Songs of Darwin’s finches diverge when a new species enters the community

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Bird species sing different songs and as a result rarely breed with each other. Species are not static but can shift in acoustic and morphological space, yet maintain their distinctiveness. Investigating such a situation in a community of Darwin’s finches sheds light on the origin and maintenance of premating barriers between species. Explanations for songs divergence generally invoke morphological changes to the sound-producing apparatus, environmental changes influencing transmitting properties of song, avoidance of acoustical interference with other species, and random processes including copying errors. We investigated changes in songs of Geospiza fortis (medium ground finch) and Geospiza scandens (cactus ground finch) from 1978 to 2010 on Daphne Major Island, Galápagos. The habitat did not change significantly; however, the finch community changed. The socially aggressive congener Geospiza magnirostris (large ground finch), singing in the same frequency band (2–4 KHz), colonized Daphne in 1983 and increased in numbers. Temporal features of the songs of G. fortis and G. scandens, especially trill rate and song duration, diverged from G. magnirostris songs as it became increasingly common. Changes in song were not a passive consequence of a change in beak morphology. Instead they arose as a bias during song imprinting and production. Sons of both G. fortis and G. scandens sang faster songs than their respective fathers and thereby differed more from G. magnirostris in their songs than did their fathers. Divergence from an aversive or confusing stimulus during learning illustrates a “peak shift” that may be a common feature of song evolution and speciation.

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ccounting for the origins, persistence, and ultimate extinction of species is one of the most challenging problems for evolutionary biologists. In most cases the divergence of species from a common ancestor happened a long time ago; therefore the process must be inferred from genetic differences between extant species, and the causes are rarely understood. The smaller are the differences between species, and the more recent is their divergence from a common ancestor, the greater is the potential for discovering how and why one species gives rise to two. There are several model systems for studying speciation across a broad taxonomic range of closely related species. From Drosophila flies (1) to Helianthus flowers (2), and each has its advantages and disadvantages. Darwin’s finches on the Galápagos islands of Ecuador are one such group. They are suitable for two reasons (3). First, the 14 species have been derived from a common ancestor comparatively recently, estimated to be in the last 2–3 My (3), and the small differences between them allow us to reconstruct the evolutionary transitions. Second, they occupy a diversity of islands, some of which are close to a pristine state, never having been inhabited, ensuring that any changes we observe are the result of natural conditions and not influenced directly by humans. The finches are easy to capture, measure, genotype, observe, and follow in successive generations.

Species coexist sympatrically as a result of barriers to the exchange of genes. The reproductive barrier between closely related species of Darwin’s finches is behavioral: They occasionally hybridize with little if any intrinsic fitness loss (4). Experiments in the field have demonstrated that closely related species in the genus Geospiza living sympatrically can discriminate between each other on the basis of song in the absence of visual cues (5, 6) and on the basis of morphology in the absence of acoustic cues or movement (7). Furthermore, discrimination between allopatric populations of the same species is possible when they differ in song (5, 8, 9) or morphology (7).

Thus differences in morphology in association with song constitute the barrier to interbreeding (10, 11). What is the origin of this premating barrier to gene exchange? Divergence takes place initially in populations on different islands with different ecologies through natural selection and random genetic drift. Islands differ in their food supplies, and the role of natural selection when feeding conditions change has been well documented (6, 7). Phenotypic differences arising in allopatry may then be enhanced by selection when populations establish sympathy on secondary contact (12, 13). Premating barriers arise when the mating signals (song and morphology) diverge in parallel.

The question of how songs diverge is not so easy to answer, although it has stimulated much research (14). Explanations generally invoke random song changes such as those caused by an accumulation of copying errors, morphological changes to the sound-producing apparatus, environmental changes influencing the transmitting properties of song, or acoustical interference with other species (13, 15, 16).

Songs may change as a result of stochastic processes such as an accumulation of copying errors between generations in a manner analogous to random genetic drift (17, 18), and differences between populations could build up over time. Random change constitutes a null hypothesis for the alternative explanations that invoke systematic factors.

Body size affects syrinx volume and hence fundamental frequencies, in Darwin’s finches (10) as well as in other species (19, 20). Beak size influences the rate of note (syllable) production and frequency range. Darwin’s finches with large beaks are constrained to have songs of lower note repetition rate and narrower frequency bandwidth than birds with smaller beaks by their inability to open and close their beaks rapidly (21–23). Changes in song can therefore occur as a passive consequence of changes in beak morphology (21, 24) due to natural selection from an altered food environment (3).

