

The secondary contact phase of allopatric speciation in Darwin's finches

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Speciation, the process by which two species form from one, involves the development of reproductive isolation of two divergent lineages. Here, we report the establishment and persistence of a reproductively isolated population of Darwin's finches on the small Galápagos island of Daphne Major in the secondary contact phase of speciation. In 1981, an immigrant medium ground finch (*Geospiza fortis*) arrived on the island. It was unusually large, especially in beak width, sang an unusual song, and carried some *Geospiza scandens* alleles. We followed the fate of this individual and its descendants for seven generations over a period of 28 years. In the fourth generation, after a severe drought, the lineage was reduced to a single brother and sister, who bred with each other. From then on this lineage, inheriting unusual song, morphology, and a uniquely homozygous marker allele, was reproductively isolated, because their own descendants bred with each other and with no other member of the resident *G. fortis* population. These observations agree with some expectations of an ecological theory of speciation in that a barrier to interbreeding arises as a correlated effect of adaptive divergence in morphology. However, the important, culturally transmitted, song component of the barrier appears to have arisen by chance through an initial imperfect copying of local song by the immigrant. The study reveals additional stochastic elements of speciation, in which divergence is initiated in allopatry; immigration to a new area of a single male hybrid and initial breeding with a rare hybrid female.

immigration | inbreeding | introgression

One hundred and fifty years ago, Charles Darwin (1859) offered an explanation for the process of speciation by which an ancestral species gives rise to one or more derived species through adaptive evolutionary divergence (1). The explanation involved colonization of a new area, adaptive divergence in allopatry, and a barrier to interbreeding when differentiated populations encountered each other in sympatry. Darwin was much clearer on the early stages of speciation than on the later ones. He wrote to one of his many correspondents "...those cases in which a species splits into two or three or more new species ... I should think near perfect separation would greatly aid in the 'specification' to coin a new word" (2). Fortunately "specification" did not catch on, and we use the term "speciation" instead, but the fundamental importance of spatial (geographical) isolation for population divergence has persisted and is incorporated in most, although not all, current models of speciation (3–6).

When divergent populations subsequently meet, their respective members do not breed with each other, or if they interbreed, they do so rarely. Differences in signaling and in response systems that function when mates are chosen arise in allopatry and constitute a premating barrier to interbreeding in sympatry. The barrier may be fully formed in allopatry, in which case no interbreeding occurs in sympatry, or it may be strengthened by natural selection that causes further divergence in sympatry, in two ways. Offspring produced by interbreeding may be relatively unfit, either because the genomes of their parents are incompatible to some degree or because they are at an ecologically

competitive disadvantage in relation to the parental populations. Discriminating among these three alternatives has been difficult, because it requires observations to be made in nature on patterns of mating at the time secondary contact is established and in subsequent generations.

We have been fortunate to witness such a secondary contact. Here, we report the origin and persistence for three generations of a premating barrier to interbreeding between two groups of Darwin's finches on one of the Galápagos islands. The barrier arose as a consequence of allopatric divergence in morphology, introgressive hybridization, and divergence of song in sympatry. The barrier has genetic and learned components. Morphology is genetically inherited, whereas song is culturally inherited. Especially noteworthy is the absence of evolutionary change in sympatry in one group in response to the other or to the ecological environment. Our example highlights a stochastic element in the process of speciation.

Results

Immigration. A long-term study of Darwin's finch populations on the Galápagos island of Daphne Major was started in 1973, and by the beginning of 1981 >90% of the two species, *G. fortis* (medium ground finch) and *G. scandens* (cactus finch), had been measured and marked with a unique combination of colored and metal leg bands. In that year, after breeding had ceased, a medium ground finch male with exceptional measurements was captured. It weighed 29.7g, which is >5g heavier than any other *G. fortis* that had bred on the island, and is at the upper end of size variation of *G. fortis* on the neighboring large island of Santa Cruz (7). An analysis of alleles at 16 microsatellite loci with a no-admixture model in the program Structure (8–11) shows that the probability of this individual belonging to the resident Daphne population is 0.088, and of being a member of the conspecific population on Santa Cruz is 0.912. Therefore, we consider it to be an immigrant. Although it is most likely to have come from the large neighboring island of Santa Cruz, we cannot be certain of the exact source (see *Methods*). Morphologically, it is similar to *G. fortis*, but with a somewhat pointed beak profile like that of *G. scandens*, and therefore possibly of mixed genetic composition. In a second analysis, using an admixture model with samples of these two species from Santa Cruz, Structure assigned a greater fraction of its genome to *G. fortis* (0.659) than to *G. scandens* (0.341) (see *Methods*). It is therefore genetically heterogeneous, and we consider it to be a hybrid.

We have followed the survival and reproduction of this individual and all of its known descendants (Fig. 1), here termed the immigrant lineage, for seven generations (F_0 to F_6) spanning 28 years.

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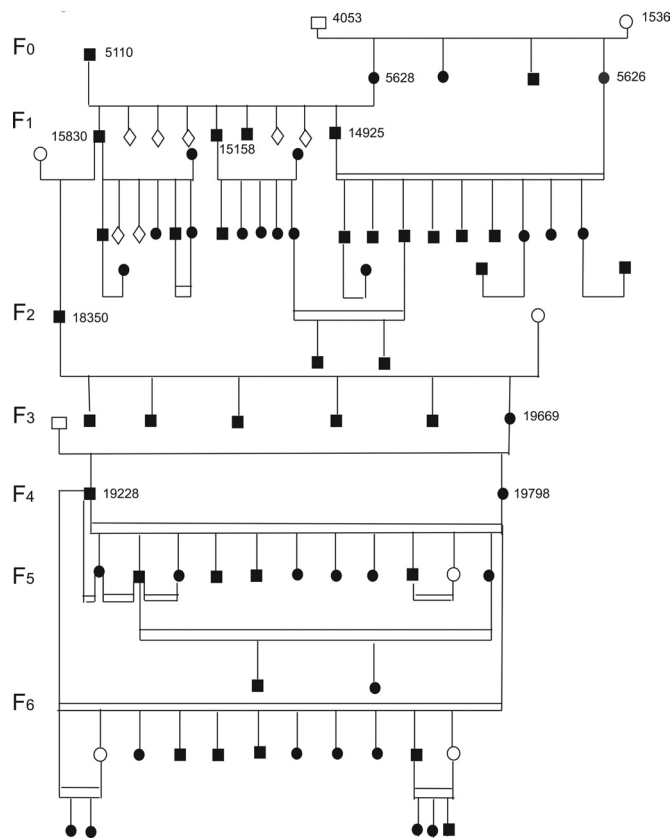


