Discrimination of geographical song variants by Darwin’s finches

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Geographical variation in the structure of mating signals can influence the processes of mate recognition and reproductive isolation. In Darwin’s finches of the Galápagos Islands, Ecuador, prior studies of geographical variation in vocal mating signals (songs) have focused on populations on small islands, on which there is presumably little opportunity for the evolution of within-island song variation or discrimination abilities. The situation on larger islands, however, is less clear. I characterized aspects of song variation and discrimination in the medium ground finch, *Geospiza fortis*, in the southeastern coastal region of the large island of Santa Cruz. Songs from two sites, Bahía Academia and El Garrapatero, separated by approximately 11 km, differed in mean minimum frequency and in a corresponding principal component factor, and could be distinguished with moderate success in discriminant function analysis. Territorial males at El Garrapatero showed strong discrimination of songs by site: in response to playback of local songs, birds showed higher rates of singing and flying, and significantly closer approaches to the playback speaker. Successful discrimination of songs by site, in the face of overlap in song structure between these sites, suggests that these birds rely on an exemplar-based mechanism of song perception. Moreover, the results support the hypothesis that birds in these populations are relatively philopatric, and thus suggest an opportunity for within-island evolutionary divergence.

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The evolutionary diversification of many animal taxa is facilitated by geographical variation in mating signals (West-Eberhard 1983; Wells & Henry 1998; Foster 1999). Divergence in mating signals among localities tends to limit interpopulation mating, and thus promotes reproductive isolation and within-species divergence (Ptacek 2000; Irwin et al. 2001; MacDougall-Shackleton & MacDougall-Shackleton 2001; Slabbekoorn & Smith 2002; Lachlan & Servedio 2004). The evolutionary impact of geographical variation in signal structure depends critically on patterns of signal perception and discrimination. If animals discriminate among geographical signal variations, then barriers to interpopulation gene flow can be introduced (Baker et al. 1987b; Wells & Henry 1998; Irwin 2000; Searcy et al. 2002). By contrast, a lack of discrimination among geographical signal variants fosters continued gene flow across localities. A comprehensive understanding of the influence of mating signals in reproductive isolation thus requires attention to both mating signal structure and signal discrimination abilities.

Key insights into the origins and consequences of geographical variation in mating signals have come from studies of birds (e.g. Marler & Tamura 1964; Baptista 1975; Slater 1986; Slabbekoorn & Smith 2002; Nelson & Soha 2004; reviewed by Edwards et al. 2005). Geographical variation in bird songs evolves as a result of numerous factors, including physical and genetic isolation among populations, limited dispersal, and divergent trajectories of sexual selection, natural selection or drift among populations (Nelson & Marler 1990; Podos et al. 2004; Podos & Warren, in press). In oscine songbirds, in which songs are learned, geographical variation in song structure also results from the accumulation of copying inaccuracies during cultural transmission, with distinct memes taking
root in partially isolated populations (Marler & Tamura 1964; Krebs & Kroodsma 1980; Slater 1989). Songbirds of some species discriminate local and foreign song types, suggesting opportunities for interpopulation genetic divergence (Baker et al. 1987b; Slabbekoorn & Smith 2002). I characterized aspects of geographical variation in song, and tested for song discrimination by site, in a species of Darwin’s finch from the Galápagos Islands. Darwin’s finches have been a central model system in studies of population evolution and dynamics (Grant 1999; Grant & Grant 2002c). Song is a key factor in territoriality, mate selection and hybridization in these birds (Grant & Grant 1997, 1998). In a series of playback studies, Ratcliffe & Grant (1985) and Grant & Grant (2002b) found that territorial male Geospiza finches responded more strongly to local song types than they did to conspecific song types from other islands. Greater responses to local songs were attributed to geographical isolation and a resulting lack of familiarity with foreign song types (Ratcliffe & Grant 1985). These studies were conducted on the relatively small islands of Daphne Major and South Plaza, and on the medium-sized islands of Genovesa and Pinta. All local song types were presumably familiar to most if not all island residents. Moreover, low interisland migration rates virtually assured that playback songs from other islands were unfamiliar. Together these interisland playback studies support an allopatric model of finch divergence and speciation (Grant & Grant 2002a, 2003).

