and captured individuals we were able to establish pedigrees, and to use them to document close inbreeding at an exceptionally high frequency as a result of random mating. Inbreeding was accompanied by an apparent loss of short-term reproductive fitness, although sample sizes were small and the loss was not supported statistically. Frequencies of three types of song, a culturally transmitted trait, changed through a process analogous to genetic drift. The population increased from three to 10 pairs, partly as a result of recruitment of locally born birds and partly through additional immigration. Over a period of 10 years the population became larger than the smallest population of congeners on the island, the small ground finch *G. fuliginosa*. Two other species on the island have much larger populations (Grant & Grant, 1996b): the cactus finch, *G. scandens* (∼20–150 pairs) and the medium ground finch *G. fortis* (∼70–300 pairs).

The genetic characteristics of the population were unknown. A blood sampling program for DNA typing was initiated in 1988, and analysis began in 1997. Some of the birds banded before 1988 were captured and sampled, and the pedigrees of these birds enabled us to reconstruct genetic characteristics of the breeding population before the blood sampling began.

Materials and methods

Details of the island, species and basic methods of study have already been published (Grant & Grant, 1992, 1994, 1995a, b). Daphne Major island has an area of ∼0.34 ha, an elevation of 120 m and is situated in the center of the Galápagos archipelago ∼8 km

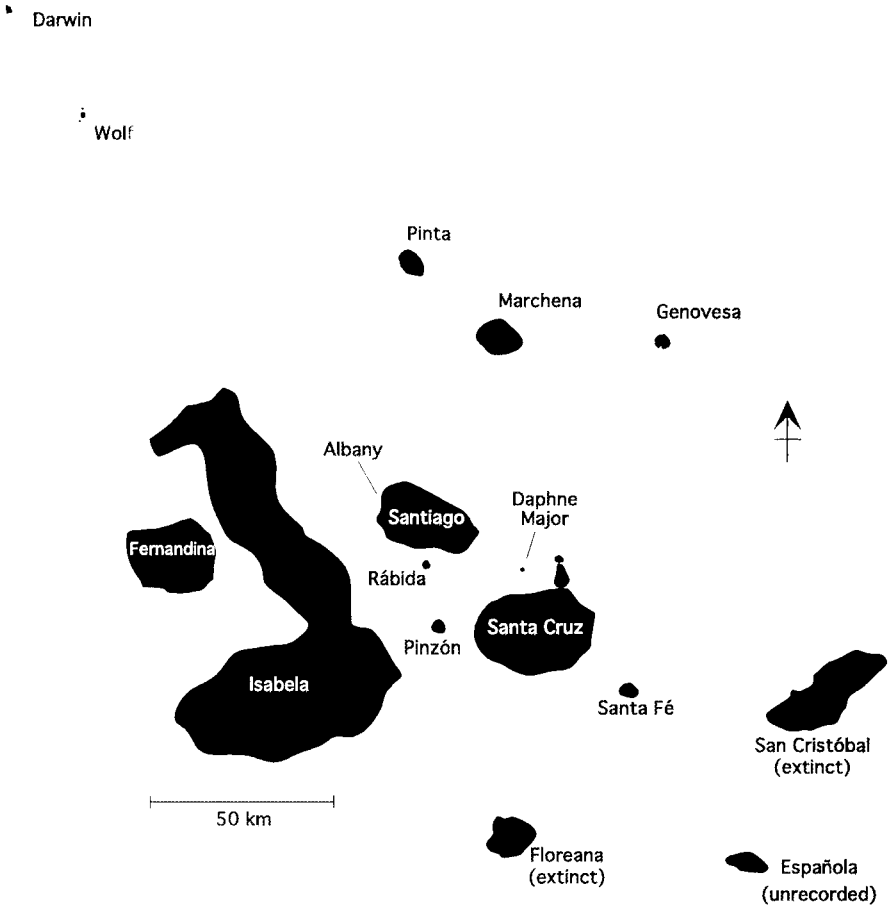

Figure 1. Galápagos islands. All islands have populations of *Geospiza magnirostris*, except San Cristóbal and Floreana, where they became extinct some time after Darwin's visit in 1835, and Española.

from the neighboring islands of Santa Cruz, Baltra and Seymour (Figure 1). Of these three islands only Santa Cruz has a resident population of *G. magnirostris*, and is believed to be the source of most immigrants to Daphne Major (Grant & Grant, 1995a).

In the breeding season, typically February–April, territories of singing males were mapped throughout the island. Nests were marked with numbered flagging, and their contents recorded repeatedly, usually every other day. Social parents were identified by behavior: incubation (female only) or feeding of the nestlings and fledglings by female and male parents. Sexes of adults were determined by plumage and behavior. Only males sing, and they gradually acquire a black plumage from an initial female-like, brown, immature plumage (Grant, 1999). Typically breeding continues for about 8 weeks, but does not occur in drought years. In the years 1976–1991 and again in 1998 almost all nests on the island were found throughout the breeding seasons. From 1992 to 1997 breeding was studied for only 3–6 weeks.

Birds were captured in mist-nets, marked with a unique combination of two or three colored plastic leg bands, and in some years (1987–1991) also with a numbered metal band, and measured before being released. Six measurements were taken; mass (weight) to the nearest gram with a 50 g Pesola^{TM} spring-balance, length of wing to the nearest millimeter with a wing ruler, length of tarsus and bill with a pair of dividers to the nearest 0.1 mm, and depth and width of bill to the nearest 0.1 mm with dial calipers (see Grant & Grant, 1989, 1994, for further details). Nestlings were banded and weighed at day 8, and some were measured when captured as adults one or more years later. Most birds (53%), and all birds captured after 1990, were measured by the senior author. Correction factors, calculated from birds measured by P.R. Grant and eight other measurers, were applied to the rest.

Table 1. Genetic characteristics of 220 *G. magnirostris* on Daphne at 16 microsatellite DNA loci

Locus	Alleles		Heterozygosity	
	Number	Size range (bp)	Observed	Expected
Gf1	11 (7)	30	0.69	0.69
Gf2	10 (7)	24	**0.39**	**0.83**
Gf3	12 (6)	22	0.69	0.75
Gf4	4 (3)	18	0.17	0.18
Gf5	5 (3)	12	0.59	0.59
Gf6	4 (3)	6	0.39	0.46
Gf7	9 (6)	24	0.69	0.73
Gf8	15 (10)	40	0.84	0.82
Gf9	4 (3)	22	0.60	0.55
Gf10	3 (3)	4	**0.02**	**0.27**
Gf11	13 (10)	52	0.86	0.86
Gf12	11 (9)	30	0.83	0.85
Gf13	10 (7)	20	0.53	0.67
Gf14	8 (3)	20	0.78	0.70
Gf15	8 (7)	16	0.68	0.72
Gf16	9 (7)	23	0.75	0.75

Expected heterozygosity is Nei's unbiassed expected heterozygosity (Nei, 1987).
Bold type signifies Z-linked loci.
bp – base pairs.
Loci Gf2 and Gf10 in boldface are Z-linked.
Numbers of alleles in the sample of breeders and their offspring are shown in parentheses.

Repeated measurements of the same bird were averaged. Repeatabilities of measurements made by the same or different measurers have been consistently found to be high in studies of Darwin's finches (Grant & Grant, 1994).

From 1988 onwards a single drop of blood was taken on EDTA-soaked filter paper by brachial vein puncture of nestlings and mist-netted adults, stored in Drierite^{TM} and transferred to the lab in Princeton for extraction and analysis of microsatellite DNA. Dinucleotide repeat alleles at 14 autosomal and two Z-linked microsatellite loci (Petren, 1998; Petren, Grant & Grant, 1999a) were used as markers to genetically characterize the birds, to compare observed with expected heterozygosities (Table 1), to identify parentage and to identify the islands of origin of the immigrants. Two hundred and thirty nine individuals from Daphne were genotyped, most (216) at 12–16 loci and the rest at 7–11 loci. For seven loci alone the probability of detecting extra-pair paternity was greater than 99.6% (Westneat, 1987). Mismatches in alleles at more than two out of 16 loci between an offspring and probable parent were considered to be evidence of incorrect identification of the parent. Two or fewer mismatches, typically of a single base-pair, were considered to be simple scoring or recording errors. In total 62 scoring errors were inferred among 113 offspring genotyped at 16 loci (3.4%). An additional 44 individuals from six islands were genotyped at all 16 loci as follows: Santa Cruz ($N = 6$), Rábida ($N = 2$), Albany ($N = 1$), northern Isabela ($N = 6$), Marchena ($N = 10$), and Genovesa ($N = 19$). Albany and Rábida are satellite islands of Santiago, and their samples were combined because we had no samples from Santiago itself (see Figure 1 for position of islands in relation to Daphne).

Analysis

An index of heterozygosity was calculated for each individual as the proportion of analyzed loci that were heterozygous. Most analyzes included birds of unknown sex and therefore only the 14 autosomal loci were analyzed. Observed and expected heterozygosities were calculated with GENEPOP (Raymond & Rosset, 1995). The squared difference in length of alleles at a locus, averaged over all autosomal loci (mean d^2), was also analyzed because of the proposed relationship between this quantitative measure of difference (or similarity) and degree of inbreeding (Coulson, Pemberton et al., 1998; Coulson et al., 1999). We used a Bayesian modelling approach to assign individuals to potential source populations (Pritchard, Stephens & Donnelly, 2000). The assumption that different island populations are distinct is generally supported by the data (Pritchard, Stephens & Donnelly, 2000; unpubl. analyzes), thus the assignment of immigrants was carried out with source populations defined. Because populations are closely related (Petren, Grant & Grant, 1999a; Sato et al., 1999) allele frequencies were treated as correlated among loci in assignment tests.

