



Asymmetric discrimination of geographical variation in song in a migratory passerine

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Variation in sexual signals across populations is a common phenomenon, and most research to date has found that individuals discriminate against nonlocal signals. However, the strength of nonlocal signal discrimination can vary asymmetrically across populations, a possibility that has received less attention. Such asymmetries can be due to recognition errors in some populations, variation across populations in the 'quality' of local individuals, such that populations with high-quality individuals respond more strongly, and/or variation in the perception of signals, with signals from some populations being perceived as more intimidating or aggressive. Here, we examine song differences and male responsiveness to local and nonlocal songs in two populations of the black-throated blue warbler, *Dendroica caerulescens*, and also explore possible reasons for geographical variation in responsiveness. In the northern population, male responses were stronger to local songs than to nonlocal songs, whereas in the southern population, male responses to local and nonlocal songs did not differ. Overall responsiveness did not differ between the populations, and songs from one population were not responded to more weakly (or strongly) across populations. Overall, our results fit best with a model of asymmetric recognition error. Asymmetries in nonlocal responsiveness across populations may be a common phenomenon, and can have profound effects on patterns of dispersal, mate choice and gene flow.

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Variation in sexual signals across populations of the same species is a common and taxonomically widespread phenomenon, occurring in organisms ranging from *Drosophila* (e.g. Routto et al. 2007) to marine mammals (e.g. Riesch et al. 2006). The functional significance of this variation has received considerable attention, and a common finding has been that nonlocal signals are responded to weakly relative to local signals (e.g. frogs: Boul et al. 2007; fish: Feulner et al. 2009; insects: Gray 2005; birds: Uy et al. 2009; snails: Johannesson et al. 2008; elephants: O'Connell-Rodwell et al. 2007), although some studies have found that nonlocal signals are responded to more strongly (e.g. Baker 1982; Balaban 1988). Such discrimination of geographical variation in sexual signals can potentially arise very

quickly (e.g. Derryberry 2007), can have important consequences for patterns of dispersal and gene flow (e.g. Irwin et al. 2001), and should play a central role in the process of speciation (Ritchie 2007).

However, geographical patterns in the response to divergent sexual signals can be complicated, as the differences between responses to local versus nonlocal signals can vary asymmetrically across populations (e.g. Coyne et al. 2002; Hoskin et al. 2005; Dolman 2008; Tinghitella & Zuk 2009). The nuances of variation in discrimination across populations remain poorly understood, at least in part because many studies have examined responsiveness in only a single population (e.g. Searcy et al. 1997; Gray & Cade 2000; Grant & Grant 2002; Podos 2007; Reynolds & Fitzpatrick 2007; Guerra & Ron 2008; Johannesson et al. 2008; Feulner et al. 2009; Uy et al. 2009).

For a male responding to a territorial challenge, variation in responsiveness can be driven by (1) variation in self-assessment (i.e. based on the quality of the respondent), and/or (2) variation in opponent assessment (i.e. based on the quality of the challenger), or (3) variation in the degree to which the challenger is perceived as a conspecific (Ryan & Rand 1993). In each of these scenarios,

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responsiveness should be dictated by the relationship between the costs and benefits of responding to a challenger. In the case of self-assessment, high-quality individuals are more willing (or able) to pay the costs of responding to challengers, and should respond more strongly to all challengers. In the case of opponent assessment, predicted responsiveness could go either of two ways. On one hand, some opponents may be perceived as more intimidating than others, such that the cost of response is greater for intimidating opponents (Arnott & Elwood 2009). In this case, responses should be stronger to weak challengers, and weaker to strong challengers. On the other hand, some opponents may be perceived as more reliably indicating their willingness to escalate a physical confrontation (Searcy & Beecher 2009). In this case, it would be more beneficial for an individual to respond to challengers that are honestly signalling aggression, and we would expect stronger responses to more aggressive signals and weaker responses to less aggressive signals. When potential challengers are perceived as nonconspecific, the costs of response outweigh the benefits (as the challenger does not represent a real competitor for mates or other resources), and thus, response strength should decrease with increasing dissimilarity of the challenger's signal (e.g. Nelson 1988; Dabelsteen & Pedersen 1992).