The situation may be more complicated, however, for finch populations on larger Galápagos islands such as Isabela, Fernandina, San Cristóbal and Santa Cruz. These islands presumably could support geographical variation in song structure. Snodgrass & Heller (1904) described minor differences in Geospiza fortis songs among the Isabela Island sites of Tagus Cove, Tagus Hill and Iguana Cove. Bowman (1979) and Ratcliffe (1981) described elevational gradients in song frequency parameters for, respectively, Geospiza fuliginosa of Pinta Island and Camarhynchus parvulus of Santa Cruz Island. The presence of geographically distinct variants would suggest limited recent dispersal tendencies among the populations in question, whereas a lack of geographically distinct variants would suggest wide dispersal and comparatively wide song meme distribution (Kroodsma et al. 1985). Similarly, greater responses to local versus foreign song types would suggest successful discrimination abilities and biased familiarity with local types, and thus some degree of philopatry. These factors (dispersal tendencies, geographical variation in mating signals and signal discrimination abilities) bear upon the possibility of within-island divergence in Darwin’s finches, and thus merit closer attention.

The first goal of this study was to quantify possible structural variation in songs of the medium ground finch, G. fortis, between two sites, El Garrapatero and Bahia Academia, separated by approximately 11 km along the southeast coastal region of Santa Cruz Island. Birds at both sites express similar ranges of variation in beak and body size, yet differ in distributions of these parameters. In particular, birds at the Bahia Academia site show fairly continuous variation in beak and body size, whereas birds at the El Garrapatero site show bimodal variation in these parameters (Hendry et al. 2006; Huber & Podos 2006), with small- and large-male ‘morphs’ producing songs with distinctive acoustic features (Huber & Podos 2006). At both sites, variation in some song features corresponds with variation in beak morphology, in a manner consistent with a hypothesis of morphological constraints on song production and evolution (Podos 2001; Podos & Nowicki 2004; Huber & Podos 2006). I quantified differences in song features at both sites to determine whether and how songs vary between sites.

The second goal of this study was to test whether song differences that might occur between these sites are functionally significant. Towards this end, I conducted a playback experiment to test the responses of territorial males at one site, El Garrapatero, to playback of both local songs and songs recorded at Bahia Academia. The reciprocal experiment (i.e. playback of songs from both sites to males at Bahia Academia) would have been additionally instructive but was not feasible because of logistical limitations (see Methods). In simulated territorial intrusion tests, high levels of response to song playback are generally regarded as evidence that songs are functionally significant (i.e. meaningful to the birds in the context of social interactions; Falls 1992; Collins 2004). In particular, I tested an a priori hypothesis that birds would respond more strongly to local songs from their site (Lemon 1967; McGregor 1983; Kroodsma et al. 1985; Baker et al. 1987b). A positive result would implicate learning and philopatry as factors in the evolution of G. fortis song preferences and discrimination abilities (Kroodsma et al. 1985; MacDougall-Shackleton & MacDougall-Shackleton 2001), and would also suggest a possible opportunity for within-island divergence among populations. A lack of discrimination among geographical song variants, on the other hand, would be more difficult to interpret but could result from a combination of factors, including a lack of discernable site-specific vocal features, low philopatry, late learning of song preferences, historical inertia in song preferences or weak selection on precise song recognition abilities (Grant & Grant 2002a).