Song features are adapted to the sound transmission properties of different habitats (25, 26). Owing to differential attenuation and degradation, songs with lower frequencies are transmitted better through dense vegetation than through open environments (10, 16, 20, 25). Rate of note repetition, or trill rate (21), also varies in relation to habitat features. Trills transmit better in open

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habitats than in forests where interference from the vegetation and reverberation blurs the distinction between notes (25). Two longitudinal studies of white-crowned sparrows (Zonotrichia leucophrys) showed that song changes were associated with more efficient transmission in altered environments over periods of 30 (27) and 35 y (28).

Competition for signal space among species using the same acoustic environment may also be a factor influencing change in song structure (14, 19). The other species may comprise closely related finch species but also distantly related species such as seabirds. Finches arriving at an island may encounter both a different habitat and a different constellation of species. The presence of congeneric species could select for divergence in song if there is a fitness penalty to interbreeding (16, 29) or more generally if there is a communication benefit from minimizing acoustical interference (30–32). Peak shift, a learning phenomenon whereby an individual responds more to an exaggerated form of its own signal in a direction away from a congeneric signal, is a mechanism that would lead to divergence between songs of related species as a consequence of direct social interactions (33–35). Therefore songs of the residents and colonists may gradually change over the next few generations under pressures for unambiguous transmission to other members of the same breeding population, both from the habitat and from other species.

**Song Acquisition and Transmission**

All Darwin’s finch males sing a single short song. Song and parental morphology are learned from day 10 to 40 after hatching through an imprinting process coinciding with the time when fathers are singing and feeding their young (10, 36). This effect of early experience was shown experimentally many years ago with finches in captivity (10). Once learned, a song is retained for life. As in other imprinting processes, learning species-specific song early in life influences mate-choice behavior later in life (37–39), resulting in individuals mating according to their conspecific song (11, 36, 37). Such vertical cultural transmission of song from father to son (6, 36) is expected to be culturally stable (16, 40), with small changes resulting from an accumulation of copying errors (18). Females do not sing, although the pattern of mating reveals that they learn their father’s song (36, 37, 41), as shown experimentally in other oscines (42). Hybrid males sing the same species-specific song as their fathers’ (36, 41) and not a mixture of songs of the parental species as occurs in a few other species (43).

**Darwin’s Finches on Daphne Major Island**

We addressed the question of what drives song divergence by studying the songs of two species of finches on Daphne Major Island (34 ha) from 1978 to 2010. The species are Geospiza fortis, the medium ground finch, and Geospiza scandens, the cactus ground finch (Fig. 1), and they differ in beak morphology and in song (Fig. 2). Even though both species vary from island to island in morphology and song features (10), song remains discretely different between species in sympathy (Fig. S1).

The study is uniquely suitable for studying two of the factors hypothesized to be important in song divergence: acoustic avoidance of other species’ songs and morphological determinants of song. A third species, Geospiza magnirostris, the large ground finch, colonized the island in 1983 and established a breeding population and numbers built up gradually over the next two decades (Fig. 3). Acoustic interference with G. fortis and G. scandens is to be expected because all three species concentrate sound energy within the same frequency band of 2–4 kHz and songs of G. magnirostris are especially loud.

**Song Changes Across Years**

All Darwin’s finch males sing one single short song, retained unaltered throughout life (36). Song variation within a population is continuously distributed. Examples in Fig. 2 reflect the range of song variation seen within G. fortis and G. scandens on Isla Daphne. We used measurements of individual songs recorded from 1978 to 2010, grouped according to the decade in which the recorded birds hatched (Table 1), to identify the song variables that changed (Methods). Song features were heterogeneous among years, in both G. fortis (MANOVA; Wilk’s $\Lambda = 0.836$, $F = 6.855$, df = 9, 808, $P = 0.0001$) and G. scandens (MANOVA; Wilk’s $\Lambda = 0.573$, $F = 13.022$, df = 9, 455, $P = 0.0001$). Component variables changed in one or both species. Changes were most pronounced in the last two decades in trill rate (number of notes per second), especially in G. scandens, and song duration (Table 1 and Table S1).