These game theoretical models suggest that, within the geographical range that signals are perceived as local (i.e. within the geographical range of the local dialect), variation in responsiveness should be primarily due to geographical variation in the quality of local individuals, and/or variation in the perceived quality of nonlocal signals. Asymmetries in nonlocal discrimination should thus be due to (1) variation in the quality of local individuals, with some populations having a higher-quality individuals (and overall responsiveness) than others, or (2) variation in how the signals from specific populations are perceived, such that signals from 'high-quality' populations should be responded to more strongly (or more weakly) across populations. Beyond the range of the local 'dialect', responsiveness to nonlocal signals should decrease with increasing dissimilarity to the local signal. For example, it is common for

individuals to respond less, if at all, to heterospecific sexual signals (e.g. Ryan & Rand 1993; reviewed for birdsong in Catchpole & Slater 2008). In these cases, asymmetries in nonlocal discrimination should arise because individuals in some populations, but not others, show reduced recognition of nonlocal signals.

The black-throated blue warbler, *Dendroica caerulescens*, is a migratory bird species that shows subtle yet important phenotypic differences across northern and southern populations, including darker plumage in the south (Grus et al. 2009), and a north–south gradient in migratory behaviour, with birds from southern populations wintering in the eastern Caribbean and those from the north wintering in the western Caribbean (Rubenstein et al. 2002). These differences in plumage signals and migratory behaviour apparently arose recently and likely in the face of ongoing gene flow, as all extant populations appear to have derived from a recent population expansion from a single glacial refugium (Davis et al. 2006; Grus et al. 2009). In this study, we were interested in determining whether any differences in song exist between the north and the south, whether males discriminate against nonlocal songs, whether such discrimination is symmetric or asymmetric, and whether variation in responses is due to variation in assessment strategy or species recognition. If discrimination is symmetric, then we would expect nonlocal signals to be responded to more weakly in both populations (Fig. 1a). If discrimination is asymmetric, asymmetric self-assessment predicts increased responsiveness (to both local and nonlocal songs) in one population versus the other (Fig. 1b), and asymmetric opponent assessment predicts that songs from one population should be perceived differently (either responded to more weakly or more strongly) in both populations (Fig. 1c). Finally, if asymmetries are due to recognition errors, nonlocal signals should be responded to less strongly than local signals in one population, but the difference in how local and nonlocal signals are perceived (and the difference in how they are responded to) should be reduced in a second population (Fig. 1d).

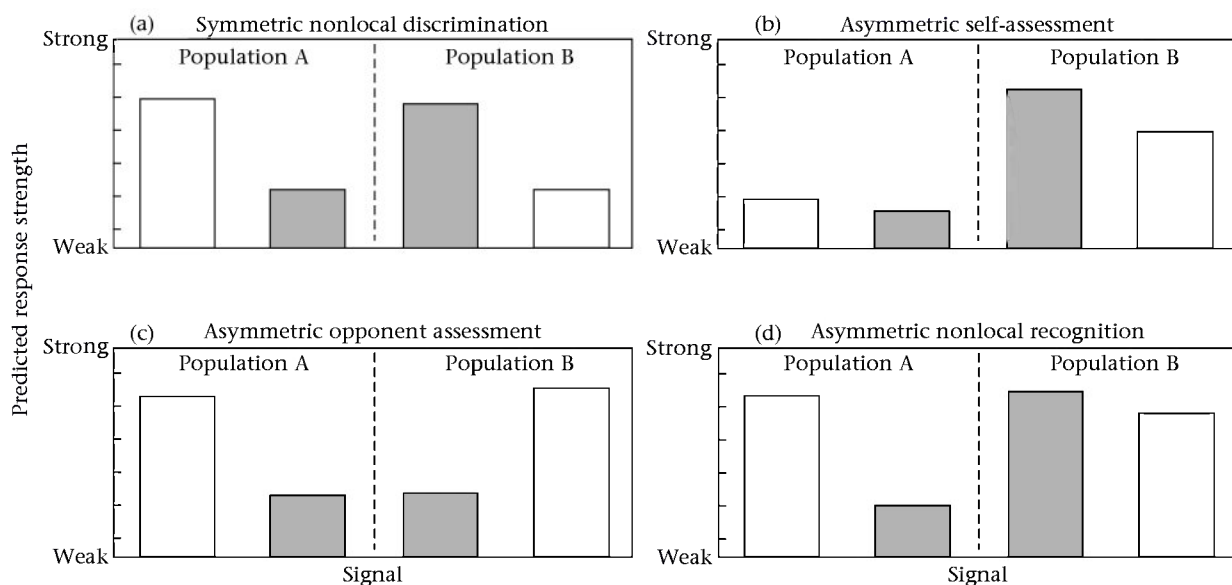


Figure 1. Models for geographical variation in nonlocal discrimination. (a) Symmetric: nonlocal signals are responded to uniformly across populations. Asymmetries could arise due to geographical variation in (b) self-assessment, when individuals in some populations respond more strongly to all stimuli, (c) opponent assessment, when individuals from some populations are perceived to be of higher quality, and are responded to more strongly (or weakly) across populations, or (d) nonlocal recognition, when the difference in how local and nonlocal signals are perceived (and responded to) is reduced in some populations. □: signals from population A; ■: signals from population B.