Fig. 1. Pedigree of an immigrant *G. fortis* male (5110) with a line of descent to an exclusively inbreeding (endogamous) group. For details of the construction of the pedigree, see *Methods*. Males are indicated by squares, females by circles, and birds of unknown sex by diamonds. Individuals of unknown genotype are indicated by open symbols, and filled symbols refer to genotyped birds. Salient individuals in the pedigree are indicated by their band numbers, e.g., the mate (5628) of the original immigrant (5110) is a backcross from *G. scandens*. Pairs of close relatives are connected by double lines. The frequency of inbreeding among close relatives in the immigrant lineage is exceptionally high. Keller et al. (12) analyzed 364 unique matings, where all four grandparents were known in the *G. fortis* population (including the immigrant lineage) up to 1992, and found that only three (0.8%) were the product of matings between first-degree relatives ($f = 0.25$). Two of the three are in the pedigree above.

Interbreeding Followed by Inbreeding. The immigrant hybrid male (5110) (Fig. 2) carrying some *G. scandens* genes (see *Methods*) bred with a female *G. fortis* also carrying some *G. scandens* genes (Fig. 1). Their sons bred with members of the resident population of *G. fortis*; no breeding females were produced by 5110. One of the sons (15830) gave rise to the next five generations (F₂–F₆) along one line of descent. The male in generation F₃ along this line was not genotyped. We strongly suspect that it was a member of the lineage, because it was seen to be unusually large (11) and sang the characteristic song of the lineage (see *Reproductive Isolation*). Members of the subsequent two generations (F₄ and F₅) bred only with each other and were thus endogamous.

The mating pattern is indicated by direct observations of pairs. Pairs may not be biological parents, however, because extra-pair mating in *G. fortis* is known to occur on this island at a frequency of 15–20% (12). Genetic evidence of paternity is more reliable and confirms our observational assessment of parentage. Genetic analysis reveals that all 25 genotyped members of the lineage in generations F₄–F₆ are homozygous (183/183) at microstallite locus *Gf.11*. The homozygote state at this locus is

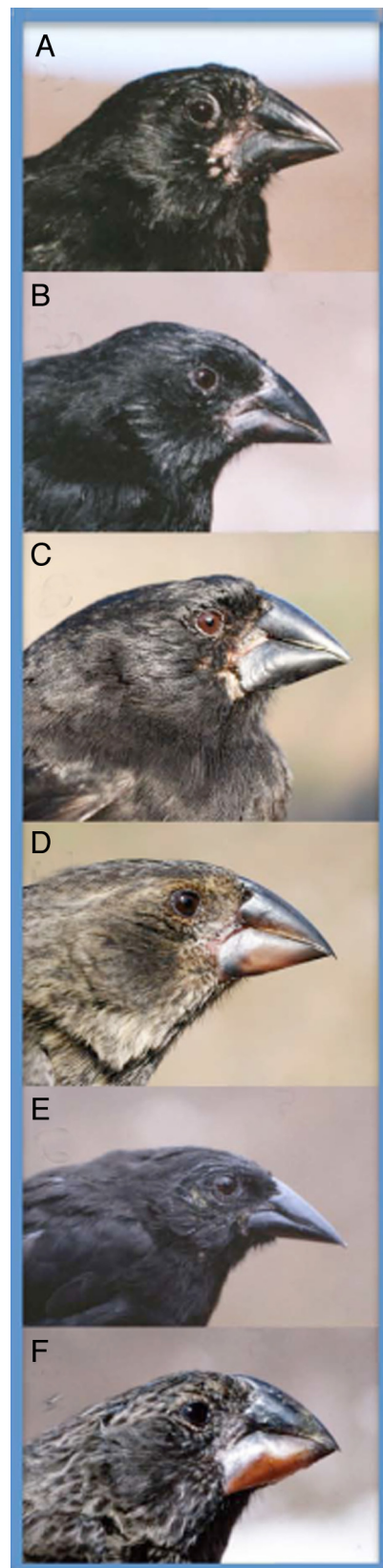


Fig. 2. The immigrant lineage contrasted with *G. scandens* and *G. magnirostris* on Daphne Major Island. (A) 5110, the original immigrant (generation F₀); (B) *G. fortis* 15830 (generation F₁), son of 5110; (C) *G. fortis* 19256 (generation F₅); (D) *G. fortis* 19566 (generation F₆); (E) *G. scandens* 15859; (F) *G. magnirostris* 17339.

highly unusual. Of 249 genotyped *G. fortis* individuals on the island from 2002 onwards that were not in the lineage, but contemporary with generations F₃–F₆, 27 carried one copy of the 183 allele and one individual carried two copies. Given a frequency of the 183 allele of 0.056 (29/498), the expected frequency of the homozygotes with random mating is 0.0032, or one individual in ≈300. Homozygotes were equally rare before 2002.

The original immigrant male (5110) was a homozygote (183/183) and his son (15830), grandson (18350), and great granddaughter (19669) in the line of descent (Fig. 1) were heterozygotes (183/-). The mate of 19669 must have carried at least one copy of the 183 allele, because their offspring were homozygous (183/183). This fact adds weight to the suggestion above that the mate of 19669 was also a member of the immigrant lineage (generation 3).

Although members of the lineage bred with each other (endogamy) in two or more generations, they might have also produced offspring by breeding with members of the resident population through extra-pair mating (exogamy). Cryptic exogamous mating can be tested by taking advantage of the fact that all endogamous parents are homozygous (183/183). Hence, if exogamous offspring are produced, they must carry at least one copy of the 183 allele at *Gf.11*. Twenty-eight individuals hatched in 2002 or later could have been produced by exogamous mating because they all had a 183 allele: One was a homozygote (183/183) and 27 were heterozygotes (183/-). However, all of these individuals were ruled out as exogamous offspring of the lineage because none of them matched any member of the endogamous group of breeders (generations F₄ and F₅) or the mother (19669; generation F₃) at all of the remaining 15 loci; mismatches of at least 4 base pairs occurred at 2–10 loci. Thus, we conclude there has been no detectable exogamous mating in the last two generations in eight years, and the immigrant lineage has been exclusively endogamous since 2002 and possibly much earlier.