Fluctuating changes in amount of vegetation also occurred as a result of pronounced annual variation in rainfall (3). The habitat is a mixture of cactus, shrubs, trees, and open areas, with no closed-canopy forest. The changes were mainly restricted to low-growing vegetation close to the ground where finches do not sing, whereas song perches are 1–5 m above ground on cactus (Opuntia echios) and trees (Bursera graveolens and Croton scouleri). Such changes in vegetation are ephemeral when considered against a finch life span. The average generation length of G. fortis is 4.5 y and of G. scandens is 5.5 y, the maximum age for both species being 16 y (3). Thus for singing finches the habitat remained the same, so changes in songs must have been caused by other factors.

**Song Changes in Response to G. magnirostris: Tests of the Acoustic Interference Hypothesis**

G. magnirostris is distinctly larger than G. scandens and G. fortis and persistently harasses both of them for nest sites in cactus bushes and when feeding. Song characteristics of the three species overlap, and therefore song divergence of the socially subordinate G. fortis and G. scandens from G. magnirostris is expected from the hypothesis of acoustic interference. The number of singing male G. magnirostris, paired and unpaired, increased from 3 in 1983 to a maximum of 51 in 2010 (Fig. 3). If G. magnirostris singing influenced the songs of G. fortis and G. scandens, we should expect their songs to change in temporal and spectral features in a direction away from G. magnirostris song characteristics. The expectation was not met with spectral features. The intercorrelated frequency variables shifted downward in all three species from the 1990s to the 2000s (Table 1 and S1 Methods).

Songs of G. fortis and G. scandens are expected to differ from the songs of G. magnirostris increasingly in time, and more in the 2000s, when there were many of them, than in the 1980s and 1990s.
when there were fewer. We tested these expectations with the five temporal variables: trill rate (number of notes per second), song duration, average interval between notes, and duration of each of the first two notes. Because these variables are positively correlated with each other, we performed a principal components analysis (PCA) with the first three variables (SI Methods).

Both univariate and multivariate analyses showed that song traits in *G. fortis* changed significantly in the expected direction away from *G. magnirostris* (one-way ANOVAs). Duration of the song as well as duration of the first two notes and the average internote interval became shorter across the four decades, whereas trill rate, illustrated in Fig. 4, increased (Table 1). The average number of notes in a song increased. Most of these changes took place from the 1990s to the 2000s (Table 1 and Tables S1 and S2). Mean principal components (PC 1) scores varied strongly among the decades (\( F_{3,332} = 27.50, P = 0.0001 \)). A post hoc test identified the decades that differed significantly at \( P = 0.01 \) as follows: 2000s > 1990s > 1980s = 1970s. Remaining variation captured by the second principal component was more homogeneous among decades (\( F_{3,182} = 2.61, P = 0.0529 \)). Thus in both species changes were in the direction away from *G. magnirostris* in acoustical space, consistent with the hypothesis of acoustic avoidance of *G. magnirostris* songs (Fig. 5).

**Song Change Related to Morphological Change Caused by Character Displacement from *G. magnirostris*: Tests of the Biomechanics Hypothesis**

A severe drought began 20 y after *G. magnirostris* arrived on the island, causing competition with *G. fortis* for the large and hard *Tribulus* seeds that constitute the diet of both species. As a result, large-beaked *G. fortis* died to a disproportionate extent, and average beak size diverged away from *G. magnirostris* (Fig. 6). This character displacement event was restricted to the influence of *G. magnirostris* on the *G. fortis* population (44); morphological features of *G. scandens* did not change.

Changes in *G. fortis* song might have been a consequence of changes in beak size (Fig. 6). A biomechanics hypothesis (21) predicts a decrease in beak size will cause an increase in frequency bandwidth and trill rate. Given the trade-off between these two song features, we tested the hypothesis with a new metric: frequency bandwidth of the first note divided by its duration as a measure of time-adjusted frequency sweep. The first note was chosen because it has an up-sweep and a down-sweep in the same note (Fig. 2). Two tests were performed with 251 *G. fortis* individuals. In the first test we compared the frequency sweep of songs grouped in the four decades, expecting no differences except between the 1990s and the 2000s, that is to say before and after the character displacement; the frequency sweep should be greater...
the 2000s than in the 1990s. Frequency sweep was heterogeneous among decades (Kruskal–Wallis ANOVA, $\chi^2 = 12.15, P = 0.0069$), as a result of a difference between the 1980s and the 1990s [Tukey–Kramer honestly significant difference (HSD) test, significant at 0.05 but not at 0.01]. However, there was no difference between the 1990s and the 2000s ($P > 0.1$). In the second test we used the dataset in a single analysis. Frequency sweeps were predicted to be inversely correlated with beak size (PC 1), but in fact the two variables were not correlated ($r = 0.08, P = 0.287$). We obtained the same results when frequency bandwidth was used without the note duration adjustment, as was done in the original development of the biomechanics hypothesis (21).