In Darwin's finches parametric correlations between all pairs of morphological traits are strong for the sexes treated separately or together (Grant & Grant, 1994, 1995b). Principal components analyses were performed with correlation matrices to reduce the six original morphological variables to three synthetic axes of variation. A body size axis was obtained from untransformed mass, wing, and tarsus in one analysis of the total sample of measurements, and beak size (PC1) and beak shape (PC2) was obtained from a separate analysis of the three beak measurements; see Table 2 for factor loadings. Beak shape

Table 2. Factor loadings from two principal components analyses of morphological variation among 345 *G. magnirostris* on Daphne Major island

	Body size PC 1	Beak size PC 1	Beak shape PC 2
Mass	0.801	–	–
Wing length	0.812	–	–
Tarsus length	0.795	–	–
Beak length	–	0.864	0.502
Beak depth	–	0.898	−0.213
Beak width	–	0.893	−0.272
Percentage variance	64.4	78.4	12.4

from the second analysis is not correlated with body size from the first analysis ($p > 0.1$). Although beak size and body size are strongly correlated ($r = 0.649$, $p < 0.0001$), each is considered to be a separate size trait in analyzes of morphological variation because less than half the variation in one is statistically accounted for by variation in the other ($R^2 = 0.408$).

We used ANOVA of PC scores for the analysis of annual change in mean morphology. Changes take place from year to year through the failure of some birds to survive locally, because some die or emigrate, and through the addition of new birds to the population that are born (hatched) on the island or immigrate from another. Annual changes can thus be analyzed independently, because their components (additions and deletions) are annually independent (e.g., Przybylo, Sheldon & Merilä, 2000a, b). Exceptions arise when there is no breeding or immigration, for then changes can only arise through loss of birds. In none of the years was local survival 100%. We used logistic regression models to estimate the separate effects of partly correlated independent variables upon survival. We sought the minimum number of variables that predicted the survival status of the maximum number of individuals.

We used linear and non-linear time-series analysis (Chatfield, 1996) as a descriptive tool to characterize the pattern of variation in mean morphology as a function of time. Data were fitted by locally weighted regression (lowess), an iterative smoothing procedure that takes into account outlying points from small samples by weighting. Tension, the smoothing parameter, was initially set at 66, residuals were checked and if they were found to depend upon morphology a new parameter value was chosen and the fitting procedure repeated (Cleveland, 1994). Statistical testing of trends in the data is undermined by the lack of independence. The same measured individual may be present in as many as eight successive years. Statistical tests that have been developed to deal with the lack of independence problem (e.g., Lettenmaier, 1976; Berryman et al., 1988) are inappropriate for our data with strong dependence persisting across several time intervals. Serial autocorrelation coefficients were calculated to estimate the dependence of samples in successive years. Statistical tests were used solely for heuristic purposes; Spearman's rank correlation test for a linear trend, polynomial regression for a non-linear trend and Mann–Whitney U test for a step function (Berryman et al., 1988). All morphological analyzes were performed in Statview 5.0. All statistical tests were two-tailed, with alpha at 0.05. Morphological and heterozygosity data were normally distributed and did not require transformation.

Results

Demography

Breeding is defined as the laying of at least one egg (females) or having a social mate that does so (males). The number of breeding pairs at first declined, then increased to a maximum of approximately 30 pairs after about 15 years (Table 3, Figure 2). The increase was slow at first, despite successful breeding, and the starting number of five breeders was exceeded only 4 years later. Immigration continued episodically after the breeding population had become established. Importantly, in most years a few new immigrants stayed to breed (Table 3). In all years of breeding except 1984 and 1986 at least one new breeder was an immigrant (Table 3). However, many immigrants disappeared at about the time that breeding began in a given year and are presumed to have emigrated, perhaps to their island of origin (Grant & Grant, 1995a).

The pattern of immigration is illustrated by the number of birds captured in mist-nets (Figure 3). The number of immigrants varied greatly among years, and to an extent that cannot be explained by variation in catching effort. This can be seen in the pulsed numbers in years of no breeding (e.g., 1988, 1989, and 1994) following productive wet years. The very large numbers of birds without bands captured in the non-breeding years of 1988 and 1989 (Grant

Table 3. Numbers of breeding immigrant and resident *G. magnirostris* on Daphne Major island

Years	Territories	Total		Breeders			
		Males	Females	New immigrants		New residents	
				Males	Females	Males	Females
1983	3	3	2	3	2	0	0
1984	4	2	2	0	0	0	0
1986	2	1	1	0	0	1	1
1987	6	3	3	2	2	1	1
1990	15	3	3	1	0	0	1
1991	5	4	5	1	0	1	3
1992	11	10	10	1	2	6	5
1993	28	9	9	1	1	1	3
1994	40	–	–	–	–	–	–
1995	26	13	13	7	6	0	1
1997	14	7	7	1	5	1	0
1998	46	30	30	2	1	2	0
2000	39	24	24	?	?	9	16

Also shown are the number of territories held by singing males (paired and unpaired).
There was no breeding in the years not shown or in 1994.

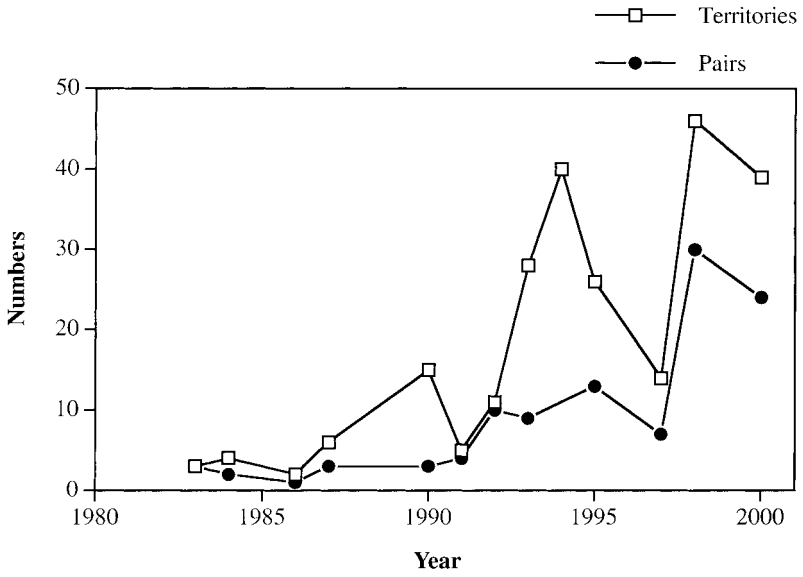

Figure 2. Increase in size of the breeding population of *G. magnirostris* on Daphne Major island. The number of territories occupied by singing males indicates the potential size of the breeding population and the number of pairs gives the actual size. Breeding did not occur in every year (see Table 3).

& Grant, 1992), and even larger numbers that were not captured in those years, can only be accounted for by immigration because all nestlings were found and banded in 1987. After 1991 a few nestlings were not found and banded, mostly in the years 1992, 1993, and 1997 of extensive breeding. In those years no more than a few captured birds could have been locally born; the large majority must have been immigrants.

Song types

Song types provide information about the occurrence of immigration and, in view of inter-island variation in song types (Bowman, 1983), the source of immigrants. Only males sing, and typically they sing a single song type unchanged throughout life (Grant & Grant, 1996c). Songs of ground finches are generally learned by juveniles from the male that feeds them in and out of the nest, but occasionally songs

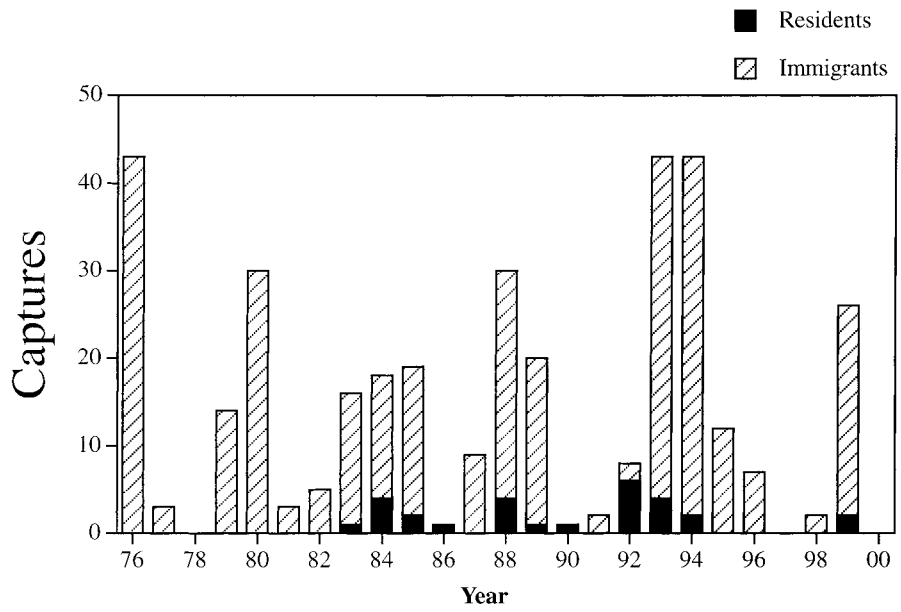

Figure 3. Mist-net captures of *G. magnirostris* on Daphne Major island in non-breeding seasons were exclusively of immigrants before 1983, when breeding began on the island, and dominated by immigrants afterwards. Netting effort varied among years.

are copied from other males (Bowman, 1983; Grant & Grant, 1996c). Thus song is a paternally inherited, non-genetic, marker or cultural signature of the island of origin of immigrants to Daphne.

Three song types, illustrated in Grant and Grant (1995b), have been heard and tape-recorded on the island. Song type A has been recorded on Santa Cruz only, type B has been recorded on Santa Cruz, Genovesa, Santiago, and Pinta (unpubl. data) and type C has not been recorded anywhere except on Daphne (Grant & Grant, 1995b). Songs have not been recorded on Marchena. Other song types not heard on Daphne have been recorded on Rábida, as well as Pinta, Genovesa, Fernandina, Wolf, and Darwin (unpubl. data, Bowman, 1983). Although song type B is not island-specific these data provide suggestive evidence that the source of immigrants to Daphne is Santa Cruz.