METHODS

Study Species

Black-throated blue warblers are small (ca. 10 g) insectivorous passerines that overwinter in the Caribbean and breed in relatively undisturbed northern hardwood forests in the eastern United States and Canada (Holmes et al. 2005). In North Carolina, U.S.A., migrants begin arriving on breeding territories in late April and initiate first broods in early May (Stodola et al. 2009), whereas in New Hampshire, U.S.A., migrants begin arriving in early May and initiate first broods by early June (Holmes et al. 2005). Adult black-throated blue warblers have very high levels of site fidelity (Silleet & Holmes 2002), but juveniles show very low natal philopatry (Holmes et al. 2005). A feather isotope analysis conducted on first-year birds indicated that most juvenile dispersal occurs within the range of the local isotopic signature, yet some individuals have isotopic signatures indicating very large (nearly range-wide) dispersal distances (M. G. Betts, N. L. Rodenhouse & R. T. Holmes, unpublished data). Although the ontogeny of song learning in the black-throated blue warbler is unknown, of 60 first-year males recorded in New Hampshire during 2004–2007, only one showed evidence of changing his song to something other than his song when he first arrived, and no returning adult male has ever changed his song (G. J. Colbeck, unpublished data). Thus, crystallized song probably develops before individuals arrive on territory in their first breeding season.

Song Variation

We recorded songs from colour-banded males in the summers of 2006 and 2007 from one population in the north (New Hampshire, Hubbard Brook Experimental Forest, 43.9°N, 71.6°W) and one population in the south (North Carolina, Coweeta Hydrological Laboratory, 35.0°N, 83.4°W). We recorded songs as uncompressed, wav files with a Marantz PMD 670 digital recorder and a Sennheiser ME66 shotgun microphone with a K6 power supply. We used one song with low background noise from each of 40 males in the north, and 38 males in the south, and we used RAVEN PRO 1.3 (Charif et al. 2008) as well as Sound Analysis Pro (Tchernichovski et al. 2000) to generate measurements (see *Supplementary Material, Table S1*, for details). We compared all individual measurements between the two sampling sites with an ANOVA. To account for correlations among song measures, we also conducted principal components analyses to extract orthogonal descriptors of the song components, and we examined differences in principal component scores between the populations with an ANOVA.

Playback Experiments

We conducted playback experiments in the spring of 2007 on colour-banded males in late April for the southern population (North Carolina, $N = 22$ males) and late May for the northern population (New Hampshire, $N = 20$ males). All experiments were conducted during the fertile period (building and/or laying) of the test subject's female. We scouted each subject the day before the first playback trial to determine the rough area of his territory, and playback arenas were set up in the approximate centre of that territory. We constructed playback loops for each locality from spectrograms with low levels of background noise recorded from individuals with no possible previous interactions with our test subjects, either from previous years or from different study plots. These constraints made it difficult to find a large number of exemplars, and we settled on six different playback exemplars from each population (Fig. 2). The specific song used in an individual

playback experiment was chosen randomly. Each playback loop consisted of 3 min of silence (the preplayback period) followed by 5 min of song, with a song rate of one song every 7 s, because this approximates the song rate during a typical song bout (G. J. Colbeck, unpublished data). Each subject received two playbacks, one with a northern song stimulus (chosen randomly from songs in Fig. 2a–f) and one with a southern song stimulus (chosen randomly from songs in Fig. 2g–l). The trial order (northern versus southern song first) was determined randomly, and the two trials were conducted 2 days apart to minimize habituation. We conducted all experiments before 1000 hours to avoid a potential midday decrease in territorial motivation. During the experiments, we played playback loops (.wav files) from an iPod through an SME-AFS portable field speaker (Saul Mineroff Electronics) placed on the ground, with fixed output levels.