The maximum frequency in *G. fortis* songs decreased in the 2000s. At this time beak sizes decreased whereas, given a decrease in maximum frequency (Table S2), the hypothesis predicts an increase in beak size. Note the average frequency bandwidth is approximately the same in *G. fortis* as it is in the much larger *G. magnirostris* (Table 1). Trill rate is hypothesized to be negatively associated with beak size. As predicted, the increase in trill rate of *G. fortis* songs from the 1990s to 2000s was associated with a decrease in beak size ($F_{(1,160)} = 13.681, P = 0.0003$). However, this is not likely to be a functional association as the trill rate in *G. fortis* is correlated so weakly with beak size ($r = -0.14, df = 1, 235, P = 0.031$); only 2% of the variation in trill rate is explained statistically by variation in beak size. A further complication is that beak size is positively correlated with body size ($r = 0.81, P < 0.0001$) and body size also decreased across the two decades ($F_{(1,117)} = 16.949, P < 0.0001$). To test the hypothesis that variation in beak size independent of body size variation differed between the decades in association with trill rate differences we compared residuals from a regression of beak size on body size (23) in the two periods. The residuals did not differ ($F_{(1,117)} = 1.7359, P = 0.1903$), which is not surprising given the strong correlation between beak and body size. The power of the test is extremely weak because there is little beak size variation left after body size variation has been statistically controlled.

We conclude that changes in *G. fortis* song did not arise from a character displacement shift in beak size (Fig. 6) caused by *G. magnirostris*. Changes in the songs of *G. fortis* and *G. scandens* reflect a change in behavior.

**Peak Shift as a Mechanism of Change**

*Males*. Changes in song might occur through a “peak shift” or learning-based bias, a common phenomenon well documented in a variety of species from insects to humans (45). During the process of discriminatory learning and production, an individual learns to respond to an exaggerated form of the desired signal in a direction away from a negative stimulus (33–35). The response can be expected to occur in two ways if there is a penalty to song transmission or in interactions with a heterospecific individual. A son, surrounded by singing heterospecifics during his sensitive period for learning or production of song, would be expected to learn and produce elements of conspecific song that were clearly distinct from sympatric heterotypic song. A female would be expected to respond to a male with a song that is an exaggerated form of a conspecific song and clearly distinct from heterospecific songs, resulting in a pattern of biased mating.

Sons learn songs early in life, mainly although not exclusively from their fathers. Consequently there is a strong relationship between the songs of sons and the songs of their fathers (36). The regression relationship (Fig. 7) is an estimate of cultural heritability, i.e., a cultural equivalent to the genetic heritability of quantitative genetics. A prediction of the peak shift hypothesis is that sons initially copy their fathers’ songs and then increase their trill rate in response to *G. magnirostris* songs. Following early learning trill rates did undergo small changes. For those individuals tested twice trill rate was faster at ages 4–14 y in the 1990s than at 2–6 y in the 1980s: in *G. fortis* (paired $t = 2.79, P = 0.021$) and *G. scandens* (paired $t = 3.12, P = 0.0168$; one outlier deleted). This trend occurred in all 10 *G. fortis* individuals (binomial $P = 0.002$) and in 8 of the 9 *G. scandens* individuals (binomial $P = 0.020$) that were recorded twice in different years. Because average song duration and interval between notes did not change (Methods), trill rate