Frequencies of the three song types on Daphne have changed markedly (Figure 4). Song type A was present for the first decade and then became extinct. Song type B became extinct after 2 years, reappeared apparently as a result of immigration in 1987, became extinct, reappeared again in 1993, became extinct again and reappeared for a fourth time in 1997. Song type C was first heard on the island in 1986. The song was not sung by a breeding bird until 1991, and then by a different individual (No. 6102). Remarkably, from then on song type C quickly became the majority song type, and in some years the only song type, owing to 6102's reproductive success and the addition of new immigrants to the population.

G. magnirostris males of all three song types produced sons whose songs were recorded. All except one of 49 sons of known paternal song type sang the song type of their (social) fathers. The exceptional male sang the majority song type (C) in 1993. Learned acquisition of song from fathers implies that the reappearance of a missing but previously present song type (B) in the population (Figure 4) is more likely to be due to new immigration than to de novo innovation.

Population establishment, genetics, and the source of immigrants

Founders

The founder population comprised three males and two females, none of which was genotyped. The genetically effective population size was two, that is, the male and the female that produced the only offspring that survived to breed. Part of the combined genotype of the founding pair can be reconstructed from the genotypes of their breeding offspring.

The second generation was started in 1986 by a sister (6509) and a brother (6501) from successive broods in 1982–1983. The genotype of 6509 was directly

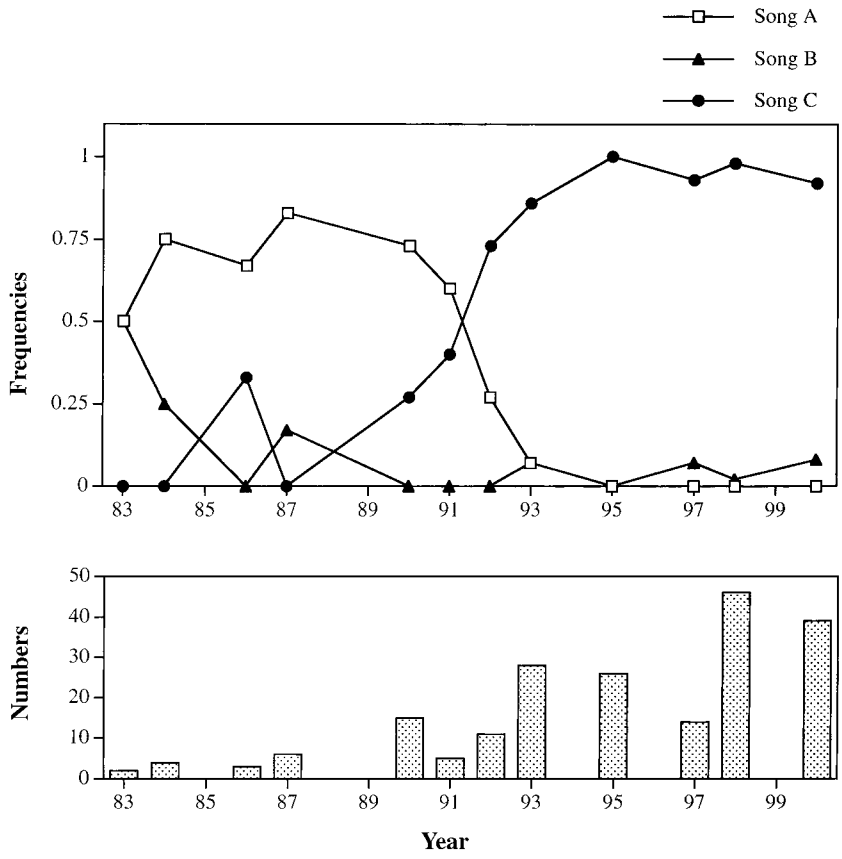

Figure 4. Changes in the frequencies of three song types of *G. magnirostris* on Daphne Major island, and the total numbers of singing males. The song type of one of the three original colonists is not known.

determined by microsatellite analysis. The genotype of 6501 was inferred from the genotypes of his offspring. The pair raised a total of 12 genotyped offspring in the years 1986–1992. All offspring genotypes matched the mother's genotype. In the combined group of 12 offspring there were no more than two alleles at each of the 16 loci that were not accounted for by the mother's alleles. These data are consistent with an hypothesis of a single father, most likely the social father (6501).

Knowing the genotypes of both 6501 and 6509 allows us to make three statements about the genotypes of the founding parents. First, in combination they had at least 37 alleles at the 16 loci. The maximum possible number would be 60 if all 14 autosomal loci were heterozygous with different alleles in the two parents, one Z-linked locus being fixed in this population and the other having a potential three alleles. Second, at least one of the parents must have been heterozygous at four loci and both parents must have been heterozygous at two more loci. Third the particular alleles give useful information for identifying the source of the two genetically effective founders. Contrary to prior expectations based on geography and song, assignment tests applied to the 14 autosomal loci of 6501 and 6509 did not identify Santa Cruz as the source of their parents. Instead, both individuals were assigned to the Santiago (Rábida–Albany) population (probabilities of 0.982 and 0.968, respectively). With Rábida and Albany treated as separate islands the tests assigned both individuals to the Albany population (probabilities of 0.982 and 0.975, respectively).

Source of subsequent immigrants

Many more immigrants arrived on Daphne than stayed to breed (Figure 3), and the genotyped sample provides much more information about the source or sources of immigrants than do the offspring of the original founders. Assignment tests applied to the 14 autosomal loci demonstrate heterogeneity in the source of immigrants. With a >50% probability criterion (majority rule) for assignment, four

Table 4. Years of arrival (capture) of genotyped immigrants on Daphne, and their islands of origin identified by assignment tests

Years	Santiago	Santa Cruz	Marchena	Isabela	Genovesa	Unassigned
1985	1	0	0	0	0	0
1987	0	0	2	0	0	0
1988	0	3	2	4	0	1
1989	3	4	7	3	0	2
1991	1	0	0	1	0	0
1992	1	1	0	0	0	0
1993	20	10	3	3	0	3
1994	11	8	6	3	1	4
1995	1	1	0	0	0	0
1998	0	0	0	0	0	1
Total	38	27	20	14	1	11

Santiago includes the satellite islands of Rábida and Albany.

islands were clearly identified as sources of immigrants; Santa Cruz, Santiago, Isabela, and Marchena (Table 4, Figure 5). Only one of the immigrants was assigned to Genovesa, at the relatively low probability of 0.582, therefore correct assignment is doubtful in this case. Approximately 10% of immigrants could not be assigned to any of the islands (all probabilities <0.50). These may have originated from an island not included in our samples, such as Pinta (Figure 1).

Immigrants arrived from more than one island in the same year, several times (Table 4). Santiago at an intermediate distance from Daphne, contributed the largest number of immigrants, followed by the near island of Santa Cruz and then the far island of Marchena (see Figure 1). By a stricter assignment criterion of >90% probability Marchena contributed as many as Santa Cruz (12) but still less than Santiago (32). By this criterion only one immigrant came from Isabela (Figure 5). The probabilities of assignment changed to only a minor degree (<2–3%) when three randomly chosen individuals were used from the potential source populations to match the small sample from Santiago.

Immigrants that bred on Daphne

Birds on Daphne were either known to have been born on the island, or not known to have been born there and hence presumed to be immigrants. Twenty three among the 117 genotyped individuals identified as immigrants bred on Daphne. Twenty two of them were fully genotyped. By the >50% criterion 20 originated on Santiago, one came from Santa Cruz and one could not be assigned but showed highest affinity to the Isabela sample (probability of 0.481). None of the Marchena sample stayed to breed (Figure 5). These proportions differ significantly from expectations based on the total sample of immigrants ($X_4^2 = 38.866,\ p < 0.0001$). The Santiago component of the breeders is significantly over-represented ($X_1^2 = 25.29,\ p < 0.0001$), and the Santa Cruz ($X_1^2 = 4.28,\ p < 0.05$) and Marchena ($X_1^2 = 4.83,\ p < 0.05$) components are significantly under-represented.

Differences in proportions did not arise from annual heterogeneity in arrival and breeding (Table 4). Most of the immigrants that bred arrived in 1993 (9) and 1994 (7). Considering only the Santiago, Santa Cruz, and Marchena samples, the excess of the Santiago component of the breeders (or deficit of the Santa Cruz and Marchena components) cannot be attributed to chance in either 1993 ($X_2^2 = 8.044,\ p = 0.0179$) or in 1994 ($X_2^2 = 12.374,\ p = 0.0021$). In neither year did any of the Santa Cruz (18) and Marchena (9) immigrants breed. Thus the breeders were not a random sample of those that arrived.

The breeders were more heterozygous than the non-breeders. For those genotyped at 12–14 of the autosomal loci the mean heterozygosity of the breeders (0.707 ± 0.125 SD, $N = 22$) was nearly 10% higher than the mean heterozygosity of the non-breeders ($0.639 \pm 0.114,\ N = 91$), and the difference is statistically significant ($F_{1,111} = 5.042,\ p = 0.0267$). The difference derives from the fact that the breeders were dominated by the sample from Santiago which were more heterozygous (0.693 ± 0.110

