Acoustic discrimination of sympatric morphs in Darwin's finches: a behavioural mechanism for assortative mating?

Jeffrey Podos*

Department of Biology, University of Massachusetts, Amherst, MA 01003, USA

Populations with multiple morphological or behavioural types provide unique opportunities for studying the causes and consequences of evolutionary diversification. A population of the medium ground finch (*Geospiza fortis*) at El Garrapatero on Santa Cruz Island, Galápagos, features two beak size morphs. These morphs produce acoustically distinctive songs, are subject to disruptive selection and mate assortatively by morph. The main goal of the present study was to assess whether finches from this population are able to use song as a cue for morph discrimination. A secondary goal of this study was to evaluate whether birds from this population discriminate songs of their own locality versus another St Cruz locality, Borrero Bay, approximately 24 km to the NW. I presented territorial males with playback of songs of their own morph, of the other morph, and of males from Borrero Bay. Males responded more strongly to same-morph than to other-morph playbacks, showing significantly shorter latencies to flight, higher flight rates and closer approaches to the playback speaker. By contrast, I found only minor effects of locality on responsiveness. Evidence for morph discrimination via acoustic cues supports the hypothesis that song can serve as a behavioural mechanism for assortative mating and sympatric evolutionary divergence.

Keywords: Darwin's finches; song playback; song discrimination; geographical variation; assortative mating; ecological speciation

1. INTRODUCTION

Evolutionary divergence and speciation often result from adaptive processes, such as when related populations that occupy different environments undergo divergent patterns of natural selection (e.g. Mayr 1963; Schluter 2000). In a growing number of systems, natural selection is seen to drive assortative mating and reproductive isolation via correlated divergence of two sets of loci: morphological or behavioural traits under natural selection, and communication signals used in mate recognition and choice (Podos 2001; Nosil *et al.* 2005; Funk *et al.* 2006; Schluter 2009). For instance, in mosquito fishes (*Gambusia*), local populations have diverged in body and head morphology in response to distinct predation risks, with divergent morphology apparently causing limits on cross-morph matings (Langerhans *et al.* 2007). Accumulating evidence of this type provides support for models of ecological speciation (Schluter 2000, 2009; Gavrilets 2004).

Populations that feature two or more morphological modes provide unique opportunities to explore the relationships between adaptation, divergence and reproductive isolation (Smith & Skulason 1996; Orr & Smith 1998; Rundle & Nosil 2005). This is because different modes in a population often represent, on incipient scales, the axes of divergence that characterize species or genus-level variations (Nosil *et al.* 2009; Peccoud *et al.* 2009). The present study focuses on a population of medium ground finches, *Geospiza fortis*, at El Garrapatero, Santa Cruz Island, Galápagos. This finch population is strongly bimodal in beak morphology (Hendry *et al.* 2006), and viability selection differentially favours birds at the two modes (Hendry *et al.* 2009). Moreover, the population shows a strong pattern of assortative mating, with females tending to pair with males of their own morph, across both dry and rainy seasonal conditions (Huber *et al.* 2007). Consistent with this observation, the two morphs show evidence of significant, albeit minor, genetic divergence (Huber *et al.* 2007; de León *et al.* 2010). At present, it is unknown whether the two modes could ever become distinct species, especially given probable limits on their future divergence imposed by both cross-mode gene flow and ecological competition from congeners at the locality (Huber *et al.* 2007; Hendry *et al.* 2009). Irrespective, the processes that maintain this population's bimodality at present are presumably indicative of processes that facilitate species divergence.

An open topic of fundamental interest here concerns the behavioural mechanisms that drive assortative mating and reproductive isolation. Behavioural mechanisms of pre-mating isolation are especially influential factors in evolution and speciation in songbirds, which typically lack strong post-mating isolating barriers (Grant & Grant 1992). Prior studies on Galápagos finches suggest two behavioural mechanisms by which *G. fortis* might recognize birds of