Playback arenas consisted of measured and flagged (one flag in each of the four cardinal directions) 4 m and 8 m radius circles around the playback speaker. We waited until the subject was seen or heard near the arena before starting the playback trial. Once the experiment had started, the observer (G. J. Colbeck for all experiments) moved to the edge of the arena and vocally dictated the behaviour of the subject into a digital recorder, in particular noting all flights, the distance of the male from the speaker after a flight, and 'dives over speaker'. The observer dictated distances outside the arena as 'beyond 8 m' and horizontal distances within the 8 m arena were estimated in approximate 2 m intervals. In addition, the observer recorded the subject male's singing behaviour (using the same recording equipment described above) so that song rate could be calculated after the experiment. To facilitate discrimination of the subject's song from that of the playback loop (as well as other males singing in the distance), the observer vocally dictated 'song' each time the subject sang. It was rare that a nonfocal male (e.g. a male from a neighbouring territory) would approach the playback arena, but in those cases we abandoned the experiment.

Data collected from each trial included length of time from initiation of playback to when the male entered the arena, closest approach to speaker, average distance from speaker, total time in arena, number of perch changes, number of dives over speaker, change in song rate in response to initiation of playback, and change in song rate in response to cessation of playback. We calculated average distance by multiplying each perch distance by the length of time the bird was at that perch, summing across all perch changes, and dividing by the total amount of time. We used a distance value of 8 for individuals that ventured outside of the 8 m arena during the experiment. We tested for an effect of treatment (local versus nonlocal) on each response variable with a nested MANOVA, in which each of the six playback exemplars (Fig. 2) were nested within local or nonlocal. Since different response variables were likely to be correlated with each other, we also included all variables in a principal components analysis (PCA) to extract orthogonal measures of male response (keeping those with eigenvalues equal to or greater than one), and the effect of treatment on each PC variable was tested with a nested ANOVA. Because between-individual variation can contribute to large variance across playbacks, and obscure the detection of differences in responsiveness (Podós 2007), we also used a paired *t* test, in which a subject's response to the local stimulus was paired to their response to the nonlocal stimulus. We tested for the overall effects of site (north versus south), playback order and local versus nonlocal upon response strength (PC1) with a MANOVA.

Ethical Note

All experiments in this study were approved by the Washington State University Animal Care and Use Committee (IACUC permit