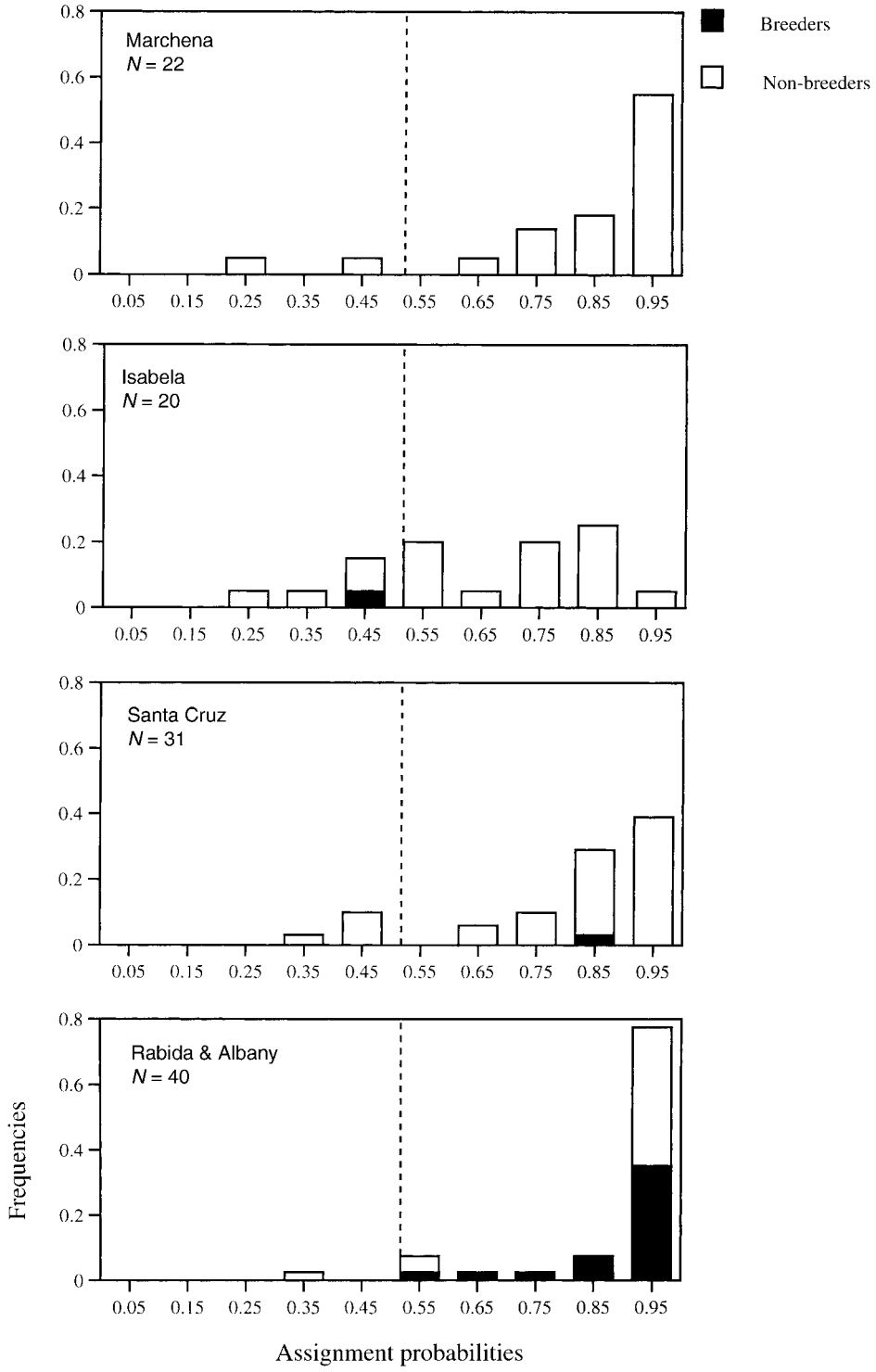

Figure 5. Maximum assignment probabilities for 113 immigrants. The 50% criterion is shown by the broken line. Individuals to the right of the line are considered assigned to the island specified, whereas individuals to the left of the line are considered unassigned to an island (this includes one of 22 breeders). Not shown is Genovesa, because only one individual was assigned to the island and at such a low probability (0.582) that the assignment is doubtful.

SD, $N = 38$) than the rest (0.630 ± 0.117 SD, $N = 75$; $F_{1,111} = 7.675$, $p = 0.0066$). Breeders and non-breeders from Santiago did not differ significantly in heterozygosity ($F_{1,36} = 0.347$, $p = 0.561$).

Genetic variation in the breeding population

Inbreeding and parentage

During the slow increase phase of the population's development, from 1986 to 1992, inbreeding occurred at a moderately high frequency; the pedigree is given in Grant and Grant (1995a). The mean coefficient of inbreeding was at its highest (0.25) in 1986 when a single pair was formed by a sister (6509) and a brother (6501). In the next year (1987) this pair bred again, a third surviving sibling (6505) bred once with 6509 and once with the daughter (14532) of 6509 x 6501 born the previous year. These three pairs comprised 60% of the breeding pairs that year, the other two pairs were immigrants and the mean coefficient of inbreeding was 0.1. The mean coefficient remained at about that level in 1990 (0.08), 1991 (0.07) and 1992 (0.11), then fell to 0.03 in 1993, 0.01 in 1995, and 0.02 in 1997. None of the known inbred birds was alive in 1998, and no inbreeding was detected that year.

Inbreeding is expected to result in a loss of genetic variation. To investigate this we first had to determine if observed breeders were in fact the biological parents of the offspring in their nests.

Both parents in 13 pairs and 41 of the offspring associated with them were genotyped. Genotypes were determined for another six female parents and 30 offspring (but no male parent) and eight male parents and 44 of their offspring (but no female parent). According to the genotypes of their assigned offspring, all female parents were correctly identified as mothers by observation. On the other hand paternity was misassigned to four offspring, from three male parents, out of a total of 85 offspring (4.7%). They exhibited mismatches at three, four, five, and six loci, respectively. The four offspring came from four out of a total of 44 families (9.1%). The genotypes of all eight offspring identified as being inbred ($f \geq 0.125$) matched the genotypes of both of their social parents.

We assume that 6501 was correctly identified as the father of 6509's 12 offspring. Addition of these 12 to the other 87 gives a frequency of cuckoldry of 4.0% in four of 48 (8.3%) families. These values of cuckoldry are low compared with *G. scandens* (~8%; Petren, Grant & Grant, 1999b) and *G. fortis* (~20.0%; Keller et al., 2001) on the same island. There are no data available for any of these species on other islands. The values imply that individuals previously believed from observations to be inbred (Grant & Grant, 1995a) were indeed probably inbred.

Inbreeding and heterozygosity

The inbreeding coefficient (f) of the 12 genotyped offspring of the sister–brother pair (6509 x 6501) was 0.25. Two of them, a sister and brother from the same nest (14952 x 14951), produced seven genotyped offspring ($f = 0.312$). Finally a pair of half-sibs produced a single genotyped offspring ($f = 0.125$). These 20 individuals were contrasted with 56 others considered to be non-inbred because their parents, although identified, were not known to be related ($f \sim 0$). For each individual the mean heterozygosity at the 14 autosomal loci was calculated. The same calculations were made for the squared difference (d^2) in length of alleles at heterozygous loci, averaged over all loci.

As expected, mean heterozygosity was significantly lower in the inbred sample (0.52 ± 0.03 SE) than in the non-inbred sample (0.68 ± 0.01, $t_{74} = 4.631$, $p < 0.0001$). Average allele length differences at heterozygous loci between inbred and non-inbred birds, and d^2 values, were not statistically different ($p > 0.1$). The inbreeding coefficient varied negatively with mean heterozygosity ($r = -0.544$, $p < 0.0001$) but not with d^2 ($r = 0.172$, $p = 0.137$).

The effect of inbreeding on allelic variation is illustrated by the reduction in number of alleles in the highly inbred lineage. The initial sister–brother pair (6509 x 6501) had a combined total of 34 alleles at the 14 autosomal loci. The daughter and son that formed a pair had 27 alleles, and the two offspring of this pair that survived to breed had a total of 22 alleles. Whereas each member of the initial sister–brother pair was heterozygous at 10 loci, their two grandchildren were heterozygous at only 4 and 5 loci respectively.

Inbreeding and fitness

Overall heterozygosity is frequently found to be positively associated with fitness (Hedrick, 1998; Mitton, 1998). Since inbred birds are less heterozygous on average than non-inbred birds they may also be less fit. Therefore, we analyzed fitness as a function of heterozygosity and of inbreeding. Fitness is measured with our data by survival to breed and by longevity;

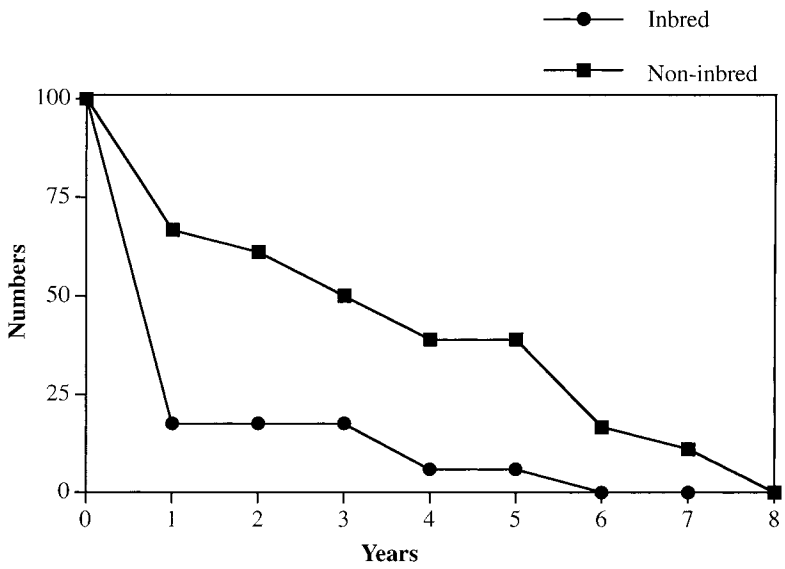

Figure 6. Survival of inbred ($f \geq 0.125$, $N = 17$) and non-inbred ($f < 0.125$, $N = 18$) members of the 1991 cohort of *G. magnirostris* on Daphne Major island, scaled to an initial 100 individuals of each.

data on number of offspring produced per pair were too incomplete for an analysis of reproductive fitness (but see Grant & Grant, 1995a).