<table>
<thead>
<tr>
<th>Species year</th>
<th>No. of notes $\times$ SEM</th>
<th>Internote interval, s $\times$ SEM</th>
<th>Trill rate, no. of notes/s $\times$ SEM</th>
<th>Bandwidth, Hz $\times$ SEM</th>
<th>Duration of song, s $\times$ SEM</th>
<th>Center frequency, Hz $\times$ SEM</th>
<th>N</th>
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<td><strong>G. fortis</strong></td>
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<td>1970</td>
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<td>0.05 ± 0.01</td>
<td>4.08 ± 0.25</td>
<td>3.1 ± 0.01</td>
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<td>3.6 ± 0.01</td>
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<td>0.04 ± 0.03</td>
<td>4.59 ± 0.13</td>
<td>3.1 ± 0.01</td>
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<td>0.03 ± 0.00</td>
<td>5.08 ± 0.15</td>
<td>3.1 ± 0.01</td>
<td>0.58 ± 0.02</td>
<td>3.5 ± 0.00</td>
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*Fig. 4.* Increase in trill rate across decades (mean $\pm$ SEM). Most SEs are too small to be shown. Sample sizes are in Table 1. *G. magnirostris* (green), *G. fortis* (red), and *G. scandens* (blue) rates are shown.
alone among the intercorrelated temporal variables increased. Thus sons (generation 1) may begin by singing a faithful copy of the song of their fathers (generation 0) but then later increase the trill rate, so that some of them will produce their own sons (generation 2) with a faster song than their father. In principle this process of song modification could continue repeatedly for several generations, resulting at each step in an increase in distance from the songs of G. magnirostris.

We tested the expectation that sons should sing faster songs on average than their fathers in successive generations by comparing the trill rate of 72 G. fortis fathers with their sons, having first averaged the trill rates of sons (one to six) of each father. In agreement with expectation, sons sang faster songs than their fathers (Wilcoxon matched pairs, $T = -506, P = 0.0031$). Sons sang faster songs in each of the 3 y of largest samples, 1983 ($n = 14$), 1987 ($n = 13$), and 1991 ($n = 31$): all $0.01 < P < 0.05$. G. scandens sons also sang faster songs than their fathers in the 3 y combined ($T = -131, P = 0.0377, n = 36$) and in 1983 alone ($T = -12, P = 0.0469, n = 6$), although not to a significant extent in 1987 ($T = -18, P = 0.1230, n = 11$) or 1991 ($T = -10, P = 0.5995, n = 13$). In all cases the difference from G. magnirostris songs increased (Fig. 5) as a result of G. fortis and G. scandens sons singing faster songs than their fathers (Fig. 7).

A second test of the peak shift hypothesis is possible by regression analysis. A regression of song characteristics of sons (generation (gen) 2) on fathers (gen 1) should have a higher intercept than a regression of fathers (gen 1) songs on the songs of their own fathers (gen 0). Our samples of the songs of grandsons of recorded fathers are too small, <10 for both G. fortis and G. scandens, to perform a sufficiently sensitive test.

**Females.** Females may amplify the peak shift by preferentially mating with males that sing an exaggerated form of a conspecific song that is clearly distinct from heterospecific (G. magnirostris) songs. A pattern of biased mating might give rise to relatively high reproductive success as a consequence. Although these ideas are plausible they are not supported by data. Seventy-one G. fortis males that hatched in 1991 and acquired mates did not differ in trill rate from 29 unsuccessful others from the same cohort that were tape recorded in the same year (1993) ($F_{1,98} = 1.990, P = 0.1609$). Lifetime reproductive success, measured as production of recruits to the breeding population after correcting for extrapair paternity, is known for members of the 1987 cohort. Nineteen G. fortis that produced recruits did not differ in trill rate from 16 that failed to produce a single recruit ($F_{1,33} = 0.346, P = 0.5605$). G. scandens show a similar pattern. Fifteen males of the 1991 cohort that bred did not differ from 10 that did not ($F_{1,23} = 0.481, P = 0.4948$), and six members of the 1987 cohort that produced one or more recruits did not differ from another 13 that failed to produce one ($F_{1,17} = 2.770, P = 0.1141$). All of the observed differences were in the expected direction away from

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**Fig. 5.** (Upper) Songs of G. fortis (red) and G. scandens (blue), in 1970–1989. All G. magnirostris songs (green) are included to indicate their relative position. (Lower) Songs of G. magnirostris, G. fortis, and G. scandens in decade 2000. Comparison of Lower with Upper shows the extent of movement of G. fortis and G. scandens songs away from G. magnirostris. The entire dataset was used in the principal components analyses. Variables were trill rate, song duration, and frequency bandwidth. For further details and eigenvector loadings see SI Methods, Fig. 6, and Table S5.