METHODS

Geographical Song Variation

Songs of birds were recorded during February and March 1999 and 2001–2004. All songs analysed in this study were from finches that had been captured and banded with unique combinations of one metal and three colour bands. Song recordings were made at two localities separated by approximately 11 km along the southeast coastal region of Santa Cruz Island: Bahia Academia (GPS coordinates 00°44′15″–30′ S, 90°18′05″–09″ W; N = 18 birds) and El Garrapatero (GPS coordinates 00°40′20″–41′20″ S, 90°13′10″–14′40″ W; N = 39 birds). Both of these sites are in the arid coastal zone and feature similar vegetation, including Opuntia echios, Acacia macracantha, Cordia lutea and Bursera graveolens. This habitat occurs continuously between the two sites, and G. fortis have been captured at regular intervals along a 4-km
Some of these features (Moran 2003). The statistical significance of each song feature and PC factor was assessed through comparisons (Bonferroni method). I did not conduct a similar test on the intersite comparison, based on crossed nature of data points in a feature-by-feature structural analysis of song exemplars to locality (Kachigan 1991), and to provide an overall view of the feasibility of classifying songs to locality based on the selected combination of acoustic features. The magnitude of any between-site song differences is presumably reflected in the accuracy with which songs can be classified correctly to site.

Third, I compared the fine-scale acoustic structure of song notes both within and between sites using a spectrogram cross-correlation method (Beeman 2002). This method calculates the structural similarity of any given pair of notes, in terms of note duration, absolute frequency and dynamic changes in frequency and amplitude (Clark et al. 1987). Notes are defined as continuous traces on spectrograms, separated by a minimum of 10 ms. Cross-correlation scores near zero indicate low similarity and suggest that the two notes being compared vary considerably in their fine-scale structure, whereas high cross-correlation scores indicate greater similarity in fine-scale structure between notes. Higher similarity scores within sites versus between sites would support the presence of geographical variation in note structure.

Cross-correlation analyses were conducted as follows. I extracted a single note from the highest-quality recordings of each of the 57 songs for this analysis (N = 18 at Bahia Academia; N = 39 at El Garrapatero). All songs in the sample consisted of two or more notes (Fig. 1). Across the sample, I chose the second note for analysis (in songs with three or more notes, the introductory note is often distinctive from remaining notes; the second note thus can be viewed as a more typical note in such songs; personal observation). Comparisons of spectrograms (FFT = 256 points, frequency range 0–10 kHz) of all note pairs across the sample generated a similarity matrix of 1596 cells, comprising 874 within-site comparisons and 722 between-site comparisons. Differences in note similarity scores for the three types of comparisons (within Bahia Academia, within El Garrapatero, and between sites) were assessed using one-way ANOVA (JMP 2002).

Numerous prior studies of vocal geographical variation, especially in species with small repertoire sizes, have examined the proportion of distinct song types produced at different localities (e.g. Slater et al. 1984; Baker & Thompson 1985). Such an approach is not feasible for G. fortis of Santa Cruz Island, however, because of the difficulty of reliably assigning songs to type. Some birds do indeed produce songs with similar overall structure. However, putative types often grade into each other, and many birds produce songs with unique features (e.g. Fig. 1). The scattered nature of data points in a feature-by-feature structural analysis (see Results, Fig. 2) supports this impression.

**Playback Study**

**Subjects**

Playback trials were conducted with nine male G. fortis at El Garrapatero, between 1 and 20 February 2004. Prior
observations indicated that territorial behaviour in ground finches at this site is associated most directly with defence of nests and nesting locations, and not with defence of food resources. Furthermore, prior observations suggested that territorial behaviour is strongest in males that are paired with a female. For these reasons, I chose as playback subjects males that had nests and that were observed to be actively paired with a female (i.e. engaged in courtship, mating, or parental behaviour). Confirmation of ‘paired’ status for playback subjects was based on observations of courtship feeding, back-and-forth calling, copulation and incubation (S. K. Huber & J. Podos, unpublished observations).