*jpodos@bio.umass.edu

different morphs. First, birds might distinguish morphs using visual cues, especially the size and shape of the beak. Experimental studies by Lack (1947) and Ratcliffe & Grant (1983) indicate that beak size and shape can indeed be used in perceptual discrimination, at least at the species level. Second, birds might distinguish morphs by song. Songs of the two *G. fortis* morphs at El Garrapatero differ acoustically, with small-morph songs showing higher frequencies and broader frequency bandwidths (as expected given our understanding of the mechanics of song production and evolution; see Podos 1997, 2001; Huber & Podos 2006; Herrel *et al.* 2009). Playback studies have illustrated that Darwin's finches can discriminate songs across three levels: heterospecific versus conspecific songs (Lack 1947; Bowman 1983; Ratcliffe & Grant 1985); conspecific songs across different islands (Ratcliffe & Grant 1985; Grant & Grant 2002b); and conspecific songs across sites within a single island (Podos 2007). In sympatric or parapatric conditions, assortative mating might be enabled by both kinds of cues, e.g. vocal cues at long distance and visual cues at closer approach (Grant 1986; Grant & Grant 1989). However, natural observations of birds that present conflicting visual and morphological cues (by virtue of having been reared by heterospecific foster parents, and thus learning heterospecific song) suggest that vocal cues take precedence over visual cues, at least in typical conspecific–heterospecific discrimination challenges (Grant & Grant 1997, 1998).

The present study asks whether *G. fortis* morphs at El Garrapatero are able to discriminate among different morphs using song alone. While patterns of mating and reproductive isolation are often determined by the behaviour of females, the behavioural responses of females in the wild, including in Darwin's finches, are notoriously difficult to quantify (e.g. Ratcliffe 1981; J. Podos & S. K. Huber, unpublished data, 2002–2004). Inferences about song discrimination abilities in birds are thus typically drawn from the responses of territorial males to song playback (Irwin 2000; Grant & Grant 2002a,b; Slabbekoorn & Smith 2002). Operationally, discrimination abilities shown in males probably occur as well in females, and probably at magnified levels. This is because sexual selection typically acts to enhance females' ability to discriminate among potential mates, with errors in mate choice holding particularly significant costs in females as compared to males (Searcy & Brenowitz 1988; Ratcliffe & Otter 1996; Holland & Rice 1998).

In this study, songs of the two morphs were played to territorial males, in simulated territorial intrusions, and birds' responses to these challenges documented. My working prediction was that territorial males would respond more vigorously to playback of songs of their own morph, because intruder males of the same morph should more probably be perceived as competitors for both food and mates (Herrel *et al.* 2005; Huber *et al.* 2007; Hendry *et al.* 2009). As part of this study, I also ask whether males respond more strongly to local songs than to songs from another locality on the same island, Borrero Bay, following the finding that birds at El Garrapatero
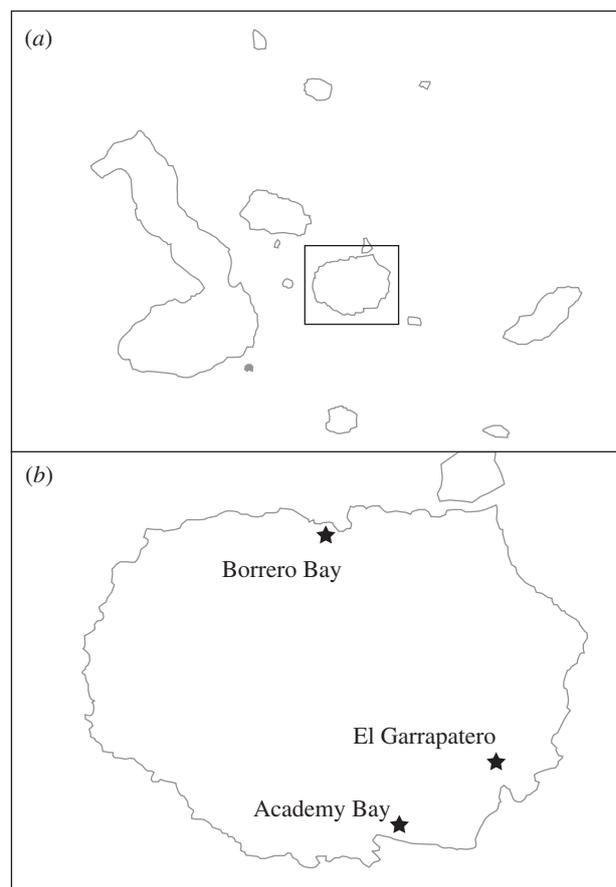

Figure 1. (a) Map of Galápagos Islands, with Santa Cruz Island in inset (b) showing the three localities referred to in this paper.

typically respond more strongly to local songs than to songs from another Santa Cruz Island locality, Academy Bay (Podos 2007; see figure 1 for localities).