Reproductive Isolation. A pre-mating barrier to the exchange of genes thus exists; an additional intrinsic post-mating barrier is unlikely because it has not been detected among any of the six *Geospiza* species (13). Furthermore, territories of the endogamous group formed spatially restricted clusters (Fig. 3) with neighbors in acoustic contact, which suggests that they recognize each other in the breeding season as members of the same group. Contrasting with this strong pattern, no more than two close relatives have been observed breeding in adjacent territories in the *G. fortis* population during 22 years (1976–98) of intensive study.

The barrier to interbreeding among *Geospiza* species has two elements, song and morphology (13). Specific features of both elements are learned during a short sensitive period early in life, while the young are dependent upon parents for food (14, 15). Male *G. fortis* sing only one song. There is individual variation on a *G. fortis* theme, which can be classified into four types on Daphne that are recognizable by sonograph and to the human ear (16, 17); females do not sing. Sixteen of 17 singing males in the lineage (94.3%), including the original immigrant (5110), sang a variant form of type III, also recognizable to the human ear: The seventeenth sang a type I song and did not breed. Eleven of them were tape-recorded and sonographed (Fig. 4). In a multiple discriminant function analysis (see *Methods*), all 11 were correctly classified as members of the immigrant lineage with probability values of 0.99 or 1.00, and 32 of 34 tape-recorded *G. fortis* males that sang type III were correctly classified ($P = 0.93$ –1.00). Songs of the 11 immigrant lineage males also differ discretely in many frequency and temporal measures from songs of all 205 tape-recorded males that sang the

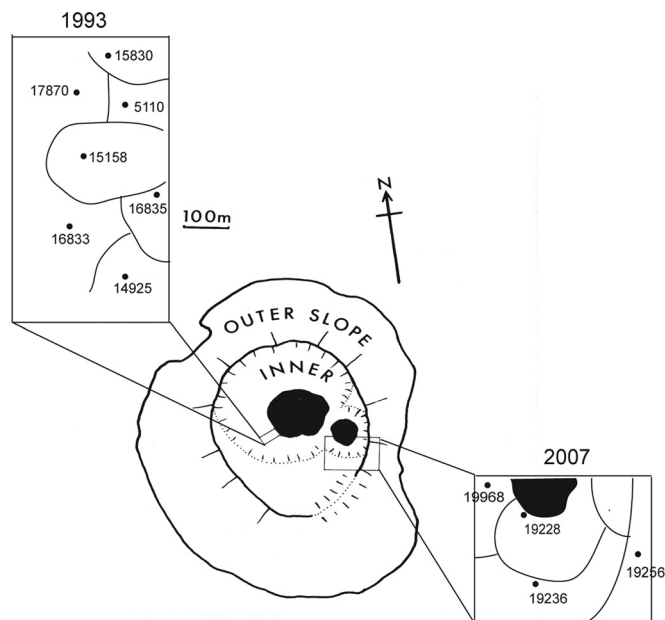


Fig. 3. Male territories of members of the immigrant lineage in two years. Note the clustering. All males sang the same song type (see Fig. 4). All members breeding in 2007 are shown, whereas in 1993, six others bred in various parts of the island. Individual 16833 paired with a sib (16834) from the same natal nest and bred next to another sib (16835). Nests are indicated by filled circles. Black areas are the floors of two craters.

other three song types. Song of the endogamous group is therefore almost discretely different from the songs of *G. fortis*.

Morphological features of the endogamous group (Fig. 5) are close to being diagnostically different from those of other *Daphne G. fortis* (Fig. 2). For example, 20 of 24 measured members of the endogamous group had wider beaks than any of the other 462 *G. fortis* on the island from 2002 onwards (Fig. 6). The remaining four overlapped only four *G. fortis* (≈1%). In average beak width (see *Methods*), the endogamous group is approximately equidistant from *G. fortis* (27.7% smaller) and *G. scandens* (25.7% smaller). It is even further from *Geospiza magnirostris* (Fig. 5), the large ground finch (37.2% larger), which established a breeding population on the island in 1983 (18). Morphological distinctness implies ecological distinctness (13).

Discussion

The Tempo and Mode of Speciation. Charles Darwin believed that evolution took place too slowly to be observed, and therefore speciation, the evolution of a new species, would take immeasurably longer (1). Evolution by natural selection is now known to occur rapidly in a variety of taxa and environments (19), including Darwin's finches in the Galápagos (20, 21), but Darwin's opinion on the slowness of speciation remains the consensus view (3, 4, 6, 22). Although generally true, it may apply more often to post-mating than to pre-mating isolation, where behavior plays an important role. Behavior has the potential to change rapidly when learned as opposed to being genetically fixed, especially in vertebrates. The origin of a pre-mating barrier between populations is a crucial component of speciation, because regardless of whether sympatric, closely-related species can or cannot produce viable and fertile offspring, the vast majority do not interbreed or do so very rarely. Our observations on the development of pre-mating isolation of two divergent lineages after secondary contact are therefore significant for two reasons. First, they show that reproductive isolation of small populations can develop rapidly. Second, they provide insight into the environmental circumstances and the relevant mechanisms.