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**Fig. 6.** Mean beak size (PC1) of adult G. fortis (sexes combined) in the years 1973–2010. Vertical lines show 95% confidence intervals for the estimates of the means. Horizontal lines mark the upper and lower confidence limits for the mean in 1973 to illustrate subsequent change. Note the decrease in beak size in 2005 due to competition for food with the much larger G. magnirostris.

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**Fig. 7.** Cultural heritability of songs: copying of fathers’ songs by sons is reflected in significant regression relationships (broken line), G. fortis $F_{1,52} = 61.40, P < 0.0001; b = 0.704 \pm 0.090$; adjusted $R^2 = 0.540$. G. scandens $F_{1,27} = 20.63, P < 0.0001; b = 0.872 \pm 0.192$; adjusted $R^2 = 0.433$. The points above the slope of one (solid line) are solid circles indicating the sons that sang a faster trill than their father.
The changes in song features we have documented over several decades are minor in comparison with the differences among populations and species that arose in the course of the radiation of Darwin’s finches (10) in the last 2–3 My (3). Nevertheless they provide some insight into long-term diversification by supporting the idea that behavioral interactions among previously separated populations cause songs to diverge. In this way they throw light on what happens at a crucial stage in speciation when formerly sympatric living populations cause songs to diverge. In this way they throw light on what happens at a crucial stage in speciation when formerly sympatric living populations cause songs to diverge. In this way they throw light on what happens at a crucial stage in speciation when formerly sympatric living populations cause songs to diverge. In this way they throw light on what happens at a crucial stage in speciation when formerly sympatric living populations cause songs to diverge.
ation (1). How rapidly do genetic incompatibilities arise and what are the consequential effects on the evolution of mate preferences? If mutations responsible for genetic incompatibilities gradually accumulate when populations are spatially separated, hybrids formed during secondary contact will have relatively low fitness, in which case differences in mating signals and responses between the populations may be reinforced by selection, resulting in diminished interbreeding and gene exchange (12, 13, 53). Typically this stage is reached in birds after many millions of years (54), much longer than the entire period of the Darwin’s finch radiation. Not surprisingly, genetic incompatibilities have not been detected in Darwin’s finches, nor reinforcement found (3).

The premating barrier to interbreeding among the finches is an interesting composite of genetic and cultural factors learned through imprinting (1). When introgression occurs, the species exchange one component (genes) but not the other (song). In this respect they differ from some other species where hybrids sing elements of the songs of both of the parental species (43, 55). Thus in contrast to many other species of birds the defining feature of a population of Darwin’s finches is a learned cultural signal.

Sexual imprinting may enhance the potential for speciation, in two ways. First, sibs with similar preferences that are learned from an atypical father may mate with each other and initiate a new lineage, as observed experimentally in virdine finches (56) and seen in the evolution of a new lineage of finches on Daphne (47). Second, paradoxically, sexual imprinting may enhance speciation when the barrier leaks through copying of heterospecific song. Hybridization with minimal fitness loss, followed by backcrossing, can lead to an increase in genetic variation. In a new environment genetically unusual individuals from such a population may begin a lineage that becomes reproductively isolated from the parental populations. The fortisclandens lineage on Daphne, a new incipient species, provides an example of reproductive isolation and shows that speciation involving hybridization can be very fast when driven by learned behavior (47).

Methods

A Sony TCM 5000 analog machine with a Sennheiser directional microphone was used to record songs of 625 birds in the years 1984–2010. Quality of songs varied owing to wind, noise of seabirds, etc. We chose for analysis the clearest recording with minimal background noise for each bird. A previous analysis showed very little variation among recordings of the same bird on different years (36). Recordings were made in 1984, 1986, 1987, 1990–1993, 2007, and 2010. In addition some G. magnirostris were recorded in 2000 and 2005. For the purpose of comparing song characteristics across years we grouped the data into four decade groups: 1970s, 1980s, 1990s, and 2000s, according to the year in which a bird had hatched and not when it was recorded.

All measurements were made in Raven (57). The following spectral and temporal song features were measured: duration of the whole song as well as the first two notes (seconds), the interval between the first and second notes (internote interval, seconds), the maximum and minimum frequency (kHz) for calculating frequency bandwidth as the difference between them, and central frequency (the frequency that divides the song into two frequency regions of equal energy; kHz). The number of notes in each song was counted, and internote intervals were calculated as the number of notes divided by duration of the song. A note is defined as a continuous sound, appearing discretely on a spectrogram.