The relationship between longevity and the two genetic measures can only be assessed with the sample ($N = 31$) from the 1991 cohort (Figure 6); genetic data are missing for most birds born before 1990, and detected inbreeding was too rare after 1991. Longevity, maximum 7 years, was a negative function of inbreeding coefficient (regression analysis, $F_{1,29} = 5.222$, $p = 0.0298$); the higher the coefficient the shorter the life, on average. A survival cost of being inbred was sustained at an early age in this cohort (Figure 6). Three of 17 (18%) highly inbred birds that fledged from five nests in the 1991 cohort ($f = 0.25$) survived at least 1 year, and hence were potential breeders, whereas 12 of 18 (67%) non-inbred birds that fledged from six nests survived at least 1 year ($X^2 = 6.744$ corrected for continuity, $p = 0.0094$). When analyzed by nests for greater statistical independence the difference is not significant (Fisher's exact probability $= 0.196$); however, for the available sample sizes the power of the test to detect a large difference in survival is too low for the test to be very meaningful. Survival beyond 1 year did not differ between inbred and non-inbred birds (Mann–Whitney U test, $z = 0.446$, $p = 0.6556$). Among a smaller cohort ($N = 22$) produced in 1987 and comprising 17 highly inbred ($f = 0.250$), one less inbred ($f = 0.125$) and four non-inbred birds ($f = 0$), survival to 1988 was almost the same for the highly inbred (18%) and non-inbred birds (25%).

Longevity of the 1991 cohort was close to being significantly related positively to heterozygosity ($F_{1,29} = 4.074$, $p = 0.0746$). As expected, inbreeding coefficient and heterozygosity are strongly and negatively related to each other in this cohort ($r = -0.740$, $p < 0.0001$). This effectively precludes a statistical assessment by multiple regression of their independent effects on longevity.

The relationship between heterozygosity and survival to breed was assessed with the samples from the two large complete cohorts, those produced in 1991 when inbreeding was at a maximum and those born in1998 when it was not known to occur. Results are mixed. For the 1991 cohort, mean heterozygosity of 14 birds that survived to breed (0.723 ± 0.126) was significantly higher than the mean heterozygosity of 17 others that failed to survive to breed (0.540 ± 0.184, $t_{29} = 3.197$, $p = 0.0033$). For the 1998 cohort there was no such effect; the 12 that bred (0.655 ± 0.129) were no different ($t_{50} = 0.502$, $p = 0.6179$) from 40 that did not (0.638 ± 0.114). The difference between cohorts is demonstrated by a significant interaction term ($F_{1,79} = 6.334$, $p = 0.0139$) in an ANOVA of survival in relation to heterozygosity in the two cohorts. The difference may have been due to the inbreeding variable. If so, the main determinant of survival in our sample was not heterozygosity as assayed but inbreeding.

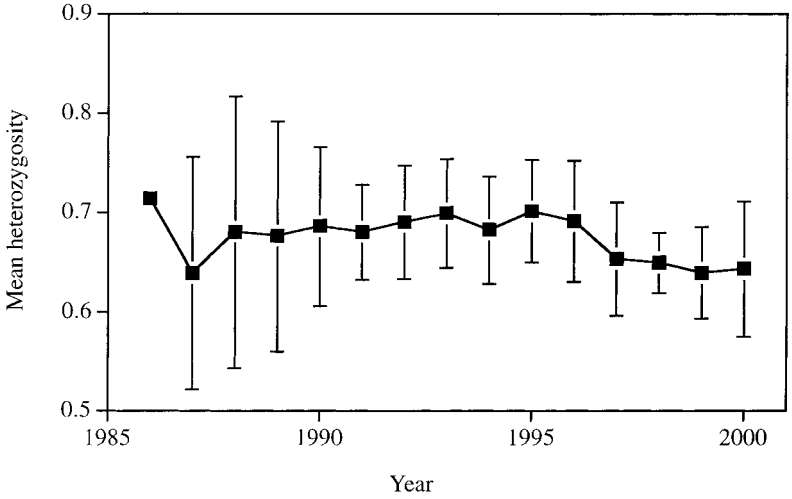

Figure 7. Annual fluctuations in mean heterozygosity at 14 autosomal microsatellite loci of *G. magnirostris* on Daphne Major island; 95% confidence bars are shown. There are no bars on the first point because the two birds then had an identical heterozygosity index. The maximum sample size is 33 in 1998.

Among the 12 offspring of the initial sister–brother pair, fitness, as measured by survival to breed, was not associated with heterozygosity at any of the autosomal loci (two-tailed binomial tests, all $p > 0.1$). It is possible that heterozygotes were selectively favored at egg and nestling stages before they entered our samples. If correct, this would have yielded an excess of heterozygotes over those expected from a random sampling of parental alleles. All observed frequencies of heterozygotes were close to those expected from random pairing of the parental alleles, with one exception. At this exceptional locus (Gf9) the observed frequency of heterozygotes (0.18) was not higher but lower than the expected 0.5, although not significantly (binomial $p = 0.066$, two-tailed).

The longest lived birds, 6501 and 6509, lived for 10 and 11 years, respectively and produced more fledglings (27) than any other pair. Seven out of their eight fledglings that survived for at least 1 year became breeders. It appears to be anomalous that the highest success was experienced by highly inbred birds ($f = 0.25$). However, their mean heterozygosities were unusually high (0.71 in each case), and they had a demographic advantage in entering the population at low density.

Genetic changes in the population

Mean heterozygosity remained approximately constant over 15 years, if anything exhibiting a slight decline in the last 3 or 4 years (Figure 7). In contrast to temporal stability in the heterozygosity index, the total number of alleles in the breeding population underwent a systematic increase. Initially there was an undocumented but almost certain decline in alleles as the breeding population dropped from five individuals of the founder population in 1982–1983 to two in the next generation in 1986 (or three in 1987). We estimate the number of alleles present in the starting population at all 16 loci to be approximately 54, on the basis of 100 random samplings (with replacement) of five individuals from the 20 immigrant breeders genotyped at all 16 loci (mean alleles 54.1 ± 0.48 SE). At the minimum in 1986 only 37 alleles were present in the breeders. Thus an estimated loss of 32% of the alleles had taken place. This is a maximum value, because the calculation ignores another son of the founder pair that bred in 1987. It was not genotyped, nor were its offspring which all failed to survive to breed.

Thereafter allelic diversity increased as a result of continued addition of breeding immigrants to the population (Figure 8). The cumulative total of alleles in the breeding population (88) is 75.9% of the total number of alleles (116) recorded in the immigrants on Daphne. This is a minimum percentage value as some of the breeders after 1993 were not genotyped. The introduction of new alleles (54) far exceeded subsequent losses (21) and the loss of two of the original alleles. Six other alleles were lost and then regained from new introductions. As a result of these dynamics the number of alleles in the breeding population approximately doubled, and increased towards an apparent plateau.

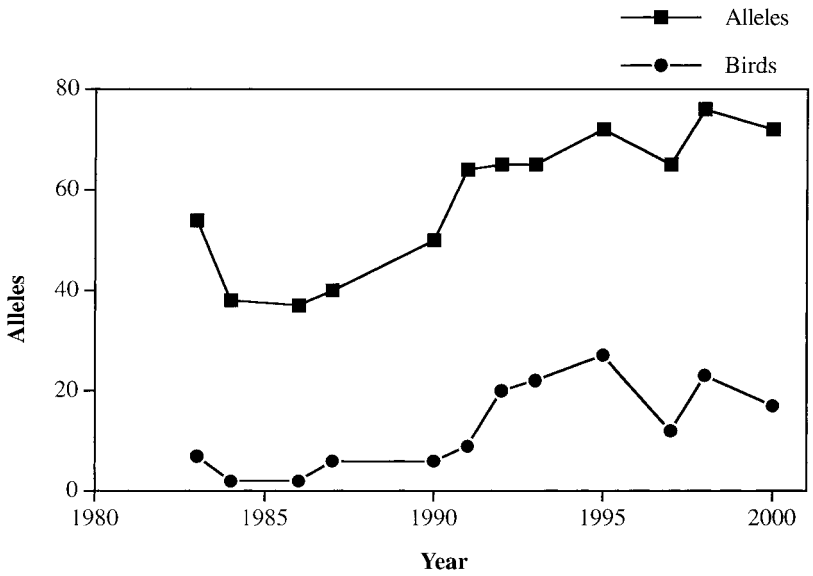

Figure 8. Changes in the number of alleles at 16 microsatellite loci in the resident population of *G. magnirostris* on Daphne Major island; that is breeders and their offspring. In the period 1986–1993 almost all alleles in the breeding population were known, either from direct genotyping or by deduction from the genotypes of their offspring. After 1993 the number of alleles is a minimum estimate of the total because not all breeders were genotyped.

Apart from one of the Z-linked loci which remained unchanged, and fixed, the total number of alleles per locus increased to as few as three and as many as nine (Table 1).

While most immigrants contributed one or two new alleles to the population two exceptional males from Santiago contributed 11 and eight in 1990 and 1995 respectively. A third male from Santa Cruz also contributed eight new alleles in 1995, and a fourth male, unassigned to island of origin but possibly from Isabela, contributed an additional six new alleles in 1998.

Morphological variation

Immigrant breeders

Twenty six measured immigrants that bred were compared with 174 contemporary immigrants that did not breed on Daphne to test the hypothesis that settlement to breed was random with respect to morphology. The hypothesis was rejected by the beak size data; the breeders were larger on average than the non-breeders ($t_{198} = 2.688$, $p = 0.0078$). The two groups did not differ in beak shape ($t_{196} = 0.935$, $p = 0.3510$) or body size ($t_{196} = 0.221$, $p = 0.8255$).

These results are probably due to heterogeneity in the non-breeders. Samples from the four islands of origin and those unassigned varied significantly in beak length ($F_{4,108} = 4.485$, $p = 0.0022$), marginally in body size ($F_{4,108} = 2.691$, $p = 0.0348$) but not in beak shape ($F_{4,108} = 1.462$, $p = 0.2188$). Deleting the unassigned category had no influence on this result.

Since most breeders were from Santiago we repeated the tests with birds from this island only and found no significant differences with either the total samples ($N = 38$) or with just the males ($N = 23$, all $p \gg 0.1$). Thus the breeders were a non-random sample of those who immigrated, but not of those who immigrated from Santiago.