**Playback stimuli**

I selected a subset of nine songs of high quality from each locality that provided a good sample of the diversity of songs. In addition, I limited my selection of songs from El Garrapatero to those of only one morph, the large-male morph, to simplify the experimental design and to facilitate preparation of playback stimuli. Males might be
expected to respond more strongly to songs of their own morphs, given the presence of acoustic differences by morph in this population (Huber & Podos 2006), and our observation that males tend to engage in territorial disputes more often with males from their own morph than with males of the other morph. In the context of the hypothesis being tested here, which is that local songs should elicit greater responses than ‘foreign’ songs, any reduction in response of small males to the playback of local large-male songs would decrease the likelihood of detecting a site-specific bias in responsiveness. Thus, the decision to use songs from only one morph was conservative with respect to the hypothesis being tested. No subjects were tested with their own songs.

I prepared 18 playback tapes, one for each of the stimulus songs, using Signal 4.0 (Beeman 2002). Each tape consisted of 18 repetitions of the song played every 10 s for 3 min. Stimulus sets (one local song, one foreign song) were assigned at random to playback birds. Playback songs were standardized for amplitude and were high-pass filtered (above 1 kHz) to reduce background noise (Beeman 2002).

**Playback method**

I used a single-speaker design to test for discrimination by geographical locality (Searcy et al. 1997, 2002; Bard et al. 2002; Lovell & Lein 2004). All trial presentations were conducted between 0600 and 0900 hours. For each trial, I placed a portable playback speaker (Saul Mineroff SME-AFS), mounted on a tripod (~1.5 m tall), within the focal male’s territory, facing and about 6–8 m from the nest. The location of the speaker was kept constant over different playback trials for each bird. Songs were played from a Sony TCD-5M cassette recorder. The amplitude of playback songs was standardized using test tones at the beginning of each tape. Test tone amplitudes corresponded to the maximum amplitude of playback songs. During playback of test tones, the output volume of the playback speaker was adjusted to a standard value, as determined using a Radio Shack sound level meter. Flagging was placed at intervals of 3 and 6 m around the speaker to assist estimates of distances between the bird and the playback speaker.

Two to three observers were posted around the focal male’s territory. Each trial consisted of 1 min of preplayback observations, 3 min of playback and 3 min of post-playback. A stereo-cassette recorder was used to record focal bird activity. One channel of the recorder received input from an omnidirectional microphone/parabola, which directly recorded the bird’s vocalizations, and the second channel of the recorder received input from a lapel microphone, into which the author, assisted by the other observers, provided a running account of the focal bird’s activity. Observers aimed to minimize the impact of their presence on the behaviour of the focal birds. Behavioural patterns noted included flights, songs and displays. After each flight, the horizontal distance of the bird to the speaker was estimated and noted. Each subject’s closest distance to the speaker during overflights was also estimated, using flagging marks as a guide. At the end of each trial, estimated distances between the speaker and the perches used by the subject were confirmed or revised using a measuring tape.

Each bird was tested with one unique local song (El Garrapatero) and one unique ‘foreign’ song (Bahia Academia). The sample size for this study thus equalled the number of birds tested (Kroodsma et al. 2001). I was able to categorize each playback stimulus as local versus foreign by ear, because of experience with individual songs. The additional one or two observers were unfamiliar with the playback stimulus categories. Trials for each bird were separated by at least 1 day to limit possible carry-over effects such as habituation or sensitization to the playback method. The order of presentation of trials was balanced across the experiment.

**Data analysis**

Annotations of playback trials were digitized in the laboratory and printed out using the ‘strip chart’ function in Signal 4.0 (Beeman 2002). This method allowed the time course of each trial to be reconstructed with precision. The focal bird’s horizontal distance from the speaker...
was tabulated on a per-second basis across the entire trial. The onset of songs and flights was also noted.

I tallied the following four dependent variables as they occurred during preplayback, playback and postplayback segments of trials: (1) flight rate (flights/min), (2) song rate (songs/min), (3) closest approach to speaker, based only on perched locations, and (4) closest approach to speaker, based on perched locations and estimated distances during overflights of the speaker. For trial segments that did not include overflights, values for (3) and (4) were identical. Within the playback trial, I also calculated (5) latency (s) to first flight, and (6) latency (s) to first song. Overall response levels were calculated using principal component analysis, PCA (JMP 2002). Response parameters were compared between stimuli (local versus foreign), using Wilcoxon two-tailed matched-pairs signed-ranks tests, on pre-, post- and playback trial segments separately. To determine which specific response parameters varied by locality, I compared raw P values to P value thresholds corrected for multiple comparisons (Bonferroni method).