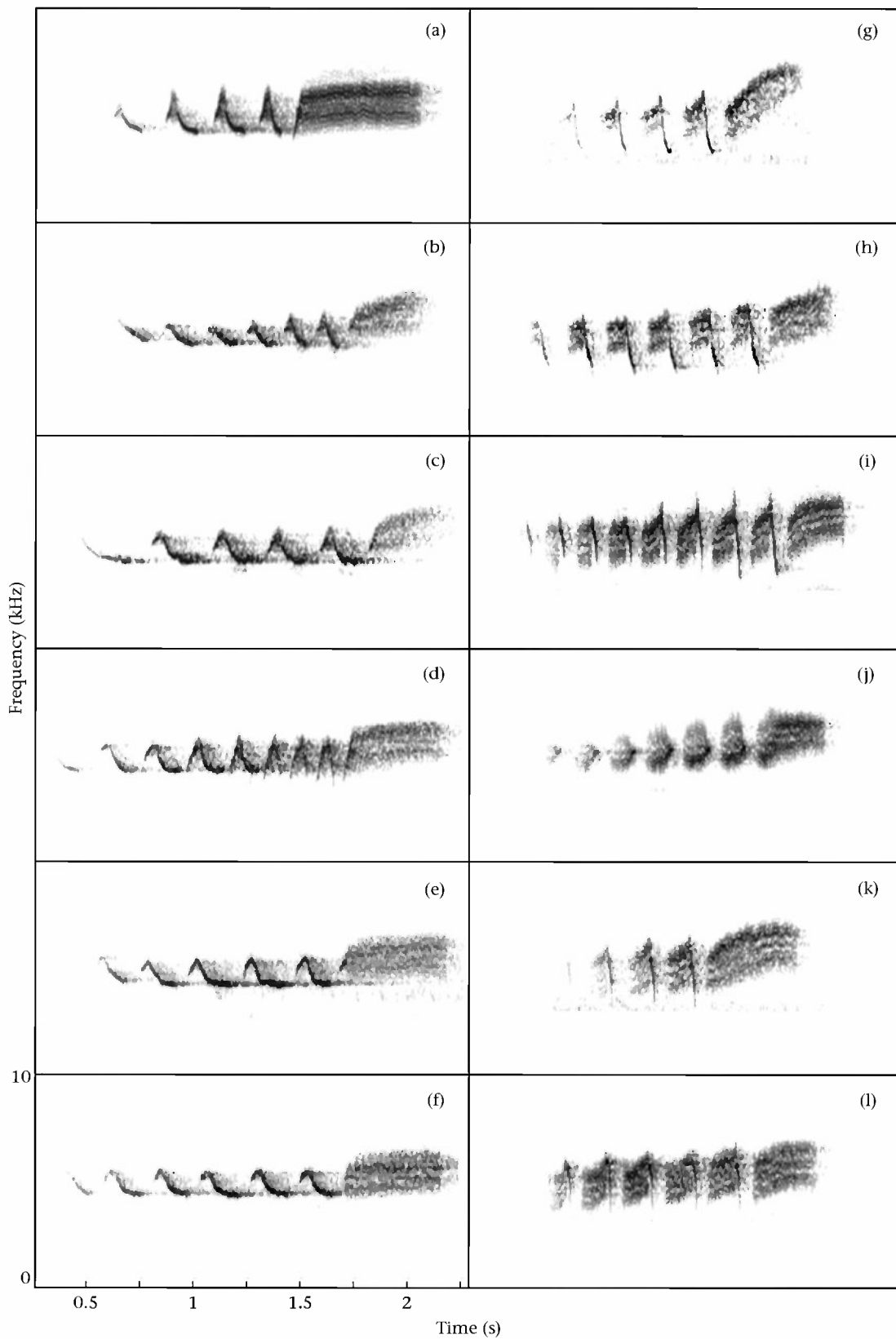


Figure 2. Example spectrograms of black-throated blue warbler songs recorded from six males at the northern study site (a–f) and six males at the southern study site (g–l). These were the songs used as stimuli in the playback experiments.

number 3067). Five minutes of playback was chosen as a minimum amount of time necessary to elicit behavioural responses. The behaviour and reproductive success of all subjects was monitored for the entirety of the breeding season and we are confident that playback experiments had no detectable lasting effects.

RESULTS

Song Variation

In an ANOVA on all PC variables with eigenvalues larger than one (Table S2), only PC1 explained a significant amount of variation between the sample sites ($\bar{X} \pm \text{SD}$: north = 2.45 ± 2.05 ; south = -2.33 ± 1.47 ; ANOVA: $F_{76,3,14} = 141.66$, $P < 0.001$), indicating that measures such as frequency bandwidth and mean entropy were larger in the south than in the north (Fig. 3). PC2 also tended to be larger in the north than in the south, suggesting that trill rate and related measures may extend to much higher rates in the north than in the south (Fig. 3), but this difference was not significant ($\bar{X} \pm \text{SD}$: north = 0.08 ± 2.76 ; south = -0.08 ± 1.36 ; ANOVA: $F_{76,4,81} = 0.10$, $P = 0.752$).

Playback Experiments

Subjects in the northern population responded much more strongly to the local song type than to the nonlocal song type (Table 1, Fig. 4). In overall tests of univariate models in the nested MANOVA, the response variables 'dives over speaker' ($F_{11,28} = 2.51$, $P = 0.024$) and 'total time within 8 m' ($F_{11,28} = 4.00$, $P = 0.001$) were significantly greater for the local stimulus than for the nonlocal stimulus, whereas all other variables were not significantly different. In contrast, subjects in the southern population showed no significant differences in responsiveness to local versus nonlocal signals (Table 1, Fig. 4). Analyses with a paired t test provided identical results, with 'dives over speaker' and 'total time within 8 m' significantly larger in response to local treatment in the north, but no significant differences in the south (data not shown). Therefore, variance in responsiveness between subjects contributed minimally, if at all, to our nonsignificant results.

The principal components analysis on response variables for these experiments revealed three principal component variables

with eigenvalues larger than one (Table 2). PC1 was loaded most heavily by negative values of variables such as 'closest approach' and by positive values of variables such as 'total time within 8 m'. Therefore, a positive score of PC1 indicates a subject that approached closely and spent more time near the speaker. PC2 was loaded most heavily by positive values of 'song rate difference – postplayback' and negative values of 'song rate difference – playback'. Therefore, a positive score of PC2 indicates that the subject responded by decreasing song rate during the experiment, and increasing song rate after the experiment. The loading scores for PC3 were much lower and more difficult to interpret, but the signs and relative magnitudes of the variables were opposite those of PC1.