2. MATERIAL AND METHODS
(a) *Subjects*
Playback trials were conducted with 10 male *G. fortis* at El Garrapatero, Santa Cruz Island, Galápagos Islands, Ecuador. Eight males were tested between 28 January and 13 February 2005, and two males were tested between 28 February and 4 March 2006. All males were colour-banded, their songs recorded and their territories mapped. All males showed evidence of being actively paired with a female, based on observations including courtship feeding, back-and-forth calling and copulation (as in Podos 2007).

(b) *Playback stimuli*
Playback stimuli were constructed from songs recorded at El Garrapatero and Borrero Bay, located approximately 24 km NW of El Garrapatero (figure 1). Recordings had been made during 2003–2005, using Sony TCD-5M stereo-cassette recorders and omnidirectional microphones (K6/ME62 or MKH20) mounted in 55.9 cm diameter Telinga Pro Universal parabolas. Birds recorded at El Garrapatero were banded and of known size, which allowed them to be classified as either 'small' or 'large' morph (Hendry *et al.* 2006; Huber *et al.* 2007). I chose high-quality

recordings of 30 songs, each from a different singer, for preparation of playback stimuli. Ten of these songs were of El Garrapatero small morphs, 10 of El Garrapatero large morphs and 10 of birds from Borrero Bay. Borrero birds recorded for this project were not banded or measured, so their morph designation could not be confirmed. However, large morphs are very rare in the Borrero Bay population (Hendry *et al.* 2006), and I thus assumed that all Borrero Bay singers were small-morphed. For each of the 30 stimulus songs, I prepared a playback sequence, consisting of 18 repetitions of each song played every 10 s for 3 min, as in Podos (2007). Playback songs were high-pass filtered above 1 kHz to reduce background noise, and were standardized for amplitude (Beeman 2002).

(c) *Playback method*

One small-morph playback sequence, one large-morph playback sequence and one 'foreign' playback sequence were assigned to each of the playback subjects. Each specific playback sequence was presented only once during the course of the entire experiment, so that the sample size for this study equalled the number of birds tested (Kroodsma *et al.* 2001). The three trials each bird received were presented on non-successive days, to limit possible carry-over effects such as habituation or sensitization to the playback method. Moreover, each bird received all three trials within a time frame of 6 days total, thus limiting possible biases in territorial responsiveness caused by seasonal variation in breeding or courting activities. The order of presentation of trials was balanced across the experiment, so as to minimize possible order effects on responses to different stimulus categories.

All trial presentations were conducted between 06.00 and 09.00 h. For each trial, a portable playback speaker (Saul Mineroff SME-AFS), mounted on a tripod (approx. 1.5 m tall), was placed within the focal male's territory, facing and about 6–8 m from the active nest. Speaker location was kept constant across the three trials for each subject. Playback sequences were presented from either a Sony TCD-5M cassette recorder (2005 trials) or from a compact disk player (2006 trials), the latter for which stimuli had been digitized at 44.1 kHz and burned onto CDs in uncompressed '.wav' format. The maximum amplitude of playback sequences was standardized to 90 dB at 1 m, using test tones and a Radio Shack sound level meter.

All trials were conducted on banded birds of known morph, and no subject was presented with playback of either his own song or that of an immediate (adjacent) neighbour. Seven study subjects were small morph, three large morph. Two or three observers were posted around the focal male's territory, and trials were initiated when birds came within approximately 25 m of the playback speaker. Each trial consisted of 1 min of pre-playback observations, 3 min of playback and 6 min of post-playback observations. Bird activity during trials was annotated using a portable digital recorder and lapel microphone. The vocal behaviour of focal birds was also recorded, using an omnidirectional microphone mounted in a parabola. Observers aimed to keep the focal bird in sight throughout the trial, while at the same time minimizing the impact of their presence on focal bird behaviour. Vegetation at El Garrapatero is typically sparse, thus facilitating behavioural observations. Behavioural patterns noted included flights, songs and displays. To be counted as a 'flight', a bird would have to move at least 1 m through the air; smaller movements ('hops') were not included in our flight tallies. After each flight, the horizontal distance between the bird and the playback speaker was estimated and noted. Sometimes, birds would respond by flying directly or near-directly over the playback speaker; birds' closest horizontal distance to the speaker during overflights was also estimated. At the end of each trial, estimated distances between the speaker and perches used by subjects were confirmed or revised using a measuring tape.