Temporal trends: residents

The null expectation is no change in mean morphology from establishment of the breeding population onwards. The data for body size and beak size are consistent with this expectation, except for small apparent deviations in the late 1980s (Figure 9). In contrast beak shape shows a trend towards more robust, less pointed, beaks. If the means were statistically independent the linear trend would be considered significant (Spearman's rank correlation, $z = 0.344$, $p = 0.0006$). Despite this the trend is best described as a couple of step-function changes, occurring first after the initial breeding and second at the mid-point of the study (Figure 10). Sample sizes are generally small and confidence intervals on the estimates of means are broad, nevertheless the beak shape stepped

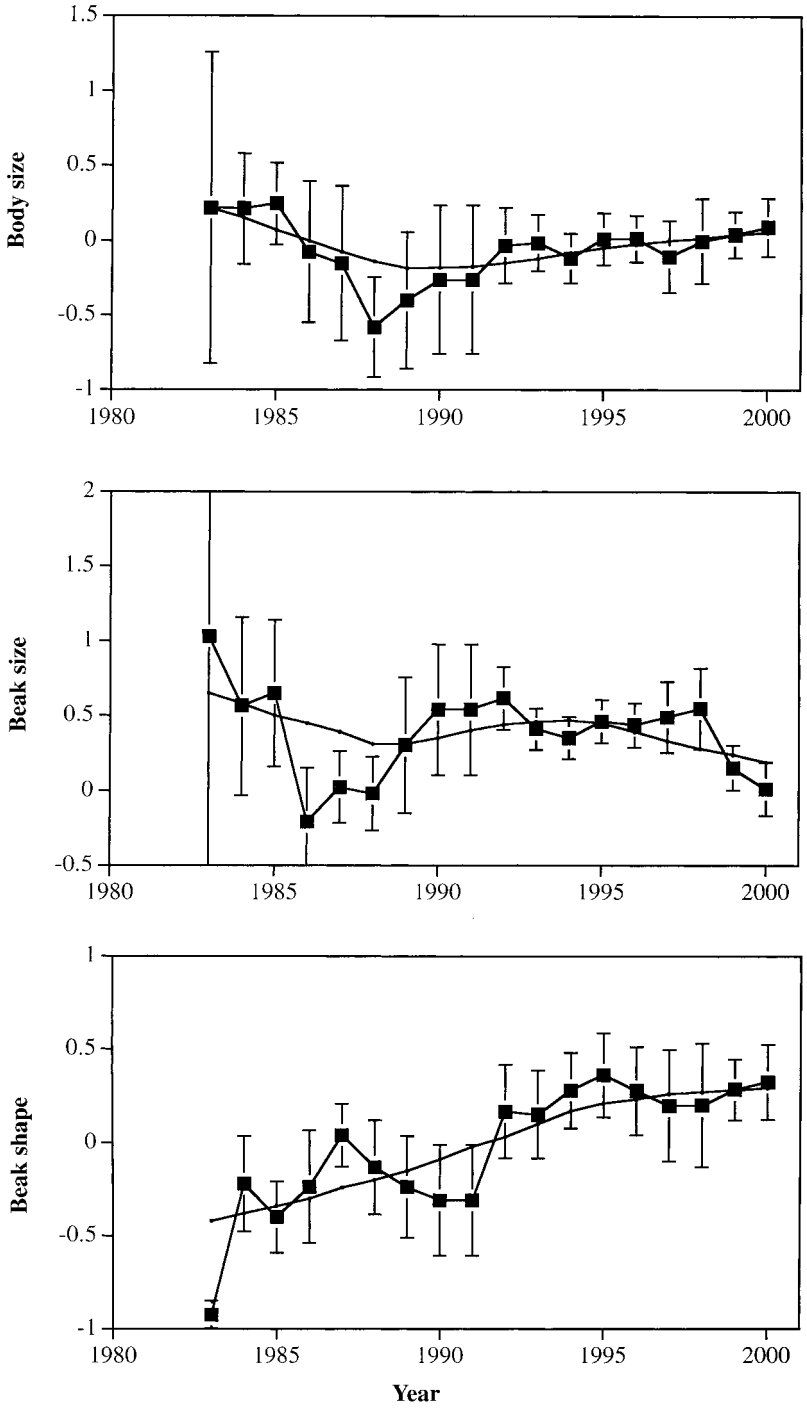

Figure 9. Morphological trajectories of the resident population of *G. magnirostris* on Daphne Major island. Annual means and one standard error are shown for body size, beak size, and beak shape values obtained from PCA. The standard error of the first beak size mean should be 2.06 and has been truncated. Serial autocorrelations are 0.512, 0.392, and 0.134, respectively for body size, beak size and beak shape. In the top two panels size increases up the axis, and in the lower one beaks become less pointed and more robust from bottom to top. Fitted curves are locally weighted regressions (lowest).

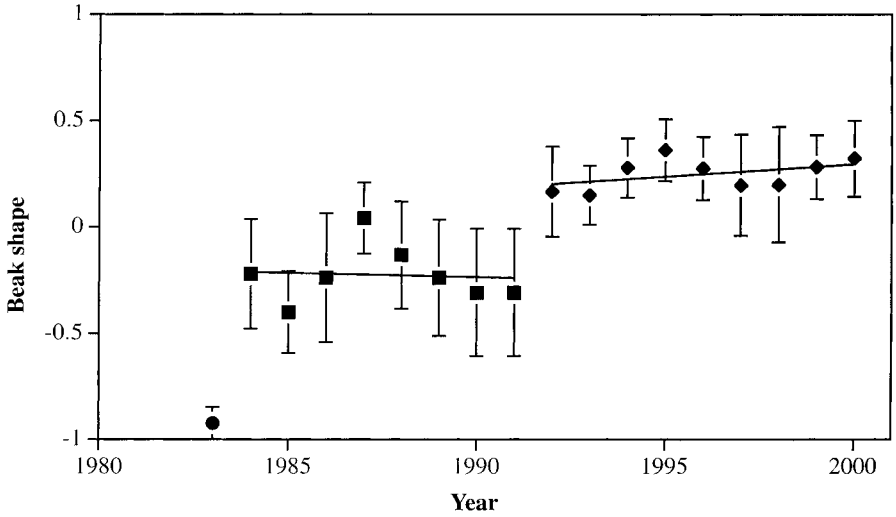

Figure 10. The beak shape trajectory taken from Figure 9 and separated at the two step transitions. Beaks become less pointed and more robust from bottom to top. Lines are least squares best fits from linear regressions, and are used heuristically to show apparently parallel and flat but displaced morphological trajectories. Annual means and one standard error are shown.

trend is strong; the second transition from relatively pointed beaks to relatively robust beaks was sustained for almost a decade, with all means after 1991 being larger than all means up to and including 1991. If the means were statistically independent the difference between the two groups would not be attributable to chance (Mann–Whitney $U = 0$, $p < 0.0001$). The combined post-1991 sample of birds ($N = 57$) had significantly more robust beaks ($t_{73} = 2.544$, $p = 0.0131$) than the preceding sample ($N = 18$) of different birds. We focus on this trend.

Inspection of the means of individual beak dimensions shows that beak length and beak width were smaller after 1991 than before (ignoring 1983 data; Figure 10), and beak depth in contrast was larger after 1991, although none of the differences are significant (but for beak length $t_{73} = 1.893$, $p = 0.0624$). The ratio of beak length/beak depth became significantly smaller after 1991 ($t_{73} = 3.084$, $p = 0.0044$), whereas the ratio of beak length/beak width remained unchanged ($t_{73} = 1.341$, $p = 0.1841$). Thus a combination of unidimensional changes in opposite directions rather than a change in a single dimension brought about the beak shape change after 1991.

The transition cannot be attributed to a shift in sex ratio as males and females do not differ in beak shape ($t_{54} = 0.432$, $p = 0.6671$). There are three possible explanations; between-measurer bias, within-measurer bias, and differential losses and/or additions to the population.

The change could be an artifact caused by a measurement bias between measurers before 1992 when birds were measured by several people and no bias afterwards when they were all measured by one P.R. Grant. This possibility can be rejected. Beak shape did not differ between the 66 birds measured by P.R. Grant and the nine birds measured by others ($t_{73} = 1.076$, $p = 0.2854$). Moreover the average of the nine birds did not differ from nine other birds measured by P.R. Grant in the same years ($t_{16} = 0.999$, $p = 0.3329$). The difference in beak shape is sustained in ANOVA of just the birds measured by P.R.Grant (Table 5). This analysis also shows that immigrant breeders and locally-born birds did not differ in their contribution to the transition.

A within-measurer P.R.Grant bias seems unlikely because it would be expected to occur slowly and gradually over many years (measurement drift) whereas the beak shape transition was abrupt. Moreover it should affect the non-breeding immig-

Table 5. Two-way ANOVA of beak shape according to years (before 1992 vs. after 1991) and origin (born on or off the island)

	df	SS	MS	*F*	*P*
Origin	1	1.648	1.648	1.844	0.1794
Years	1	5.492	5.492	6.147	0.0159
Interaction	1	2.233	2.233	2.5	0.1190
Residual	62	55.391	0.893		

rants as well as the residents, but there is no evidence for this in a comparison of the non-breeders measured before and after the transition ($t_{172} = 0.514$, $p = 0.6078$). The direct test of within-measurer bias cannot be done; none of the birds was measured both before and after the transition.

The transition from 1991 to 1992 could have been the result of selection, random drift, or both. It involved the loss of two birds and the addition in 1992 of one immigrant and 10 offspring born in 1991. The offspring had relatively more robust beaks than the seven measured parents (out of nine) that produced them, but in a two-way ANOVA the difference is not significant ($F_{1,13} = 1.147$, $p = 0.3036$) nor is there a significant interaction with sex ($F_{1,13} = 0.659$, $p = 0.4315$). The 35 members of the 1991 cohort may have been subject to beak-shape selective mortality before the survivors were measured.

Changes in successive years

Annual changes in mean morphology can arise from morphologically selective losses or gains to the population. If both selective processes occur, but in opposite directions, the result could be no net change. We therefore analyzed components of change separately. In most years, including 1991 and 1992, sample sizes of survivors, non-survivors, or both were inadequate for testing the hypothesis of selective mortality. Survivors from 1998 to 1999 ($N = 29$) were larger in beak size ($t_{33} = 2.460$, $p = 0.0193$) than non- survivors ($N = 6$), but did not differ from them in the other two traits ($p > 0.1$). This may reflect a higher survival of males than females, because among males alone 10 survivors did not differ from five non-survivors in beak size ($t_{14} = 1.432$, $p = 0.1758$). Two other comparisons revealed no difference between survivors and non-survivors. Thirteen survivors from 1996 to 1997 were compared with 12 non-survivors, and 19 survivors from 1999 to 2000 were compared with 10 non-survivors (all $p > 0.1$).