A possible stretching of stored tapes could introduce heterogeneous measurement errors across years. To examine this possibility we took advantage of measurements made from the spectrograms in the 1980s and 1990s. We had previously used a Kay Elements DSP model 5500 Sona-Graph for producing spectrograms (36) and measured temporal and frequency features of songs by hand. We first had to calibrate the Kay and Raven systems. We obtained help from Dean Hawthorne, Tim Krein, Dounan Hu, and Russ Charif at the Cornell University (Ithaca, NY) Laboratory of Ornithology to match Kay and Raven machine parameters. To match theKay setting of DC-8 kHz with a sampling rate of 20,480 Hz the Raven software was adjusted to record at 22,050 Hz, using a device setting of 44,100 Hz and an decimation factor of 2. The window was set to 6.25 ms or 138 samples, producing a 3-dB filter bandwidth of 208 Hz. A Hamming window was used with 50% overlap. We then compared song measurements of 88 G. fortis individuals made from Kay Sona-Graph spectrograms and from Raven software. They were almost identical. Mean duration of songs measured from the Kay spectrograms (mean ± SD, 0.5959 ± 0.082 s) is almost identical to the mean measured in Raven (0.5966 ± 0.081 s).

We performed a second test by comparing the songs of 10 G. fortis males and 9 G. scandens males recorded twice, 4–9 y apart. The first recordings were made in 1984–1987 and the second ones were made in 1990–1993. All of the birds except for one G. fortis individual were first recorded at a minimum age of 2 y, having had experience of at least one breeding season. There were no differences in song lengths between recordings, in either G. fortis (paired t = 1.16, P = 0.2725) or G. scandens (paired t = 1.42, P = 0.1926). There were also no differences in internote duration in either G. fortis (paired t = 0.15, P = 0.8890) or G. scandens (paired t = 0.63, P = 0.5447). However, in both species stall reproduction rate increased from the first to the second recordings, in G. fortis (paired t = 2.79, P = 0.021) and G. scandens (paired t = 3.12, P = 0.0168; one outlier deleted). This trend occurred in all 10 G. fortis individuals (binomial P = 0.002) and in 8 of the 9 G. scandens individuals (binomial P = 0.020). These results show there was no stretching of tapes in the intervening years, but a change in stall rate with age.

Two hundred nineteen G. fortis and 114 G. scandens were both tape recorded and measured morphologically when captured in mist nets (3). The measurement comprises body mass (weight, grams), wing length, tarsus length, beak length, beak depth, and beak width (all in millimeters). At the time finches were captured or when they were banded as nestlings, a single drop of blood was taken from the brachial vein and stored on EDTA-soaked filter paper in Drierite for later genetic (microsatellite) analysis (46). Hybrid backcrosses (n = 9) from G. fortis to G. scandens were identified from pedigree and confirmed genetically with assignment tests (58) applied to variation at 14 autosomal microsatellite loci. Their song characteristics were tested against the characteristics of contemporary G. scandens. None of the differences were significant by t tests (all P > 0.20); therefore the backcrosses were included in the G. scandens samples. G. magnirostris has never hybridized with either species on Daphne.

Principal components analyses were performed on song, body size, and beak measurements separately. Song principal component scores were generated in analyses of six song temporal parameters and, separately, four frequency parameters. The percentage of variance explained by the first two components varied from 80 to 90% (for further details and the eigenvector loadings see SI Methods and Tables S3 and S4). Body size principal component scores were generated from weight, wing length, and tarsus length, and beak size principal component scores were generated from beak length, beak depth, and beak width. The percentage of variance explained by the first two components was 94% in the body analysis and 97% in the beak analysis. In the body size analysis loadings were 0.59 (0.87) for length, 0.64 (0.65) for wing, and 0.57 (0.78) for tarsus length. In the beak analysis loadings were 0.41 (0.9) for length, 0.64 (0.31) for depth and 0.65 (0.27) for width. Two G. scandens (numbers 19527 and 19518) were statistical outliers, up to 5 SDs from means, and were removed from analyses.

Statistical analyses were performed in JMP 7.0 (59). For ANOVA we checked for equal variances with Levene’s tests and used a Welch ANOVA when variances were unequal. In multiple-group analyses we used a Kruskal–Wallis ANOVA when skewness was strong and Tukey–Kramer post hoc HSD tests at P = 0.05 or 0.01 to identify which groups differed significantly. All statistical tests were two-tailed.

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