(d) *Data analysis*

Annotations of playback trials were printed using the 'strip chart' function of 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 all songs and flights was also noted to the nearest second. Six response parameters were tallied for the playback segment of each trial, as in Podos (2007): minimum distance (perched), minimum distance (including overflights), latency to first flight, flight rate (flights/min), latency to first song and song rate (songs/min). For trials in which birds did not respond with any flights or songs, latencies were coded as 180 s, the total time of the playback presentation. Three of these six response parameters (minimum perched distance, flight rate and song rate) were also tallied for pre-playback and post-playback trial segments. I excluded data from one of the test subjects, a large-morph bird, who neither flew nor sang during any of his three playback trials. Of the remaining nine test subjects, seven were small morph and two were large morph.

Three sets of statistical comparisons were conducted. First, I compared birds' responses to playback of 'same-morph' versus 'other-morph' stimuli. This test addresses the primary hypothesis of interest, i.e. whether birds can distinguish among morphs based on song alone.

Second, I compared birds' responses to playback of 'small-morph' versus 'large-morph' stimuli. This comparison represents a statistical control of the same- versus other-morph stimuli comparison, by asking whether all birds, as a group, respond more strongly to songs of one morph or the other. The songs of small and large morphs differ in vocal performance levels required for their production (Huber & Podos 2006), and territorial male songbirds of several species have been shown to respond differentially to intraspecific song variants with varying levels of vocal performance (Illes *et al.* 2006; Cramer & Price 2007; de Kort *et al.* 2009). This comparison also allowed

me to assess whether my uneven effective sample size, with only two large-morph birds included in the analysis of playback responses, would bias the outcome of the first comparison. In particular, if birds of both morphs turn out to respond more strongly to the songs of small morphs, my small-morph-biased sample could lead me to an erroneous inference of greater salience of same-morph stimuli.

Third, I compared birds' responses to playback of El Garrapatero songs ('local') versus playback of Borrero Bay songs ('foreign'). For this latter comparison, for the local stimulus condition, I only considered responses to small-morphed playback stimuli, i.e. I excluded from consideration birds' responses to large-morphed local playback songs. This is because the *G. fortis* population at Borrero Bay consists almost entirely of small-morphed birds (Hendry *et al.* 2006), and the comparison of El Garrapatero large-morph stimuli and Borrero Bay stimuli thus presumably differs in both morph and locality. Focusing on the small-morph stimuli here thus allows a more precise test of the possible impacts of locality on song discrimination.

All three sets of statistical comparisons were conducted using Wilcoxon two-tailed matched-pairs signed-ranks tests on playback trial segments (all response variables), both for individual response parameters and for overall response scores, the latter calculated for each condition using principal component analysis (PC1 and 2 only, JMP 2002). For comparisons 1 and 3, I also analysed how response parameters varied across condition for pre- and post-playback trial segments (minimum perched distance, flight rate and song rate response parameters only). Analysis of pre-playback trial segments aimed to detect possible initial biases in birds' overall responsiveness, and analysis of post-playback trial segments aimed to detect possible carry-over effects of song playback on subsequent behaviour.

3. RESULTS

(a) *Discrimination of songs by morph: same versus other*

Territorial males responded significantly more strongly to songs of their own morph than to songs of the other morph, according to four of the six raw response parameters (figure 2). Approaches to the speaker were significantly closer during playback of same-morph song stimuli than during playback of other-morph song stimuli (minimum distance perched, $t = 2.375$, $n = 9$, $p = 0.008$; minimum distance with overflights, $t = 2.371$, $n = 9$, $p = 0.008$). For same-morph stimuli, birds initiated flights, typically towards the speaker, within an average of only 10 s of playback onset. By contrast, flight latencies for playback of other-morph stimuli averaged nearly 90 s ($t = 2.429$, $n = 9$, $p = 0.006$). Birds also flew more than twice as often when hearing same-morph songs as opposed to other-morph songs ($t = 2.205$, $n = 9$, $p = 0.016$). No significant differences were detected for the two-song response parameters (song latency, $t = 1.183$, $n = 9$, $p > 0.1$; song rate, $t = 1.338$, $n = 9$, $p = 0.1$). Responsiveness also differed by morph according to PC1 (same morph = $-0.886 \pm$

1.122 s.d., different morph = 0.886 ± 1.267 s.d., $t = 2.666$, $n = 9$, $p = 0.004$), but not according to PC2 ($x \pm$ s.d. response: same morph = -0.196 ± 1.120 s.d., different morph = 0.196 ± 1.267 s.d., $t = 1.007$, $n = 9$, $p = 0.36$, see table 1 for PC loadings). PC1 loaded fairly strongly and evenly for the approach and flight parameters (table 1). Notably, all birds (nine of nine) showed higher PC1 response scores to same-versus other-morph stimuli. The four raw and one composite (PC1) response parameters that showed significant differences in same- versus other-morph stimuli classes all retained their significance after a sequential Bonferroni correction at $p < 0.05$.