An alternative to selective mortality of the parental generation is selective addition from the offspring generation. The evidence for this is stronger. Sex-specific analyses were conducted with data from 1991 and 1998, the two most productive years of the decade, to compare adults with birds born in those years and measured in subsequent years when fully grown. In both years daughters were significantly larger in mean body size than the breeding females (1991, $t_8 = 2.490$, $p = 0.0375$; 1998, $t_7 = 2.867$, $p = 0.0241$). In 1991, but not in 1998, the adults were the mothers of all of the daughters. There were no significant differences in beak size or shape changes in females, or any differences in the traits of males.

Temporal trends: non-breeding immigrants

We analyzed temporal variation in morphology in the 13 years between 1976 and 1995 when sample sizes were greater than 10 individuals, and found no systematic trends. This result, conforming to the expectation of random fluctuations about a long-term mean, contrasts with the temporal trends in resident mean morphology.

Nonetheless local survival (persistence) from 1 year to the next was influenced by morphology in 4 years out of the six in which minimum sample requirements ($N = 5$) were met (Table 6), and by island of origin. All survivors from 1993–1994 were from Santiago, and eight out of 11 in 1994–1995 were from Santiago. Non-survivors may have died or emigrated.

We used logistic regression models to assess the relative importance of these factors as predictors of survival. The models showed that island of origin was always the most important predictor. In 1993–1994 it alone was sufficient to account for the pattern of survival, correctly predicting the status of 28 out of 30 individuals (93.3%) (likelihood $X^2_4 = 26.221$, $p < 0.0001$), while the beak variables made no improvement to the model. In two other years beak size and shape but not body size made significant contributions to the models. For the 1989–1990 data the status (survivors or non-survivors) of 35 out of 41 (85.37%) was correctly predicted by a model that included island of origin and the two beak variables (likelihood ratio $X^2_7 = 26.907$, $p = 0.0003$). Beak shape made a significant contribution ($X^2_1 = 7.650$, $p = 0.0057$), whereas the contribution of beak size fell short of statistical significance ($X^2_1 = 2.765$, $p = 0.0963$). Island of origin was the single best predictor (31/41, 75.61%). In 1994–1995 island of origin and both beak variables were required for correct prediction of all 43 individuals (likelihood $X^2_7 = 55.618$, $p < 0.0001$), although island of origin by itself correctly predicted the status of 39 of the 43 individuals (90.70%).

Microsatellites and morphology

Genetic variation and morphological indices have been considered independently. However there is one indication that they are associated. Homozygotes and heterozygotes differ in morphology in four compar-

Table 6. Morphological differences between non-breeding immigrants that survived on Daphne for 1 year and those that did not

		Survivors			Non-survivors		
		N	Mean	SE	*N*	Mean	SE
1985–1986	Body size	7	−0.120	0.422	13	0.018	0.205
	Beak size	7	−0.020	0.295	13	0.632	0.249
	Beak shape	7	0.643	0.367	13	−0.384	0.273
1989–1990	Body size	21	0.001	0.155	20	0.291	0.277
	Beak size	21	0.022	0.167	20	0.162	0.237
	Beak shape	21	−0.537	0.208	20	0.569	0.234
1993–1994	Body size	9	0.115	0.211	21	−0.406	0.459
	Beak size	9	−0.749	0.395	21	0.454	0.234
	Beak shape	9	0.088	0.398	21	−0.188	0.177
1994–1995	Body size	32	−0.093	0.321	11	0.422	0.186
	Beak size	32	−0.640	0.315	11	0.319	0.177
	Beak shape	32	0.305	0.322	11	−0.145	0.153

Differences were not significant in two other years tested, 1988–1989 and 1995–1996.
Negative values for body and beak size indicate large size, and negative values for beak shape indicate robustness.

Table 7. Morphological heterogeneity between homozygotes and heterozygotes at three of 14 autosomal loci

Locus	Trait	*N*	Mean	SE	*t*	*P*
Locus Gf3	Beak size					
	Homozygote	5	−1.055	0.454	2.787	0.0085
	Heterozygote	32	0.016	0.198		
Locus Gf12	Body size					
	Homozygote	7	−1.189	0.437	3.169	0.0032
	Heterozygote	29	0.152	0.180		
Locus Gf12	Beak shape					
	Homozygote	7	−1.181	0.560	2.530	0.0163
	Heterozygote	28	−0.367	0.171		
Locus Gf13	Beak shape					
	Homozygote	18	−0.589	0.214	3.502	0.0013
	Heterozygote	18	0.532	0.238		

Negative values for body and beak size indicate large size, and negative values for beak shape indicate robustness.

isons at three of the 14 autosomal loci (Table 7). Two differences at $p < 0.05$ might have been expected by chance in 42 comparisons (three morphological variables, 14 loci), but the four differences (∼10%) are significant at $p = 0.0013$–0.0163. The associations are therefore probably real. They may reflect linkage between particular microsatellite marker alleles and genes affecting morphology, or joint covariation with other variables. We use one example to illustrate this point. At locus Gf12 homozygotes and heterozygotes differ in bill shape (Table 7). The two classes of genotypes differ markedly in frequencies of alleles. The commonest allele in heterozygotes (165) occurred in 18 of 28 individuals, and in none of the seven homozygote individuals. Carriers of this allele differ in bill shape from those without it to a degree that approaches statistical significance ($t_{32} = 1.961$, $p = 0.0586$). This is notable in view of the probable polygenic influence over the beak trait. Associations like this through linkage imply an opportunity for selection on morphological traits to alter allele frequencies at neutral genetic loci, with the result that fitness is correlated with microsatellite variation (e.g., Coulson, Albon et al., 1998).

Discussion

This study confirms some expectations from population genetics theory of what happens in the founding of a new population from an extremely small number of individuals: a loss of alleles, inbreeding at a high frequency as a result of random mating while numbers remain low, and low fitness of the inbred offspring. The first two can be attributed to random processes, as can minor fluctuations in morphological traits and major changes in the frequencies of song types.

It is well recognized that selection may modify the outcome of colonization (e.g., Mayr, 1963), but difficult to anticipate and be precise about the effects because they are likely to be situation- specific. In view of such uncertainties, simplifying assumptions are often made in theoretical treatments of colonization. For example, it is generally assumed in models of gene flow that colonization is random with respect to genotype and phenotype (e.g., Rannala, 1996; Johnson, Adler & Cherry, 2000). Although reasonable, neither assumption is supported in our study. The individuals that stayed to breed were more heterozygous on average than those that did not stay to breed, and they were larger in beak size. In the breeding population there is further evidence for selection in morphologically non-random survival of adults in 1 year and non-random addition to the population of large daughters in 2 years. An increase in beak robustness after 9 years may have been caused at least partly by selection, although chance is a more parsimonious explanation. The group of immigrants that did not stay to breed were also subject to selection: persistence (local survival) to the next year was non-random with respect to beak morphology in 4 years out of 6.

These non-random characteristics of the immigrants became interpretable with the discovery that immigrants were heterogeneous with respect to island of origin. Rather than coming entirely from neighboring Santa Cruz, as previously believed on the basis of geographical proximity and song characteristics (Grant & Grant, 1995a), they were found to have originated on Santiago, Isabela, and Marchena as well as Santa Cruz. Yet those that stayed to breed were mostly from Santiago, and the non-breeders that stayed from 1 year to the next were also predominantly from Santiago. Thus the breeders and the surviving non-breeders were a non-random sample of the total immigrants in terms of genotype and phenotype, but a random sample from the subset of immigrants from Santiago. Only in this latter restricted sense is the assumption of randomness supported.

Repeated immigration from heterogeneous sources explains why we did not confirm some other expectations from population genetics theory: heterozygosities were not reduced in generations after the founding event and the population did not diverge genetically from the parent population.

At least some of the original founders and 20 out of 22 immigrant breeders that arrived later came from Santiago (Rábida–Albany). There are several possible explanations for this selectivity. First, immigrants from Santiago may have been the only ones on Daphne when the heavy rains arrived in late 1982. This seems unlikely because immigrants were heterogeneous with respect to island of origin in most of the years when sampling was carried out after 1987. Second, the Daphne habitat may be more suitable for birds from Santiago than from another island. Inter-island differences in vegetation and food supply certainly exist (Grant, 1999). Comparisons of quantitative data from Daphne, Santa Cruz, and Rábida show that vegetation on Rábida is no more similar to Daphne's than is the lowland Santa Cruz vegetation (Abbott, Abbott & Grant, 1977). Therefore habitat appears not to have been an important factor, and our unquantified observations of the vegetation on Isabela, Marchena, and Santiago support this view. A third possibility is it was a matter of chance that birds from Santiago rather than those from another island stayed to breed in 1982, and thereafter new arrivals from Santiago were attracted to stay by the (Santiago) songs they heard on the island. In the absence of information to the contrary this combination of initial chance and subsequent determinism seems the most plausible explanation to us. The Santiago origin of the songs needs to be confirmed.

Thus the characteristics and fate of the population were molded by an interplay of stochastic and deterministic processes affecting genotype, phenotype, and a culturally inherited trait, song. The interplay governed which particular individuals stayed to breed, and their subsequent success.