For experiments conducted in the north, response values of PC1 were larger for local stimuli compared to nonlocal stimuli, whereas PCs 2 and 3 did not differ significantly between the stimuli (Fig. 4; $\bar{X} \pm \text{SE}$: PC1: local: 1.22 ± 0.58 ; nonlocal: -1.09 ± 0.37 ; $F_1 = 18.31$, $P = 0.002$; PC2: local: -0.17 ± 0.32 ; nonlocal: -0.38 ± 0.17 ; $F_1 = 0.37$, $P = 0.559$; PC3: local: 0.33 ± 0.20 ; nonlocal: -0.15 ± 0.26 ; $F_1 = 18.31$, $P = 0.076$). These results indicate that northern males approached more closely and spent more time near the speaker in response to the local stimulus than to the nonlocal stimulus, but their singing behaviour did not differ between stimulus types. For experiments conducted in the south, none of the principal components differed significantly between local and nonlocal stimuli (Fig. 4; $\bar{X} \pm \text{SE}$: PC1: local: -0.22 ± 0.46 ; nonlocal: -0.39 ± 0.37 ; $F_1 = 0.18$, $P = 0.680$; PC2: local: 0.11 ± 0.20 ; nonlocal: 0.32 ± 0.23 ; $F_1 = 0.72$, $P = 0.415$; PC3: local: 0.36 ± 0.16 ; nonlocal: 0.43 ± 0.15 ; $F_1 = 0.07$, $P = 0.793$).

For all experiments, there was no effect of site (north versus south) on PC1 response score ($F_{1,2,87} = 0.68$, $P = 0.413$), nor was there an effect of playback order ($F_{1,0,77} = 0.181$, $P = 0.157$). The only significant effects were for local versus nonlocal ($F_{1,31,93} = 7.52$, $P = 0.008$) and the interaction between site and local versus nonlocal ($F_{1,24,1} = 5.68$, $P = 0.02$).

DISCUSSION

In this study we found strong differences in song between a northern and a southern population, accompanied by stronger responsiveness to local songs in the northern population but no difference in responsiveness between local and nonlocal songs in the southern population. We detected no differences in overall responsiveness between the populations, and thus, our findings do not support a model of asymmetric self-assessment (see Fig. 1b). Similarly, a model of asymmetric opponent assessment was not supported: responses to northern and southern songs did not differ in the south, meaning that songs from one population were not responded to more strongly (or weakly) in both populations (see Fig. 1c). Instead, our findings best support a model of nonlocal recognition error: males in the north appear to recognize southern song less well than local song, whereas males in the south perceive northern song more equally to their own song.

Recognition Error

One of the main mechanisms through which geographical variation in sexual signals is thought to evolve involves the local acquisition of signals, followed by limited dispersal between populations (e.g. Endler & Houde 1995). This process is thought to be particularly common for learned song in songbirds (reviewed in Podos & Warren 2007). Similarly, recognition errors associated with foreign signals should arise when dispersal is limited between populations, and individuals in each population have limited opportunities to perceive or learn foreign signals (e.g. Wright &

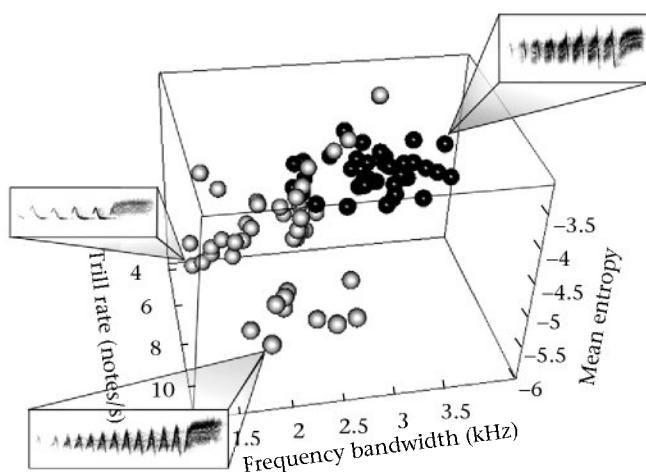


Figure 3. Scatterplot of black-throated blue warbler songs from the north (grey spheres) and the south (black spheres) along three axes of song variation. Songs from the south have large measures of frequency bandwidth and entropy, while songs from the north tend to be the opposite. In the north, a minority of individuals have trill rates that are up to three times faster than those in the south.