No statistical differences were detected across same-morph and other-morph playback conditions in pre-playback behaviour (minimum approach perched, $t = 0.534$; flight rate, $t = 0.535$; song rate, $t = 0$, all $n = 9$, all $p > 0.3$). This indicates no bias among conditions in birds' baseline behaviour. However, significant differences among conditions were detected post-playback, with birds showing more active behaviour after having heard same-morph stimuli, in two of three measured response parameters: minimum approach distance, same morph = 4.33 ± 3.71 m (s.d.), other morph = 8.11 ± 4.57 m (s.d.); $t = 1.829$, $n = 9$, $p = 0.039$; and song rate, same morph = 3.59 ± 3.25 (s.d.) songs/min, other morph = 1.00 ± 1.93 (s.d.) songs/min; $t = 2.530$, $n = 9$, $p = 0.016$.

(b) *Discrimination of songs by morph: small versus large*

In contrast to the same- versus other-morph designation, the comparison of responses to small- versus large-morph stimuli yielded few significant results. Most notably, neither PC1 nor PC2 response parameters differed by morph size (PC1, small morph = -0.564 ± 1.68 s.d., large morph = 0.564 ± 1.948 s.d., $t = 1.481$, $n = 9$, $p = 0.068$; PC2, small morph = -0.277 ± 1.018 s.d., large morph = 0.196 ± 1.267 s.d., $t = 1.244$, $n = 9$, $p = 0.11$). Uncorrected p-values were significant for three of six raw response parameters (minimum approach perched, $t = 1.781$, $n = 9$, $p < 0.04$; flight rate, $t = -2.375$, $n = 9$; $p < 0.01$; song latency, $t = -1.690$, $n = 9$, $p < 0.05$). None of these values retained significance, however, after a sequential Bonferroni correction at $p < 0.05$.

(c) *Discrimination of songs by locality*

Results from this comparison are shown in table 2. Birds flew more often in response to local songs than in response to 'foreign' (Borrero Bay) songs. The remaining five raw response parameters did not differ by condition. Overall responsiveness did not differ by locality according to either PC1 or PC2 (table 2, PC1, $n = 9$, $p = 0.57$; PC2, $n = 9$, $p = 0.25$; see table 3 for PC loadings). The difference by condition for flight rate did not retain significance once a Bonferroni correction was applied.