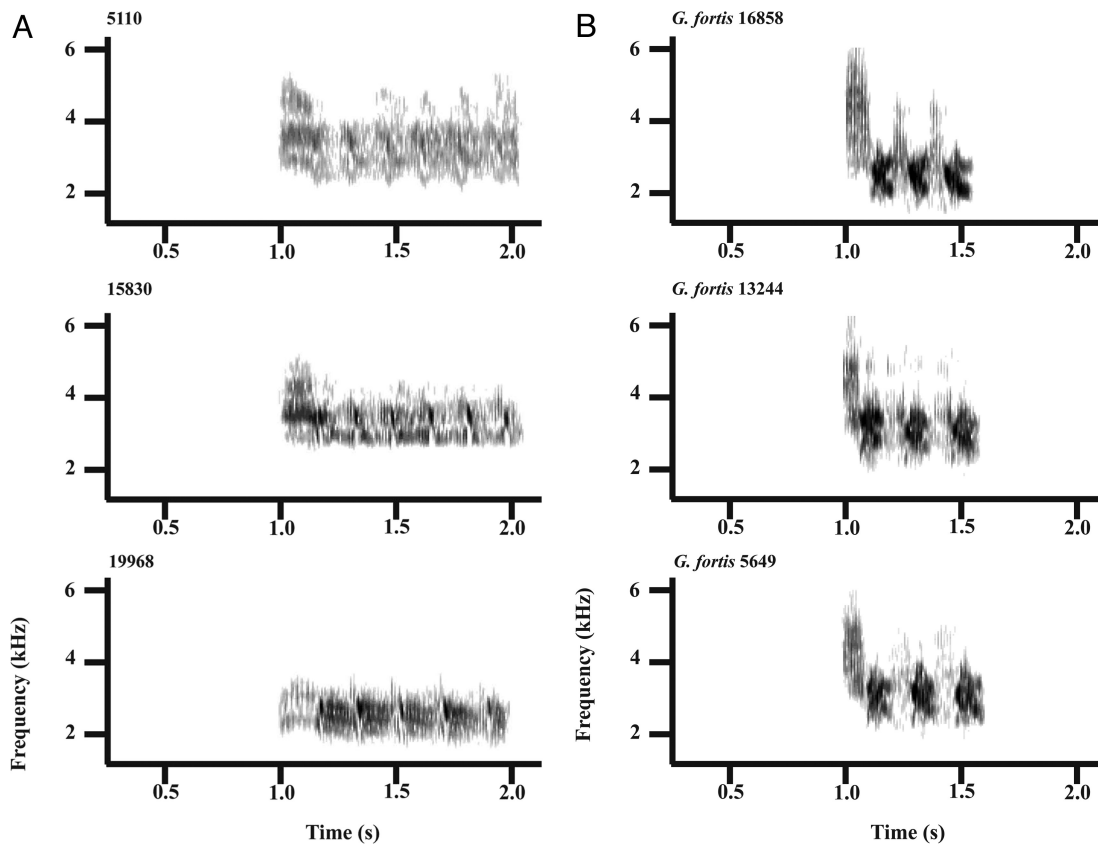


Fig. 4. Songs of the original immigrant (5110), a son (15830) and a fifth generation descendant (19968), compared with three *Daphne G. fortis* individuals that sang a standard form of type III. Immigrants differ from residents statistically in lower maximum frequency and higher note repetition rate (see *Discussion; Behavior and Speciation*). A wideband setting and a Hamming window with DFT 256 were used.

The events we have described demonstrate one way in which speciation proceeds by a combination of stochastic and deterministic processes. The development of reproductive isolation involved rare chance events of immigration and introgressive hybridization and an initial mating between two hybrid individuals. The breeding of immigrants on *Daphne* is rare; we have identified by microsatellites two *G. fortis* immigrants and one hybrid (5110) in 18 years and one *G. scandens* immigrant in 24 years (23). Hybridization, leading to gene exchange between residents of the two species on *Daphne*, is not much more frequent; 13 occurrences have been identified in 21 years (23). It generally results from the learning of the song of another species during the early sensitive period of song learning. This misimprinting can occur through cross-fostering, when an egg remains in a nest after the nest is taken over by another species, or after the death of the father, when the young hear a neighbor of another species (13). Hybridization has resulted in backcrossing to one parental species or the other depending on the song sung by the hybrid's father (15), but, unlike the case with the immigrant lineage, no close inbreeding has ensued. This outcome underscores the uniqueness of endogamy in the immigrant lineage and the special circumstances that gave rise to it.

Ecology of Speciation. Stochastic elements are inevitably present in allopatric speciation, owing to different mutations occurring at random in separate environments (24, 25). In the words of Hermann Muller (24) “Thus a long period of non-mixing of two groups is inevitably attended by the origination of actual immiscibility, i.e., genetic isolation.” There is also an inevitable element of determinism in allopatric speciation arising from ecological differences between separate environments, because

no two environments can be exactly the same, and therefore selection pressures must differ. Each class of factors, stochastic and deterministic, could be vital or trivial in particular cases. There is no single mechanism of speciation (3–6). The challenge for evolutionary biologists is to identify and assign importance to each contributing factor when accounting for the causes and circumstances of speciation in particular cases, and then to seek generalizations. Theories serve as a guide.

One theory of speciation proposes a completely allopatric origin of a barrier to interbreeding with different emphases on random processes and selection (3, 5, 24, 25). A second theory proposes a major genetic change, shortly after the founding of a new population by a few individuals, in which random drift plays an essential, but not exclusive, role (26). Neither is applicable to our study, because the barrier originated partly in sympatry with song (see *Behavior and Speciation*) but without genetic change in sympatry.

Instead, our observations are largely consistent with an ecological theory of speciation (27–30) in which a barrier to interbreeding arises as a behaviorally flexible correlated effect or byproduct of adaptive divergence of an ecologically selected trait (22, 27, 28). Beak size, with known ecological function of food handling (31, 32), is also a key component of the barrier as it signals species identity in a reproductive context (33). Divergence in beak size of the immigrant lineage and the residents occurred in allopatry. The immigrant lineage did not diverge from the residents in beak size in sympatry, as would be expected if selection minimized ecological competition between them (character displacement theory; ref. 21) or minimized the probability of interbreeding (reinforcement theory; refs. 34, 35).