Fragmented populations and the gene flow-selection balance

The situation we have investigated may be seen as an empirical model for partially isolated demes in either insular or continental settings, rather than as a model of events on strongly isolated islands. The paradigm for such situations is evolution governed by opposing

processes; local adaptation in the isolate is counterbalanced by continuing gene flow from the source population (Mayr, 1963; Barton, 1992; García & Kirkpatrick, 1997; Holt & Gomulkiewicz, 1997; Kirkpatrick & Barton, 1997; Fleming et al., 2000; Nuismer, Thompson & Gomulkiewicz, 2000; Hendry, Day & Taylor, 2001; but see Ehrlich & Raven, 1969; Irwin, Irwin & Price, 2001; Smith, Schneider & Holder, 2001). The net result could be a maladaptive state (Crespi, 2000; Nuismer, Thompson & Gomulkiewicz, 2000), with a local phenotypic optimum approached but not reached. Theoretical studies have examined how an equilibrium is determined by the strength of selection, the amount of genetic variance and the rate of gene flow (King & Lawson, 1995; Kirkpatrick & Barton, 1997; Case & Taper, 2000; Hendry, Day & Taylor, 2001). The strength of selection is a function of the ecological difference between source and recipient populations. The genetic variance is assumed to be low because the population size is small. The rate of gene flow determines the degree to which the population is held permanently off a local optimum.

The theoretical models imply first, that in the face of continuing immigration the population experiences continuing directional selection, and second, that cessation of gene flow results in a pronounced response to selection, leading potentially to speciation (García-Ramos & Kirkpatrick, 1997).

Our field study is relevant to the first of these implications, in three ways. First, continuing gene flow contributed to a lack of phenotypic and genetic divergence. Second, despite continuing immigration, the population did not experience *continuing* directional selection, at least not at the level of detectability. Nevertheless, at the mid-point of the study a transition did occur to more robust beaks, even in the face of gene flow and not caused by it.

These observations are not entirely in accord with theory. A possible reason for the discrepancy is that the Daphne environment is not so different from the Santiago environment in critical resources to set up different selection pressures on *G. magnirostris*. If this is true it is all the more surprising that immigrants did not stay to breed in the years 1976–1982, and that a transition occurred to more robust beaks in 1991. We cannot associate the transition with a change in the environment. Either it was the result of chance, or it was produced by selective additions to the population rather than by selective losses; the statistical evidence is ambiguous. Robustness of the beak signifies an enhanced ability to crush hard seeds at the base (Bowman, 1961). The dry season diet of birds throughout the study period was dominated by the large and hard seeds of *Tribulus cistoides*. Possibly the survival of young birds in late 1991 was affected by their ability to crack these seeds, but we have no quantitative data to test this hypothesis.

There are other important factors to consider in building a theory of gene dynamics in partially isolated populations. Heterogeneity in the source of genes is one such factor. Gene flow from heterospecific or differentiated conspecific sources will elevate genetic variances, alter genetic correlations for continuously varying phenotypic traits, and enhance the responsiveness of the population to selection (Grant & Grant, 1994, 1995b). The demonstration of multiple sources of colonists in our study and others (see e.g., Phillips et al., 1989; Klein & Brown, 1994) emphasizes the potential for heterogeneous gene flow to have an enhancing effect. Kirkpatrick and Barton (1997) have pointed out that enhancement may still be overwhelmed by the swamping effects of gene flow under some circumstances. It thus becomes an empirical question whether gene flow swamps local adaptation, facilitates it or has no effect.

The answer to the last question may be all three, depending upon which particular alleles are added to the population. We believe that insufficient attention has been paid to the nature and fate of the genes that are brought to a semi-isolated population by immigrants that stay to breed. Certain individuals may bring a large number of new genes, and when those individuals come from different source populations, as occurred in our study, they facilitate the assembly of favorable new combinations. Such combinations may have a much higher chance of establishment in peripheral isolates than in large central populations. This could be one way in which speciation begins (Grant & Grant, 1994). On the other hand ecological or social circumstances may limit the degree of penetration of immigrant alleles into an isolated population. For example, Verhulst and van Eck (1996) showed that immigrant great tits to a Dutch island mated assortatively and had lower lifetime reproductive success than residents because they left the island after breeding. There is no evidence that this happened in our population, although there is evidence of selective gene flow mediated by song.

Finally, in environments experiencing fluctuating climates with strongly oscillating effects on the biota there may not be a single local optimal phenotypic state except as an average over a very large number of

years. Selection on the other resident species finches has oscillated (Grant & Grant, 1995b). This suggests that the fates and effects of immigrants are not fixed, but are determined by the dynamic state of the environment and the size of the population at the time of immigration (Holt & Gomulkiewicz, 1997; see also Reznick & Ghalambor, 2001), as well as the degree to which it is inbred (Kaufman & Wool, 1992; Ball et al., 2000). Rather than being held permanently off a local optimum by gene flow the population, even a small one, may periodically track it as it moves.

Implications for speciation

Founder effects and random genetic drift are frequently invoked to explain genetic and morphological divergence in peripheral populations of birds and even speciation itself (e.g., Baker & Moeed, 1987; Baker et al., 1990; Dennison & Baker, 1991; Merilä, Björklund & Baker, 1996a, b; Omland, 1997), especially in phylogeographic studies that use neutral genetic markers. The evidence is relatively low genetic variation in a population, which might have been caused in the initial stages of colonization (e.g., Seutin et al., 1994). Our study differs from others in directly investigating the dynamics of presumably neutral genetic markers and at the same time morphological and behavioral traits likely to be of biological significance in the lives of birds (see also Degnan, 1993). We found that during the first three to six overlapping generations mean heterozygosity remained more or less constant as the population size increased. It did not increase, in contrast to what happened in small populations of the Laysan finch translocated to unoccupied islands (Tarr, Conant & Fleischer, 1997). Although allelic diversity initially fell in the bottleneck, then rose to approximately double the minimum value, we observed no phenotypic manifestation of these and other possible genetic changes apart from two small morphological trends that might have been caused by chance. Nevertheless, results of the study support the more general idea that drift and selection jointly cause population divergence in the first few generations after a new peripheral area is colonized.

The absence of pronounced change after the founding of the population could be explained by recurrent immigration, with gene flow ameliorating the effects of inbreeding and shortening the period over which they occurred (Keller et al., 1994). With recurrent immigration the important events with long-term consequences may occur a few generations after the founding of a population rather than in the founding itself. For example, a single immigrant male, 6102, had a disproportionate effect on the genetic and song characteristics of the population from 1991 onwards, a little more than 8 years after a breeding population was founded. He introduced 11 new microsatellite alleles and a new song type into the population. The song type rapidly increased in frequency, not because it was under direct selection but because other members of the population with a different song type experienced relatively low fitness as a result of inbreeding. Song, a cultural trait, is relevant to speciation because it is involved in pre-mating isolation of closely related species (Grant & Grant, 1996a, c; Irwin, Irwin & Price, 2001). Chance has been implicated in song diversification of populations of sharp-beaked ground finches, *G. difficilis*, in both the initial colonization of different islands and subsequently (Grant, Grant & Petren, 2000). Interestingly, the song established by 6102 appears to have caused gene flow of *G. magnirostris* to be selective on Daphne, thereby setting up subtle pre-mating isolation of birds from different island sources. This can be considered a first step in speciation, initiated by chance.

Implications for conservation

Small and newly established populations have a high chance of becoming extinct for reasons associated with a loss of genetic diversity, ecological factors that differ from the source environment, and externally driven population fluctuations (Lande, 1988, 1993; Woodworth et al., 1994; Frankham, 1995; Saccheri et al., 1998; Ripa & Lundberg, 2000). Small populations have thus received a large amount of attention from conservation biologists in the hope of gaining insights, not only into the predisposing factors leading to extinction but also into factors that favor long-term persistence (Caughley, 1994; Pimm, Jones & Diamond, 1988). In this context it is helpful to consider why the study population was successful in establishing itself in a community of two common and one rare congeneric species on a small island. A strongly fluctuating climate causes strongly fluctuating population sizes (Grant & Grant, 1996b; Grant et al., 2000). Thus the odds against success would seem to have been large. We can identify three important factors in the success; two ecological and one genetic.

First, ecological factors were unusually favorable at three important points. An environmental change associated with the extraordinary el Niño event of

abundant rain in 1982–1983 allowed a breeding population to become established (Grant & Grant, 1995a). The crucial factor may have been a rapid growth of *Croton scouleri*, a shrub or small tree that produces an abundant supply of seeds during the birds' breeding season that are a major source of food for offspring. Later, an exceptionally prolonged el Niño event in 1991–1993 provided the conditions for multiple breeding, favored high survival in the dry (non-breeding) seasons, and resulted in an expansion of the population. Later still another intense el Niño event in 1997–1998 produced the same favorable conditions for population expansion (Grant et al., 2000).

Second, the population started genetically rich. The only pair among the founders to produce breeding offspring were genetically diverse, as inferred from the above-average levels of heterozygosity in their offspring. A brother and sister started the F_1 generation. Both were highly heterozygous, implying genetically diverse founder parents.

Third, and perhaps most important, immigration was recurrent. The population increased from one to about 30 pairs over a period of 15 years as a result of both recruitment of locally born birds and repeated addition of new immigrants to the breeding population. Augmentation of the breeding population by new immigrants appears to have offset the deleterious effects of inbreeding, in two ways. It resulted in the introduction of many new alleles, thereby counteracting the tendency for the population to lose alleles through drift. Replacement of lost alleles was actually documented in six cases. Recurrent immigration also contributed to the increase in population size. This had the important effect of decreasing the chances of inbreeding through random pairing, and helped to end the period of time when the population sustained a genetic load through close inbreeding. Addition of immigrants to the breeding population occurred repeatedly because the habitat was suitable and the breeding population remained small for several years.

The population has either stabilized or is continuing to grow slowly. If we have identified the causes correctly reversing the conditions will reverse the population's trajectory. If climatic conditions return to those prevailing before 1982, and particularly before the current warming trend in tropical Pacific waters began in the mid-1970's (Guilderson & Schrag, 1998; Zhang, Rothstein & Buscalacchi, 1998), then population sizes generally in the archipelago are likely to decrease, Daphne may no longer have a suitable habitat for the breeding of *G. magnirostris*, immigration will decline and the population may become extinct. This apparently happened once last century (Grant & Grant, 1995a). However, in the near future global warming may prevent a return to preceding conditions by intensifying el Niño events, lengthening them, or increasing their frequency.

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