No statistical differences were detected by locality in pre-playback behaviour (minimum approach perched, $t = 0.210$; flight rate, $t = 1.342$; song rate, $t = 1.206$, all $n = 9$, all $p > 0.15$), indicating no bias by condition in birds' baseline behaviour. Similarly,
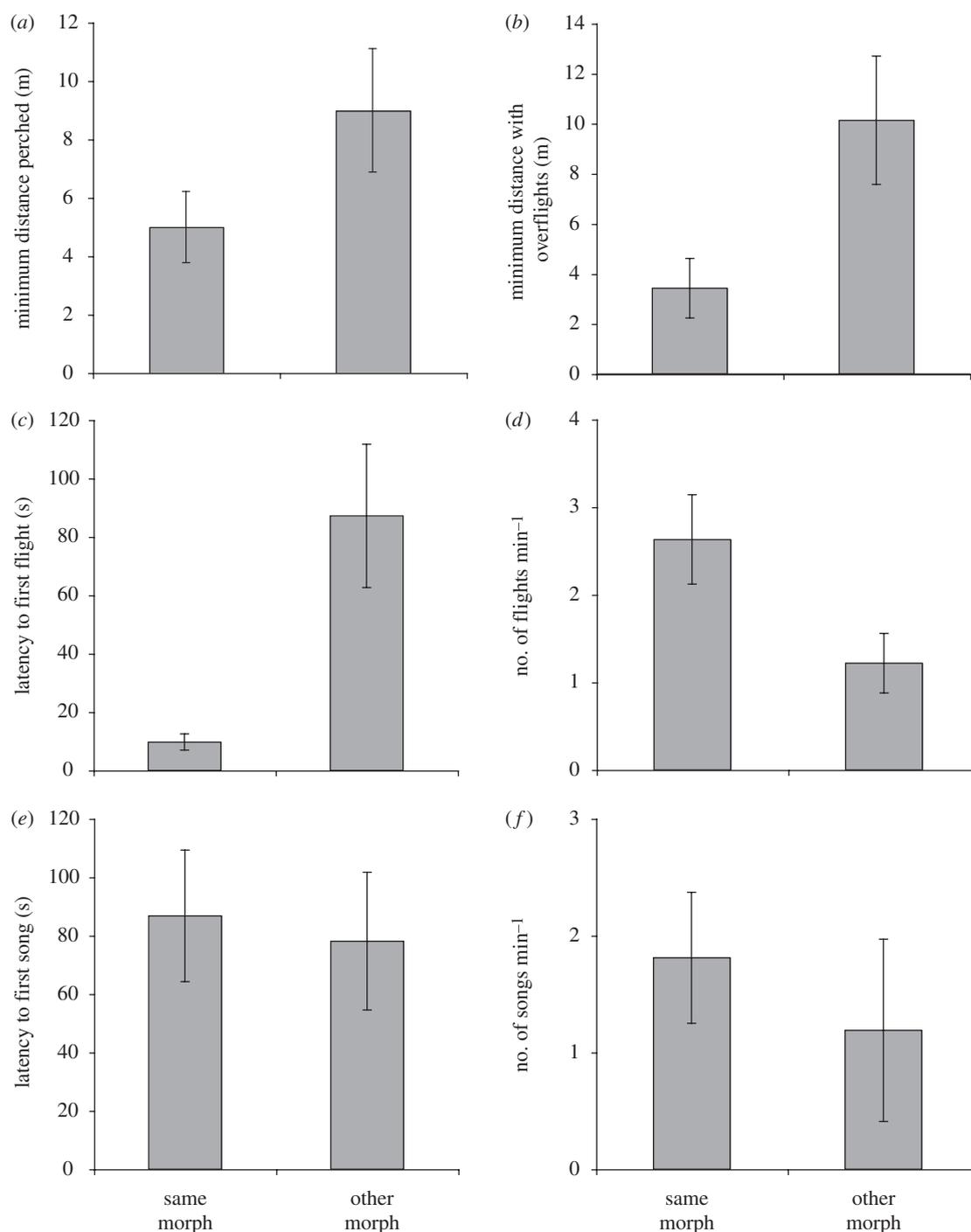
Figure 2. Responses of territorial male *G. fortis* ($n = 9$) at El Garrapatero to playback to songs of the same morph and of the other morph, across six raw response parameters (a–f). Error bars show standard error. Statistically significant differences by condition were found for the distance and flight parameters (see text).

no statistically significant differences were detected by locality in post-playback behaviour (minimum approach perched, $t = 1.420$; flight rate, $t = 0.968$; song rate, $t = 0.140$, all $n = 9$, all $p > 0.05$).

4. DISCUSSION

(a) *Discrimination of songs by morph*

The main result of this study is that, in the focal bimodal population of *G. fortis*, males discriminated song stimuli by morph, reacting with greater vigour to playback of songs from their own morph than to playback of songs from the other morph (figure 2). This

discrimination was evident both in an overall response score (PC1) and for individual flight and approach parameters. Birds hearing songs of their own morph typically flew in towards the speaker almost immediately upon song playback, in about 10 s after playback onset. By contrast, birds hearing other-morph songs typically lingered on their perches, for an average of nearly a minute and a half after playback onset. Similarly, birds hearing same-morph songs flew significantly more often, and approached the playback speaker more closely. Carry-over effects were also evident, in that birds stayed closer to the speaker and sang at higher rates after having heard same-morph songs,

Table 1. Principal component loadings of response parameters from the same-morph versus other-morph test. Percentage of variation across all parameters explained by each PC factor is shown in parentheses.

response parameter	PC1 (57.5%)	PC2 (23.1%)
minimum distance (perched)	0.483	0.168
minimum distance (with overflights)	0.520	0.115
latency to first flight	0.461	0.178
flight rate	−0.427	−0.126
latency to first song	0.284	−0.602
song rate	−0.142	0.741

Table 3. Principal component loadings of response parameters from the locality test. Percentage of variation across all parameters explained by each PC factor is shown in parentheses.

response parameter	PC1 (50.6%)	PC2 (29.8%)
minimum distance (perched)	0.449	0.393
minimum distance (with overflights)	0.470	0.336
latency to first flight	0.488	−0.021
flight rate	−0.385	−0.099
latency to first song	0.349	−0.552
song rate	−0.264	0.646