Table 1
Comparisons of responses by black-throated blue warblers to local and nonlocal stimuli

Response variable	North				South			
	Stimulus		Between-stimulus comparison		Stimulus		Between-stimulus comparison	
	Local	Nonlocal	$F_{11,28}$	P	Local	Nonlocal	$F_{11,32}$	P
Number of perch changes	8.65±8.35	2.05±3.33	1.34	0.255	4.27±5.1	2.64±3.58	0.43	0.929
Total time within 8 m (s)	158.5±149.29	28.7±42.65	4.00	0.001	86.91±109.47	61.14±96.03	0.50	0.890
Dives over speaker	2.7±2.8	0.15±0.37	2.51	0.024	0.86±1.36	0.64±1.0	0.60	0.813
Song rate difference (postplayback) (notes/s)	0.72±1.8	−0.69±1.58	1.97	0.072	0.59±1.96	0.46±2.3	1.08	0.406
Song rate difference (playback) (notes/s)	0.8±2.14	0.6±1.88	0.58	0.829	0.77±1.36	0.25±1.68	1.47	0.191
Average distance (m)	5.41±1.9	7.32±1.79	1.34	0.255	6.72±1.87	7.0±1.98	0.42	0.938
Time of first approach (s)	82.45±287.09	255.75±287.07	0.58	0.826	192.77±300.17	176.73±265.52	0.45	0.922
Closest approach (m)	3.1±3.58	6.7±2.77	1.48	0.193	5.27±3.52	6.09±3.29	0.34	0.970

Values (northern population: $N = 20$; southern population: $N = 22$) are means ± SD. F and P values come from overall tests of univariate models in the nested MANOVA.

Dorin 2001). For the black-throated blue warbler, this could indicate that individuals in the north have had limited opportunity to perceive and/or learn southern song, but the same is not true in the south. One way that this could happen is asymmetrical exposure during migration. For example, if other populations in the north sing songs similar to those in New Hampshire, and individuals from those populations interact with individuals from North Carolina during migration (while males are still actively singing), then North Carolina males may have had previous exposure to northern song. However, if individuals from New Hampshire never interact with males that sing North Carolina-like songs (e.g. because of variation in migration routes amongst northern populations), then they would not have any previous exposure to southern song. Previous research indicates a north/south gradient in migratory behaviour (Rubenstein et al. 2002), so populations south of New Hampshire (that sing songs similar to New Hampshire) may have more opportunities to interact with southern populations. Correspondingly, 'northern' singing populations further to the south should show increased responsiveness to southern song. This possibility will require further knowledge of the extent of variation in song, and the extent of variation in responsiveness across populations.

Given the pronounced differences in song between populations, it is perhaps not surprising that individuals in the north responded less strongly to southern song. What is more surprising is that individuals in the south responded equally to both song types. A lack of differential responsiveness in the face of strong differences in song between populations has also been reported by Nelson (1998) for Gambel's white crowned sparrow, *Zonotrichia leucophrys gambelii*. One of the more plausible explanations for Nelson's findings is that individuals are capable of differentiating amongst different parts of a song, and while the latter portion of sparrow songs differs considerably between populations, song features in

the beginning are similar between populations. Correspondingly, sparrows attend primarily to the beginnings of songs (Nelson 1998). Black-throated blue warbler song variation follows a similar pattern, with the strongest differences between populations in introductory notes, and more subtle differences for terminal trills (Table S1). Thus, it is possible that individuals in the north attend more to introductory notes, while individuals in the south attend more to terminal buzzes. This possibility will require further investigation.

Variation in Assessment Strategy

The possibility of recognition errors between populations is more plausible if each population occupies a distinct dialect (discrete variation in signals between adjacent populations; Podos & Warren 2007). Despite strong differences in song between populations, we are not in a position to determine whether the northern and southern populations represent different dialects, as there is a continuum of unsampled populations that connect them. If black-throated blue warbler song variation is gradual across populations, we might have expected variation in responsiveness to follow models of contest escalation, in which individuals are expected to respond strongly to 'weak' opponents and weakly to 'strong' opponents (Arnott & Elwood 2009), or strongly to 'aggressive' opponents (Searcy & Beecher 2009). The fact that males in the north responded less strongly to nonlocal songs could suggest that they perceived southern songs as 'more threatening' than local songs. This may be a plausible scenario in that southern songs have larger frequency bandwidths, including lower 'low frequencies', than northern songs, and the subjects of our experiments would have been able to discern northern from southern