RESULTS

Geographical Song Variation

Songs from the two localities did not differ in trill rate, duration, maximum frequency or frequency bandwidth (Table 1), but songs from Bahia Academia had significantly lower minimum frequencies than those from El Garrapatero. Differences in song structure between localities were also evident in the PCA results; loadings for PC2 differed significantly between sites, according to Bonferroni corrected P values (Table 1). The probability of two of three PC factors differing across sites (uncorrected P values) by chance is approximately 0.0086 (Bernoulli equation; Moran 2003). PC1 was best represented by duration and maximum frequency, and PC2 by trill rate and minimum frequency (Table 2). Songs from the two localities showed considerable overlap in plots of PC1 × PC2 and of PC1 × PC3 (Fig. 2).

Discriminant function analyses (DFA) on the raw song variables classified 12 Bahia Academia songs correctly to site, but misclassified an additional six Bahia Academia songs to El Garrapatero. Furthermore, 33 El Garrapatero songs were correctly classified to site, but an additional six songs were misclassified to Bahia Academia. The DFA on PC factors was slightly less accurate, misclassifying the same songs as in the raw song variable DFA, but also misclassifying an additional two songs from Bahia Academia and an additional three songs from El Garrapatero.

Spectrogram cross-correlation scores differed significantly between the three types of comparisons (X ± SD: within Bahia Academia = 0.452 ± 0.102; within El Garrapatero = 0.404 ± 0.107; between sites = 0.402 ± 0.105; $F_{1,1593} = 16.393, P < 0.0001$). Post hoc tests revealed that cross-correlation scores were similar for comparisons within El Garrapatero and between sites (Tukey test: $P > 0.05$), but higher for comparisons within Bahia Academia (Tukey tests: $P < 0.001$).

Playback Study

During song playback, birds at El Garrapatero responded more strongly to local songs than to foreign songs from Bahia Academia, for all six raw response variables examined (Fig. 3). Birds flew significantly closer to the speaker during playback of local song than they did during playback of foreign song when overflights were included (Wilcoxon matched-pairs signed-ranks test: $T = -2.524, N = 9, uncorrected P = 0.008$, significance retained after Bonferroni correction), but not when overflights were excluded (perched distances: $T = -2.316, N = 9, uncorrected P = 0.016$). Differences in the birds’ responses to local and foreign songs for the other four variables showed trends in the same direction, although none was significant after Bonferroni correction. Birds flew more often during playback of local song ($T = 1.622, N = 9, uncorrected P = 0.055$), and showed a shorter latency to first flight after playback onset ($T = -1.365, N = 9, uncorrected P = 0.102$). Similar results were found for song rates ($T = 1.866, N = 9, uncorrected P = 0.039$) and latency to first song ($T = -1.521, N = 9, uncorrected P = 0.078$). The level of response to local versus foreign playback songs did not differ between small-morph subjects ($N = 5$, birds B, C, D, G and I; Fig. 1) and large-morph subjects ($N = 4$, birds A, E, F and H; Fig. 1; all Mann–Whitney U values < 1.15, all $P > 0.26$).