Table 2. Responses of territorial males to playback of local songs and Borrero Bay songs, compared across six response parameters and two composite response measures (PC1 and PC2). Wilcoxon t-values are shown. *$p < 0.01$, otherwise p-values are n.s. Comparable data for the morph discrimination are presented in figure 2 and in the text.

response parameter	small-morph songs	foreign songs	t-value
minimum distance perched (m)	9.51 ± 7.44	10.27 ± 7.04	0.350
minimum distance with overflights (m)	4.71 ± 5.41	7 ± 2.77	0.837
latency to first flight (s)	33.4 ± 51.5	57.29 ± 66.59	1.007
flight rate (flights/min)	2.67 ± 1.48	1.62 ± 1.11	2.446*
latency to first song (s)	102 ± 62.1	80.8 ± 70.5	0.980
song rate (songs/min)	1.74 ± 1.71	2.26 ± 2.05	0.341
PC1	−0.323 ± 1.78	0.323 ± 1.75	0.652
PC2	−0.349 ± 0.96	0.349 ± 1.61	1.244

as compared with their behaviour after presentation of other-morph songs. The conclusion that birds discriminate same- versus other-song morphs is reinforced by two additional findings: (i) all birds (nine of nine) responded more strongly to their own morph type during song playback, according to PC1; and (ii) the pooled sample of playback subjects, which included both morphs, did not discriminate as a group between small- and large-morph stimulus classes, according to all raw and composite response measures.

The finding that birds respond more strongly to playback of same-morph stimuli, as compared with other-morph stimuli, implies that similarly morphed intruders present territorial males with the more salient threat. Intruders of the same morph might represent special threats to territory holders for at least two reasons. First, territory holders and intruders of the same morph may compete directly for food resources, given the observation that finches with divergent beak morphologies tend to specialize on

distinct food types (Abbott et al. 1977; Schluter & Grant 1984; Schluter 2000; L. F. de León, A. P. Hendry & J. Podos 2004–2007, unpublished data). Second, males of the same morph may compete directly in mate attraction, given the observation that females from this population typically mate to type (Huber et al. 2007). These expectations, and the morph playback results presented here, are consistent with observations of finches of this population in natural interactions, in which birds of different morphs are seen to nest in close proximity and to maintain territories with minimal conflict, whereas birds of similar morph are often seen to engage in territorial disputes and chases (Huber et al. 2007; J. Podos & S. K. Huber 2006, unpublished data).

While my results indicate that birds can discriminate morphs by song, they do not reveal the extent to which song is actually used for morph discrimination in natural interactions. Perceptual discriminations might also rely on visual cues such as body size, beak size and beak shape, which also differ noticeably by morph (Huber & Podos 2006; Foster et al. 2008). Expectations about the relative contributions of song and morphology to morph discrimination might be guided by observational studies from Daphne Major, which suggest that song cues normally take precedence over visual cues, at least in cross-species discrimination (Grant & Grant 1997, 1998). In brief, the Daphne Major studies followed *Geospiza* nestlings that were reared by heterospecific parents. Because finches learn their songs from their social fathers (Grant & Grant 1996), male finches raised by heterospecific parents learned to sing heterospecific song, and upon maturation were found to attract heterospecific mates, in spite of the visual cues available in their genetically inherited morphology (Grant & Grant 1997, 1998). For *G. fortis* morphs at El Garrapatero, documenting the relative salience of vocal and visual cues will require additional field observations, particularly to evaluate whether individuals ever learn heteromorph songs, and how cross-morph song learning (if it ever occurs) influences patterns of mating.

Irrespective of the relative salience of vocal versus visual cues, both typically provide redundant, complementary information about morph identity, especially given the proximate influence of beak form and function on vocal mating signal structure (Podos

2001; Huber & Podos 2006; Herrel *et al.* 2009). In the El Garrapatero *G. fortis* population, large morphs are expected to face greater challenges in vocal performance, and these birds indeed show lower values for song frequency bandwidth, an index of vocal performance (Podos 1997, 2001; Huber & Podos 2006; Herrel *et al.* 2009). As the two *G. fortis* morphs undergo adaptive evolution in beak morphology (Hendry *et al.* 2009), the structure of their vocal signals should thus be displaced along performance-based axes, as an incidental consequence (Podos 2001; Podos & Nowicki 2004). Redundancy across multiple modalities, and any ability of birds to associate cues across multiple modalities (e.g. Matyjasiak 2005), would presumably facilitate morph discrimination.