However, the resident population of *G. fortis* did diverge from

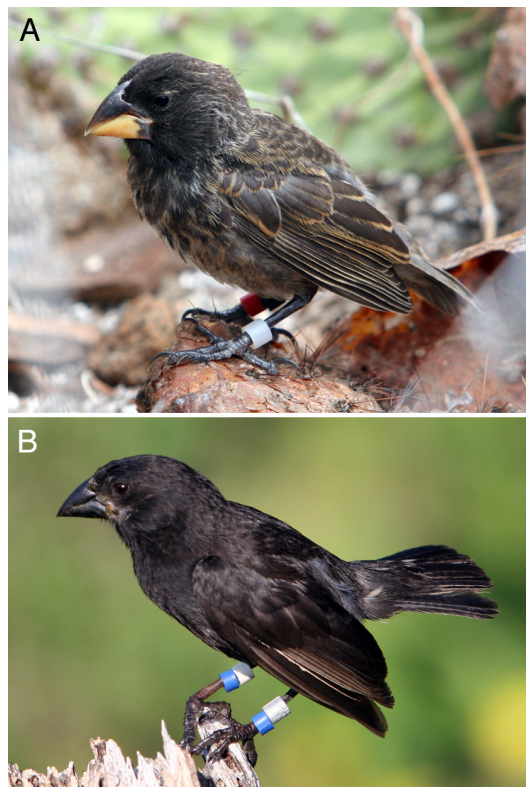


Fig. 5. Immigrant and resident *G. fortis*. (Upper) 9807, member of the immigrant lineage (generation F₅). (Lower) 19181, contemporary member of the resident population of *G. fortis* on Daphne Major Island.

the immigrant lineage, and divergence may have facilitated intra-group mating. Divergence was caused by natural selection during the drought of 2004, when small members of the *G. fortis* population survived best, in part because large-beaked members suffered in competition for food with *G. magnirostris* (21). The sole surviving brother and sister of the immigrant lineage (F₄ generation) bred with each other in 2005, when large members of the *G. fortis* population were scarce. Intra-group mate recognition and endogamy in generations F₄ and F₅ may have been facilitated by the morphological divergence of resident *G. fortis* from *G. magnirostris*. In the five years after the natural selection event in 2004, the endogamous group was almost completely separated in morphology from the residents (Fig. 4). Ecological differences associated with morphological separation probably contributed to their coexistence. The same has been argued for double-invasion species-pairs of birds on islands, in which a mainland species colonizes an island twice. Successful establishment of the second population depends upon prior divergence of the first in morphology and ecology and population-specific mating (36).

Behavior and Speciation. The other component of behavioral isolation is song. The song of the immigrant male 5110 was acquired initially by learning from early exposure to songs on Santa Cruz. Then it appears to have been modified by imperfect copying of the type III song of Daphne *G. fortis* during the crystallization phase of song production in his first breeding season in 1983. The alternative possibility of an allopatric origin is not supported by any of the >100 spectrograms of tape-recorded song in the published literature from Santa Cruz (14, 37) and other islands (14). We have not heard the type III song or 5110's at locations on both the north and the south coasts of the adjacent Santa Cruz Island or on any of the other major

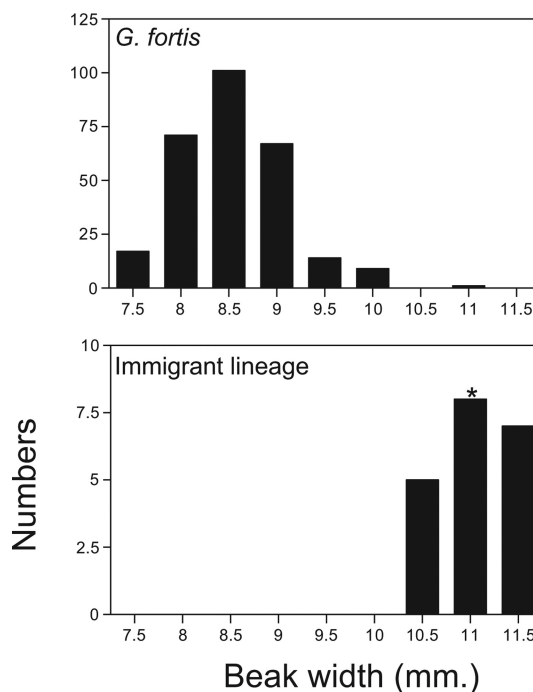


Fig. 6. Morphological contrast between the immigrant lineage ($n = 20$) with other *G. fortis* ($n = 280$) on Daphne Major Island in the years 2005–09. The position of the original immigrant (5110) is indicated by an asterisk.

islands of the archipelago (Santa Fe, Floreana, and those listed in *Methods*).

The imperfect copying of a resident's song appears to be a stochastic element in the development of reproductive isolation, and a nonecological component of the barrier to interbreeding. An alternative possibility is that the particular characteristics of the immigrant song could be a correlated effect of allopatric divergence in beak morphology and therefore part of the syndrome of ecological speciation (38). Song characteristics can be affected by beak morphology for biomechanical reasons; the larger the beak the slower the production of repeated notes and the smaller the range of their frequencies (39). Thus, a large bird like 5110 and its descendants might sing a slower version of the type III song over a smaller range of frequencies. However, the former expectation is not upheld; the mean repetition rate of notes is not slower, but faster, in songs of the immigrant lineage ($n = 11$ birds) than in type III songs of *G. fortis* ($n = 34$ birds; $F_{1,43} = 19.45$, $P < 0.0001$). In contrast, and consistent with the biomechanics hypothesis, the maximum frequency is reduced in the first note of songs of the immigrant lineage (Fig. 3) compared with type III songs ($F_{1,43} = 62.59$, $P < 0.0001$) and so is the frequency range of this note ($F_{1,43} = 54.19$, $P < 0.0001$). However, reduction does not appear to be due to mechanical constraints, because the largest species of ground finch on Daphne, *G. magnirostris* (Fig. 5), sings a song with a large frequency range in its initial note (18) like the type III song of *G. fortis*.

We conclude that song features of the immigrant lineage are not a by-product of beak divergence in allopatry. Reproductive isolation depended in part on ecological factors associated with beak size and in part on chance behavioral factors associated with song learning independent of ecology. The importance of beak size in mate choice has been emphasized in a study of assortative mating in a population of *G. fortis* on Santa Cruz Island (40). Chance factors have been invoked to explain the

large differences in songs between populations of *Geospiza difficilis* on adjacent islands (41).

Future Prospects of Incipient Species. These observations provide important insight into the process of speciation at the beginning of the sympatric phase following divergence in allopatry. They also raise a question that is rarely if ever asked: How many generations of exclusively within-group mating are needed before the group is recognized as a separate species that deserves taxonomic status? There is no nonarbitrary answer. We treat the endogamous group as an incipient species because it has been reproductively isolated from sympatric *G. fortis* for three generations and possibly longer.