Table 1. Descriptive statistics (means ± SD) for acoustic features and principal components (PC1–PC3) of *G. fortis* song from two sites, El Garrapatero and Bahia Academia

<table>
<thead>
<tr>
<th>Acoustic feature</th>
<th>El Garrapatero (N=39)</th>
<th>Bahia Academia (N=18)</th>
<th>Mann–Whitney U score</th>
<th>Uncorrected P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trill rate (Hz)</td>
<td>2.29 ± 0.61</td>
<td>2.06 ± 0.60</td>
<td>-0.876</td>
<td>0.381</td>
</tr>
<tr>
<td>Duration (s)</td>
<td>0.84 ± 0.14</td>
<td>0.88 ± 0.24</td>
<td>-0.241</td>
<td>0.810</td>
</tr>
<tr>
<td>Minimum frequency (kHz)</td>
<td>2.21 ± 0.23</td>
<td>1.92 ± 0.24</td>
<td>-4.080</td>
<td>0.001*</td>
</tr>
<tr>
<td>Maximum frequency (kHz)</td>
<td>4.27 ± 1.02</td>
<td>4.63 ± 0.91</td>
<td>1.743</td>
<td>0.081</td>
</tr>
<tr>
<td>Frequency bandwidth (kHz)</td>
<td>2.07 ± 0.99</td>
<td>2.71 ± 1.04</td>
<td>2.258</td>
<td>0.024</td>
</tr>
<tr>
<td>PC1</td>
<td>-0.31 ± 1.32</td>
<td>0.66 ± 1.52</td>
<td>2.481</td>
<td>0.013</td>
</tr>
<tr>
<td>PC2</td>
<td>0.18 ± 0.13</td>
<td>-0.39 ± 0.76</td>
<td>-2.721</td>
<td>0.007*</td>
</tr>
<tr>
<td>PC3</td>
<td>0.14 ± 0.93</td>
<td>-0.31 ± 1.18</td>
<td>-1.039</td>
<td>0.299</td>
</tr>
</tbody>
</table>

*Statistically significant at $P < 0.05$ after Bonferroni correction.
Principal component analysis revealed that responses to playback stimuli differed significantly by site according to PC1 (X/C6 SD response: El Garrapatero stimuli = –0.932 ± 1.342; Bahia Academia stimuli = 0.932 ± 1.966; T = –2.666, N = 9, uncorrected P = 0.004), but not according to PC2 (El Garrapatero stimuli = 0.151 ± 0.890; Bahia Academia stimuli = –0.151 ± 1.255; T = 0.776, N = 9, uncorrected P = 0.496; Table 3). PC1 explained approximately 60% of the variation in raw response parameters. Loadings for PC1 were consistent with the finding that birds responded to local songs with lower minimum approach distances, higher rates of singing and flying, and shorter latencies to first songs and flights.

To examine whether subjects differed in their responses during pre- or postplayback of local and foreign songs, I compared subjects’ responses based on three dependent variables (song and flight rate, and perched minimum distances) that were expressed during pre- and postplayback trial segments. There were no statistical differences in subjects’ responses during preplayback (song rate: T = 0.943, N = 9, P = 0.438; flight rate: T = –0.184, N = 9, P = 0.876; perched minimum distance: T = 0.658, N = 9, P = 0.742) or postplayback (song rate: T = –0.421, N = 9, P = 0.742; flight rate: T = –0.425, N = 9, P = 0.688; perched minimum distance: T = –1.183, N = 9, P = 0.218).

**DISCUSSION**

Acoustic analyses revealed statistically significant differences in song structure by site (Table 1). Songs at Bahia Academia had significantly lower minimum frequency values than those at El Garrapatero (Table 1). This result mirrors the finding of Ratcliffe (1981) that minimum frequency varies along an altitudinal gradient on Pinta.
The percentage of variation across all songs explained by each PC factor is shown in parentheses.