The informational redundancy of vocal and visual signals, together with the intermorph discrimination abilities demonstrated here, offers a mechanistic basis for observed patterns of assortative mating (Huber *et al.* 2007). The ability of birds to identify morphs by song over long distances, and then to confirm identities at close range using visual cues, should help birds to avoid cross-morph matings. Strong assortative mating, in turn, could power intermorph reproductive isolation and genetic divergence (Huber *et al.* 2007). A parallel argument had been made previously for the *Geospiza conirostris* population on Isla Genovesa (Grant & Grant 1979), which in initial years of study showed vocal morphs and some bimodality in morphology. However, initial observed patterns of assortative mating broke down over subsequent years (Grant & Grant 1989). For the El Garrapatero *G. fortis* population, evolutionary trajectories of the two morphs will ultimately be determined not only by patterns of assortative mating, and by the persistence of the mechanisms that underlie it (i.e. signal redundancy, morph discrimination abilities), but also by factors such as patterns of demography, survival selection and cross-species hybridization, all of which are shaped by variable and sometimes unpredictable ecological parameters including food availability and the size and distribution of sympatric congener populations (Huber *et al.* 2007; Grant & Grant 2008*a*,*b*; Hendry *et al.* 2009). Future work might examine how fission facilitated by morph discrimination abilities is offset by processes facilitating intermorph fusion (Grant & Grant 2008*a*,*b*; Hendry *et al.* 2009). Future work should also aim to characterize perceptual differences among male and female finches, in terms of their capacities for morph discrimination. Females typically are expected to evolve the ability to make especially stringent discriminations, because of the high potential costs to females in choosing sub-standard mates (Trivers 1972). This is in contrast to the situation in males, in which males responding to songs of the wrong morph or species would face only fleeting costs of territorial conflict. I thus predict that the discrimination abilities shown here for males will extrapolate to, and probably be magnified in females (Searcy & Brenowitz 1988; Ratcliffe & Otter 1996).

(b) *Discrimination of songs by locality*

The secondary result of this study is that birds failed to discriminate songs by locality, responding with equal vigour to songs from El Garrapatero and Borrero Bay. A significant difference in responsiveness was found according to only one response score (flight rate), and this difference no longer held once *p*-values were corrected for multiple comparisons (table 1). These data contrast with those from a prior study of male *G. fortis* in the same El Garrapatero population, which asked whether birds discriminate local songs from those from another locality 11 km to the SW, Academy Bay (Podos 2007). Birds in that study were observed to respond more strongly to local songs, according to a first PC axis and approach distance. Interpreting the conflicting outcomes of these results is partly impeded by differences across the two studies, which emerged in spite of the fact that the identical playback protocol was used in both. More specifically, absolute responses to local songs differed in the two studies, with birds in the Podos (2007) study showing stronger reactions to local songs in all response parameters. To illustrate: minimum distance approached to local songs averaged 7 m in Podos (2007) yet 9.51 m here; and flight rate to local songs averaged 2.89 in Podos (2007) yet 2.67 here (for further details, compare table 2, column 2, with fig. 3 of Podos 2007; note that the upper label for songs/min in that figure should have read '3' instead of '9'). Differences in birds' responsiveness across the two studies might derive from interannual differences in breeding ecology, e.g. associated with drought or rainfall, or from random differences in the overall responsiveness of individual subjects chosen. The weaker baseline responsiveness of birds in the present study may have diminished the probability of finding an effect of locality on song discrimination.

Nevertheless, it is possible that birds indeed show differential responses to songs from different foreign localities. Based on distance alone, we might have expected a trend in reverse of that observed, with more stringent discriminations against songs from the more distant foreign population (Borrero Bay). Further resolution of discrimination abilities by geographical locality would ideally include repeated presentation designs of songs from multiple foreign localities, analysis of acoustic variation across localities (as in Podos 2007) and reciprocal experiments in which birds from multiple localities are presented with exemplars of each others' songs, and relative responsiveness evaluated.

To conclude, it is instructive to note that discrimination abilities by morph far exceed discrimination abilities by locality, as shown both here and in Podos (2007). Perhaps, the ability to use song to discriminate morphs is superior because birds grow up with the opportunity to associate both classes of song types to known individuals (of either morph), who may impose varying degrees of threat. By contrast, birds may have more difficulty assessing the relative threat of territorial intruders from foreign localities, because of unfamiliarity with foreign song types and characteristics of the birds who sing them. Irrespective of the perceptual mechanisms involved, greater discrimination by morph than by locality is consistent with our recent finding that, in Santa Cruz *G. fortis*, genetic

isolation is markedly higher between morphs than across localities (de León *et al.* 2010).

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