Many episodes of incipient speciation probably fail for every one that succeeds in reaching complete genetic isolation due to incompatibility factors. In the present case, it is too early to tell whether reproductive isolation is transitory or likely to be enduring. The odds would seem to be against long-term persistence of the immigrant lineage as a reproductively isolated population. First, numbers are small and stochastic fluctuations in population size may result in extinction. Second, the new populations might run the risk of competitive exclusion from *G. fortis* and/or *G. magnirostris* if the food environment changed. Third, it might disappear through interbreeding with *G. fortis* and/or *G. scandens*, an example of reproductive absorption of one species by another (30), initiated perhaps by extra-pair mating or misimprinted song. Fourth, it might suffer from inbreeding depression.

With regard to the last possibility, a small closed inbreeding population is expected to lose alleles by chance, leading to extreme homozygosity, which makes the population more vulnerable to extinction. However, the history of another episode of immigration shows that neither extreme homozygosity nor extinction is inevitable. A breeding population of *G. magnirostris* was established on Daphne in 1983 by two immigrant females and three males (18). Inbreeding depression was moderately severe two generations later (42, 43), but subsequent immigration alleviated the effects and the population has persisted (23, 42). In the present case genetic heterogeneity of both the immigrant male and his mate due to gene mixing with *G. scandens* (see Fig. 1 legend and *Methods*) makes it likely that the population is open to genetic input from resident or immigrant *G. fortis* and *G. scandens*. The outcome, fusion or persistence, will depend on rates of introgression and fates of introduced genes (23, 44, 45). Divergence in beak size has increased the chances of long-term ecological coexistence.

Conclusion

Our observations provide insight into speciation and hence, into the origin of a new species. They show how a barrier to interbreeding can arise behaviorally and without genetic change in sympatry. A necessary condition was prior ecological divergence, and introgressive hybridization was possibly another. Evidently it takes only a single diploid immigrant to start the process by breeding with a resident, and tolerance of the effects of inbreeding is needed to complete it.

Methods

Assignment Tests. We used genotypic information from blood samples to identify individuals with version 2.2 (8) of the program Structure (9, 10). Individuals were assigned to specified groups with a probability estimated by a Bayesian analysis of frequencies of microsatellite alleles at 16 loci (11). We applied the majority rule ($P > 0.500$) to assign individuals to groups. Following the authors' recommendations we used a burn-in of 50,000 iterations, a run length of 100,000, and for each new analysis we repeated the procedure once to make sure results were consistent. We used a No Admixture model for questions about population membership of an individual and an Admixture model for questions about the fraction of an individual's genome attributable to each of two populations. The correlated alleles option was used through-

out. An individual for assignment was given a value of zero in the Popflag column, and all individuals from defined islands were given a value of 1, which allowed repeated updating of allele frequencies of all groups except the targeted individual. We split the birds into an early (up to 1998) and a late group (1999–2008) because full pedigree information was available up to 1998, and only partial information was available afterward. There was almost no breeding between 1999 and 2001.

To identify F_1 hybrids and backcrosses we used an ancestry model with two prior generations. This procedure gives an estimated probability that an individual belongs to another species (generation 0), having a parent (generation -1) or having a grandparent (generation -2) from another species. The last two are almost equivalent to F_1 hybrid and a backcross generation (first or higher).

Source and Identity of the Original Immigrant. The individual 5110, captured in a mist net in 1981, was initially suspected of being an immigrant. It was much larger than any resident member of *G. fortis* on Daphne and more similar in size and proportions to *G. fortis* on other islands. Moreover, we could not identify potential parents at a time when 90% of *G. fortis* and *G. scandens* were banded. On geographical grounds the large, neighboring island of Santa Cruz is the most likely source (30). Furthermore, allele 183 at locus *Gf.11*, which is homozygous in 5110, has a frequency of 0.077 in the Santa Cruz population of *G. fortis*, but is not present in any of our admittedly small samples from other islands. Assuming Santa Cruz is correctly identified as the source, we performed an assignment test with alleles at 16 microsatellite loci using a no-admixture model in the program Structure (8–11). The probability of this individual belonging to the resident Daphne population ($n = 77$) was found to be 0.088, and the probability of being a member of the conspecific population on Santa Cruz ($n = 39$) was 0.912. Therefore, 5110 was a probable immigrant.

We used the following samples of *G. fortis* genotypes from defined populations in an attempt to identify the source island of 5110 with Structure: Santa Cruz ($n = 39$), Santiago ($n = 9$), Rábida ($n = 3$), Marchena ($n = 17$), San Cristóbal ($n = 4$), Pinta ($n = 12$), Isabela ($n = 11$), and Daphne ($n = 77$). The defined Daphne population comprised only those individuals that hatched on the island. A sample of 12 birds captured on Daphne without bands and therefore potential immigrants, including 5110, comprised an undefined population. All but three were assigned to the Daphne population at $P > 0.950$. Assignment probabilities of 5110 were 0.427 to Isabela, 0.282 to Santiago, and 0.244 to Santa Cruz, but 0.000 to Daphne. Sequential deletions of the other populations with small samples (Rábida, San Cristóbal, Santiago) gave similar results with the probability of assignment to Isabela being the highest and to Daphne always being 0.000. These results support the immigration hypothesis. However, the analysis failed to identify the source island, probably because the *G. fortis* populations are too similar genetically (23).

Although clearly referable to *G. fortis*, 5110 has a somewhat pointed beak profile like that of *G. scandens* (Fig. 2) and is therefore possibly of mixed genetic composition. In an analysis using an admixture model with samples of these two species from Santa Cruz, Structure assigned a large fraction of its genome to *G. fortis* (0.659) and a smaller fraction to *G. scandens* (0.341). Therefore, 5110 is genetically heterogeneous. The homozygous condition of the 183 allele at locus *Gf.11* is further evidence of 5110 being a hybrid because the allele is at a much higher frequency in the Santa Cruz population of *G. scandens* (0.361) than in *G. fortis* (0.077). There is indirect genetic evidence of rare interbreeding on this island between *G. fortis* and *G. scandens* (46). These facts, together with the exceptional morphology, support the hypothesis that the immigrant was a *G. fortis* \times *G. scandens* hybrid or backcross.