Table 3. Principal component loadings (eigenvectors) for playback response parameters

<table>
<thead>
<tr>
<th>Response parameter</th>
<th>PC1 (59.79%)</th>
<th>PC2 (18.97%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum distance (perched)</td>
<td>0.466</td>
<td>-0.238</td>
</tr>
<tr>
<td>Minimum distance (with overflights)</td>
<td>0.466</td>
<td>-0.181</td>
</tr>
<tr>
<td>Flights/min</td>
<td>-0.388</td>
<td>0.469</td>
</tr>
<tr>
<td>Latency to first flight</td>
<td>0.376</td>
<td>-0.080</td>
</tr>
<tr>
<td>Songs/min</td>
<td>-0.379</td>
<td>-0.512</td>
</tr>
<tr>
<td>Latency to first song</td>
<td>0.334</td>
<td>0.650</td>
</tr>
</tbody>
</table>

Mechanism of Song Discrimination

A number of playback studies have shown that Darwin’s finches discriminate between conspecific and sympatric heterospecific songs (Lack 1945; Ratcliffe 1981; Bowman 1983; Ratcliffe & Grant 1985; Grant 1999). In one such study, Ratcliffe & Grant (1985) found that South Plaza Island *G. fortis* and *G. fuliginosa* expressed strong species-level discrimination in song playback, although songs of the two species were statistically indistinguishable. To explain this apparent paradox, Ratcliffe & Grant (1985, page 303) hypothesized that these two species successfully discriminate each other’s songs using ‘subtle (as yet unidentified) common cues’ (see also Grant 1999). A similar explanation might apply to the results of the present study. Songs from El Garrapatero and Bahia Academia may have one or more song features that are diagnostic but that were overlooked in the present analysis. (The only variable that differed statistically in the present study, minimum frequency, showed extensive overlap between sites and thus cannot be regarded as diagnostic.) According to this view, birds may learn one or more diagnostic features of different song stimulus classes, and subsequently categorize novel stimuli based on the presence or absence of such features. Success in geographical song discrimination would thus be contingent on the ability of birds to form and apply precise categorical distinctions (e.g. Harnad 1987).

An alternative mechanism of geographical song discrimination worth considering, alluded to by Ratcliffe & Grant (1985), is exemplar based (e.g. Trainor et al. 2004).
According to this view, birds may be able to recognize specific sets of song types that they hear and memorize, and perhaps only when these songs are heard during the sensory phase of song learning. Subsequent responses to songs, such as during territorial interactions, would then depend on the relative similarity of song stimuli to previously memorized song exemplars. Experience with particular song exemplars, such as in association with territorial disputes, may give familiar songs greater communicative salience. Recent experimental support for familiarity as a factor in geographical song discrimination comes from O’Loghlen & Beecher (1999), who found that female song sparrows, Melospiza melodia, displayed with greater intensity during playback of familiar than of unfamiliar song types, even when both kinds of song types were recorded from strangers. Given the extensive degrees of within-site variation and between-site overlap detected in the present study, local song discrimination seems most consistent with an exemplar-based model of song perception, even though discrete song type categories are not readily apparent.

Evolution of Geographical Variation in Song

Geographical variation in song, and the ability of birds to discriminate songs by locality, often evolves in songbird populations in which at least three criteria are met (Lemon 1975; Krebs & Kroodsma 1980; Nelson & Soha 2004). The first is that vocal learning occurs mainly through imitation rather than improvisation (e.g. Marler & Tamura 1964; Kroodsma 1996). Imitative learning enables particular song types to persist in given locations. The second criterion is that vocal learning is sufficiently plastic to allow for the occurrence and retention of minor inaccuracies in imitation (Marler & Peters 1988). ‘Mutations’ in song memes can thus be retained and integrated into vocal repertoires, and geographical variation generated. The third criterion is that song learning occurs in locations where birds eventually breed. Nelson (1999), for example, has shown that early song learning in white-crowned sparrows facilitates geographical song differentiation in sedentary populations, but may impede geographical divergence in song structure in populations that engage in long-distance migrations away from their natal grounds (Nelson 1998). Geographical variation in song also evolves in populations that learn their songs later in life on their breeding territories following dispersal (e.g. Rothstein & Fleischer 1987; Warren 2002).