Identity of Birds Breeding with Members of the Immigrant Lineage. In generations F_0 – F_2 (Fig. 1), two male and five female resident *G. fortis* that mated with members of the immigrant lineage were genotyped. Their identities were first established by their measurements (47), then assessed with a no-admixture model in Structure. In the analysis of 1423 *G. fortis* and 504 *G. scandens* present in 1978–98, a genotyped sib 5627 was entered in place of the missing 5628. Structure assigned six of the targeted individuals to *G. fortis* with probabilities of 0.998 or 1.00. The remaining two, 5626 and 5627, were assigned to *G. fortis* with probabilities of 0.858 and 0.794, and to backcrosses from *G. scandens* to *G. fortis* with probabilities of 0.142 and 0.205, respectively. The sib that was not genotyped (5628) was therefore probably genetically heterogeneous also. We have no reason to suspect extra-pair paternity, as all three sibs were similar morphologically. For example, the beak depths and widths of 5626, 5627, and 5628 were respectively 8.9 and 8.2, 8.0 and 7.9, and 8.6 and 8.5 mm. One of them (5628) bred with the immigrant male (5110) and another (5626) bred with a son (14925). Their father (4053) sang a *G. fortis* song (type I) but was not genotyped. He was considered to be an F_1 hybrid

because his measurements were on the borderline between those of the two species (7), but he may have been a first generation backcross.

Parentage. Parentage was initially inferred by observing adults attending a nest. Adults were identified by their color bands or their observed large size (11). To construct the pedigree of the immigrant lineage, we used allele lengths at 16 microsatellite loci (11). We allowed 2bp differences (and no more) between offspring and presumptive parents as being within the range of scoring variation (11, 12, 43). Almost all offspring matched both parents at all loci.

Construction of Fig. 1. The line of descent to the endogamous group is as follows. The immigrant male (5110), F_0 generation, bred with a genetically heterogeneous *G. fortis* female (5628). Although her parents were not genotyped, she was most likely to have been a backcross from *G. scandens*; see *Identity of Birds*. A son (15830), F_1 generation, bred with a *G. fortis* female of unknown genotype. She could not have been a sib, because all of 15830's sibs that attempted to breed were males. A grandson (18350), F_2 generation, might have bred with a sib; the genotype of his mate is not known. In the next generation (F_3), a daughter (19669) bred with a male that was, like her, unusually large and therefore may have been a sib. He was not captured and genotyped but was observed (and heard) repeatedly. The female 19669 was originally thought to be an immigrant (11), but we have since discovered a complete genetic match at 16 loci between 19669 and 18350. Generations F_4 – F_6 comprise the endogamous group.

Generation F_5 is shown at two levels corresponding to early (2006–07) and late (2008–09) production of offspring. The first level is known with certainty because only two members of the pedigree, 19228 and 19798, could have produced them. The parents of those at the second level could not be identified genetically, because more than one generation was present at that time. Because there are many offspring, it is likely the parents were members of both F_4 and F_5 generations. The unknown genotypes of two females in the fifth generation were inferred from the genotypes of their offspring and the offspring's known fathers. A third of unknown genotype was seen to be very large (11).

The immigrant 5110, after breeding with 5628, bred with two banded *G. fortis* females. In both cases their offspring did not breed. They have been omitted from the figure for simplicity.

Song and Morphology. Song was recorded with a Sony (TCM 5000) tape recorder and a Sennheiser AKG D900 microphone (17). Two to 15 songs per bird were recorded. Because songs remain unchanged throughout life (17), only the first one recorded for each bird was included in analyses of songs performed in Raven version 1.3, Beta version (48). The following were measured for each song: number of notes, number of notes/sec, central frequency, frequency at which maximum energy was produced, and for each of the first two notes the duration, minimum and maximum frequency, and frequency range. Five uncorrelated variables were entered simultaneously into a two-group discriminant function analysis performed in JMP 7.0 (49). These variables were number of notes, number of notes/sec, duration of first note, and maximum frequency of the first and second notes. The groups were 11 males of the immigrant lineage that sang the type III variant and 34 *G. fortis* that sang the type III song, which is the most similar type to the immigrant's song. All immigrant males were classified correctly ($P = 0.99$ or 1.00). All but two of the 34 type III songs were classified correctly ($P = 0.93$ –1.00). The other two were misclassified as songs of the immigrant lineage at $P = 0.93$ and 0.97, respectively. For a test of the fit of the discriminant function, Wilk's lambda = 0.2010, Exact $F_{5,34} = 31.003$ ($P < 0.0001$). Songs of the immigrant group differed significantly from *G. fortis* type III songs in maximum frequency, frequency range, and note repetition rate (see *Results; Reproductive Isolation*), but did not differ in minimum frequency, central frequency, or frequency at which maximum energy was produced ($P > 0.1$).

Morphological measurements were made as described in ref. 47 and illustrated in ref. 13. Beak-width means in millimeters and standard deviations for the samples of birds on the island from 2002 onwards are 10.82 ± 0.432 for the immigrant lineage ($n = 21$), 8.47 ± 0.616 for all other *G. fortis* ($n = 462$), 8.61 ± 0.546 for *G. scandens* ($n = 291$), and 14.85 ± 0.870 for *G. magnirostris* ($n = 241$). Beak-depth means are slightly larger in each case.

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- Darwin C (1859) *On the Origin of Species by Means of Natural Selection* (J. Murray, London).
- Darwin C (1878) Letter to K. Semper, Nov. 26 in *The Life and Letters of Charles Darwin. Including an Autobiographical Chapter. Vol. III*, ed Darwin, F. (D. Appleton and Co., New York, 1919), p 160.
- Mayr E (1963) *Animal Species and Evolution* (Belknap, Cambridge, MA).
- Coyne JA, Orr HA (2004) *Speciation* (Sinauer, Sunderland, MA).
- Gavrilets, SS (2004) *Fitness Landscapes and the Origin of Species* (Princeton Univ Press, Princeton, NJ).
- Price T (2008) *Speciation in Birds* (Ben Roberts, Greenwood, CO).
- Grant PR (1993) Hybridization of Darwin's finches on Isla Daphne Major, Galápagos. *Philos Trans R Soc London Ser B* 340:127–139.
- Pritchard JK, Wen X, Falush D (2007) *Documentation for structure software: Version 2.2*. Available at <http://pritch.bsd.uchicago.edu/software>. Accessed May 23, 2008.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure: Extensions to linked loci and correlated allele frequencies. *Genetics* 164:1567–1587.
- Grant PR, Grant BR (2008) Pedigrees, assortative mating and speciation in Darwin's finches. *Proc R Soc London Ser B* 275:661–668.
- Keller LF, Grant PR, Grant BR, Petren K (2001) Heritability of morphological traits in Darwin's finches: Misidentified paternity and maternal effects. *Heredity* 87:325–336.
- Grant PR, Grant BR (2008) *How and Why Species Multiply* (Princeton Univ Press, Princeton, NJ).
- Bowman RI (1983) in *Patterns of Evolution in Galápagos Organisms*, ed Bowman RI, Berson M, Leviton AE (AAAS Pacific Division, San Francisco), pp 237–537.
- Grant BR, Grant PR (1998) in *Endless Forms: Species and Speciation*, eds Howard DJ, Berlocher SH (Oxford Univ Press, New York), pp 404–422.
- Gibbs HL (1990) Cultural evolution of male song types in Darwin's medium ground finches, *Geospiza fortis*. *Anim Behav* 39:253–263.
- Grant BR, Grant PR (1996) Cultural inheritance of song and its role in the evolution of Darwin's finches. *Evolution* 50:2471–2487.
- Grant PR, Grant BR (1995) The founding of a new population of Darwin's finches. *Evolution* 49:229–240.
- Hendry AP, McKinnon MT, eds (2001) *Microevolution. Rate, Pattern, Process* (Kluwer Academic, Boston).
- Grant PR, Grant BR (2002) Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296:707–711.
- Grant PR, Grant BR (2006) Evolution of character displacement in Darwin's finches. *Science* 313:224–226.
- Dobzhansky T (1937) *Genetics and the Origin of Species* (Columbia Univ Press, New York).
- Grant PR, Grant BR (2009) Conspecific versus heterospecific gene exchange between populations of Darwin's Finches. *Philos Trans R Soc London Ser B*, in press.
- Muller H (1940) *The New Systematics*, ed Huxley JS (Clarendon, Oxford), pp 185–268.
- Mani GS, Clarke B (1990) Mutational order – a major stochastic process in evolution. *Proc R Soc London Ser B* 240:29–37.
- Mayr E (1954) in *Evolution as a Process*, ed Huxley J, Hardy AC, Ford EB (Allen and Unwin, London), pp 157–180.
- Lack D (1947) *Darwin's Finches* (Cambridge Univ Press, Cambridge, U.K.).
- Grant PR (1986) *Ecology and Evolution of Darwin's Finches* (Princeton Univ Press, Princeton, NJ).
- Schluter D (1996) Ecological causes of adaptive radiation. *Am Nat* 148(Suppl):S40–S64.
- Schluter D (2009) Evidence for ecological speciation and its alternative. *Science* 323:737–741.
- Schluter D, Grant PR (1984) Determinants of morphological patterns in Darwin's finch communities. *Am Nat* 123:175–196.
- Herrel AJ, Podos J, Huber SK, Hendry AP (2005) Bite performance and morphology in a population of Darwin's finches: Implications for the evolution of beak shape. *Funct Ecol* 19:43–48.
- Ratcliffe LM, Grant PR (1983) Species recognition in Darwin's finches (*Geospiza*, Gould) I: Discrimination by morphological cues. *Anim Behav* 31:1139–1153.
- Johnson MS, Murray J, Clarke B (2000) Parallel evolution in Marquesan partulid land snails. *Biol J Linn Soc* 69:577–598.
- Nosil P, Yukilevich R (2008) Mechanisms of reinforcement in natural and simulated polymorphic populations. *Biol J Linn Soc* 95:305–319.
- Grant PR (2001) Reconstructing the evolution of birds on islands: 100 years of research. *Oikos* 92:385–403.
- Huber S, Podos J (2006) Beak morphology and song features covary in a population of Darwin's finches. *Biol J Linn Soc* 88:89–498.
- Seddon N (2005) Ecological adaptation and species recognition drives vocal evolution in neotropical subsocial birds. *Evolution* 59:200–215.
- Podos J (2001) Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* 409:185–188.
- Huber SK, De León LF, Hendry AP, Bermingham E, Podos J (2007) Reproductive isolation of sympatric morphs in a population of Darwin's finches. *Proc R Soc London Ser B* 274:1709–1714.
- Grant BR, Grant PR, Petren K (2000) The allopatric phase of speciation: The sharp-beaked ground finch (*Geospiza difficilis*) on the Galápagos islands. *Biol J Linn Soc* 69:287–317.

42. Grant PR, Grant BR, Petren K (2001) A population founded by a single pair of individuals: establishment, expansion, and evolution. *Genetica* 112/113:359–382.
43. Keller LF, Grant PR, Grant BR, Petren K (2002) Environmental conditions affect the magnitude of inbreeding depression in survival of Darwin's finches. *Evolution* 56:1229–1239.
44. Bolnick DI, Caldera EJ, Matthews B (2008) Evidence for asymmetric migration load in a pair of ecologically divergent stickleback populations. *Biol J Linn Soc* 94:273–287.
45. Grant BR, Grant PR (2008) Fission and fusion of Darwin's finches populations. *Philos Trans R Soc London Ser B* 363:3821–3829.
46. Grant PR, Grant BR, Petren K (2005) Hybridization in the recent past. *Am Nat* 166:56–67.
47. Boag PT, Grant PR (1984) The classical case of character release: Darwin's finches (*Geospiza*) on Isla Daphne Major, Galápagos. *Biol J Linn Soc* 22:243–287.
48. Charif RA, Clark CW, Frisrup KM (2006) Raven 1.3 User's Manual (Cornell Laboratory of Ornithology, Ithaca, NY).
49. SAS Institute (2007) JMP 7.0 (SAS Institute Inc., Cary, NC).