The present description of vocal geographical variation and discrimination suggests that these three criteria are met to some extent in Santa Cruz G. fortis. In Darwin’s ground finches, as in most emberizids, song learning relies largely on imitation, and occurs early in life (Bowman 1983). The observation that young males normally learn their songs from their fathers is most likely explained by early learning (Grant & Grant 1996, 1997, 1998). An early window for vocal imitation in G. fortis, coupled with the present observation of geographical song variation and discrimination, suggests that these birds breed on their natal grounds (i.e. that they show some degree of philopatry). The actual degree to which G. fortis on southeastern Santa Cruz are philopatric is presently unknown, although three lines of evidence from our research program support a hypothesis of philopatry (S. Huber, A. Herrel, A. Hendry & J. Podos, unpublished data). First, only one of the approximately 1000 birds banded at the two study sites (~500 at each site) between 1999 and 2006 has been recaptured or resighted between localities. Second, the between-year recapture/resighting percentage of G. fortis at El Garrapatero, the site of our most intensive current field effort, is substantial (at least 40% from 2003 to 2004, and again from 2004 to 2005). Third, birds with known breeding sites have regularly been observed in the same or adjacent territories over subsequent years. This evidence refers most directly to breeding philopatry; less is known about natal philopatry for these populations.

Within-island Divergence?

Geographical variation in song, between-site song discrimination, early song learning, and indications of philopatry, together suggest an opportunity for local adaptation and divergence among G. fortis populations on southeastern Santa Cruz. The results of playback tests suggest that males dispersing to neighbouring localities enjoy fewer challenges from other males during territory acquisition and maintenance. However, females at these sites presumably also fail to respond to the songs of newcomers, because of a parallel failure to recognize, and thus prefer to mate with local males (e.g. Searcy et al. 2002). Female songbirds tend to show stronger discrimination of song variants than do males, perhaps as a result of their generally greater reproductive and parental investment (Ratcliffe & Otter 1996). Searcy et al. (2002), for example, showed that male song sparrows tend to be more catholic than females in the range of geographical song variants to which they respond. For Darwin’s finches, data from the responses of males to simulated territorial intrusions have been used to draw inferences about speciation, in part following the assumption that stringency in responses expressed by males will likely carry over, and perhaps be amplified, in females (Grant & Grant 2002a, b). Tests of geographical discrimination in females (e.g. using a copulation solicitation display assay; Baker et al. 1987a; Searcy 1992), would ultimately provide more direct insights into the role of geographical song variation as a potential deterrent in cross-population breeding.

Actual probabilities of interpopulation divergence depend on numerous additional factors not yet characterized. Interpopulation divergence may be counterbalanced by even low levels of gene flow between finch populations (Hendry et al. 2002; Emelianov et al. 2004; see also Petten et al. 2005). To test the possibility of local adaptive divergence, it might be best to start with an assessment of gene flow between such populations. Geographical differences in genetic structure, however, even if present and identified, may not necessarily persist over time. Studies of G. conirostris (large cactus finch) on Genovesa Island indicate that initial within-island divergence in Darwin’s finch morphology and song can be counteracted by subsequent
nonassortative mating and resulting interpopulation gene flow (reviewed by Grant & Grant 1979, 1989). The possibility of within-island divergence has thus been largely discounted for Darwin’s finches (Grant 1999; Grant & Grant 2003). A recent study by Kleindorfer et al. (2006), however, provides quantitative evidence for within-island morphological divergence in one of Darwin’s ground finches, *G. fuliginosa*, between upland and coastal sites on Santa Cruz Island.

The conclusions of the present playback study are also necessarily tempered by limits on the study design, which did not include the reciprocal experiment (playback of El Garrapatero songs at Bahia Academia), and which used only large-morph songs from El Garrapatero as playback stimuli. Future progress in evaluating probabilities of intersite divergence in Santa Cruz *G. fortis* will also require further studies on song variation, perception and discrimination at multiple sites (e.g. Grant & Grant 1989; Grant et al. 2004; Patten et al. 2004). Information about genetic diversity and gene flow would be well complemented by studies of dispersal and philopatry, insofar as high philopatry would suggest a greater opportunity for within-island divergence.

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