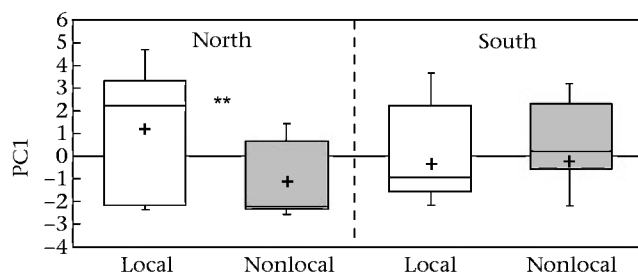


Figure 4. Box and whisker plots of PC1 scores from playback experiments. □: responses to local stimuli; ▒: responses to nonlocal stimuli. Plots include the mean (+), median, interquartile range and full range. ** $P = 0.002$.

Table 2
Correlations between initial playback response variables and principal components

Response variable	PC 1	PC 2	PC 3
	(53.87%)	(14.84%)	(11.28%)
Number of perch changes	0.886	−0.120	−0.283
Total time within 8 m	0.881	−0.069	−0.238
Dives over speaker	0.769	−0.070	−0.169
Song rate difference (postplayback)	0.233	0.744	−0.157
Song rate difference (playback)	0.198	−0.743	−0.154
Average distance	−0.922	−0.098	−0.147
Time of first approach	−0.927	−0.028	−0.132
Closest approach	− 0.944	−0.067	0.298

The variable that loaded most heavily onto each principal component is in bold. The percentage of response variation explained by each principal component is given in parentheses.

songs based on frequency. In the event that low-frequency calls are associated with body size, and body size is associated with quality (e.g. Bee et al. 2000; Price et al. 2006; Vannoni & McElligott 2008), northern males may have perceived the southern calls as higher quality, and therefore, may have approached them more reluctantly. However, if this were the case, we would have also expected males in the south to respond more strongly to northern song than to their own song. Equal responsiveness to local and nonlocal songs in the south argues against the idea that southern songs were perceived as more threatening in the north.

Nevertheless, asymmetric nonlocal discrimination based on opponent assessment may be a common phenomenon. For example, in the event of geographical variation in size, individuals from across populations might respond differently (either more weakly or more strongly) to large individuals. Variation in the perceived 'quality' of nonlocal individuals may also vary across populations if there is geographical variation in the conditions that affect the expression of sexual signals, such as predators or resources (reviewed in Price 1998). Geographical variation in the morphological traits that constrain signal expression (reviewed for birdsong in Podos et al. 2004) may also lead to asymmetric discrimination. For example, some species of birds show variation in bill morphologies across populations due to ecological adaptation, and these adaptations subsequently affect vocal performance (e.g. Ballentine 2006). In these cases, 'high performance' variants may be preferred (or perceived as threatening) in both local and nonlocal populations.

Female Preferences and Dispersal

For a male, the ability to recognize and distinguish closely related species is thought to be beneficial because it prevents interactions with individuals that do not represent 'real' sexual competitors (Ryan & Rand 1993). In other words, the costs of responding to a 'heterospecific' (i.e. expending energy on territory defence) outweigh the benefits. For males, benefits often take the form of access to fertile females, so if females are less likely to mate with 'heterospecifics', then a male has less incentive to respond to a heterospecific challenger. From this perspective, male black-throated blue warblers in the north may be responding less to southern songs because northern females are less likely to mate with southern males. If this is the case, a south to north barrier to gene flow may exist (without a barrier from north to south). Such asymmetries in female preferences may evolve when new populations are founded by a few individuals, such that selection favours 'nondiscriminating' females in the new populations (Kaneshiro & Boake 1987). A Kaneshiro effect for southern populations of the black-throated blue warbler may be unlikely, as phylogeographical analyses indicate that all current populations expanded from a single refugium during the recent Pleistocene (Davis et al. 2006; Grus et al. 2009). This interpretation also relies on the assumption that females prefer the same signals that elicit strong responses from males, which may (e.g. Searcy et al. 1997) or may not (e.g. Nelson & Soha 2004) be the case. Knowledge about the preferences of females for local versus nonlocal song variants is crucial for inferences of gene flow, and will require further investigation.

In the absence of female discrimination, variation in discrimination by males (by itself) can, in theory, also have important consequences for nonlocal dispersal (Ellers & Slabbekoorn 2003). Populations where nonlocal signals are responded to more strongly might be populations where nonlocal males have a harder time establishing territories. For example, in red-collared widowbirds, *Euplectes ardens*, males with dull or experimentally reduced collars elicit more territorial aggression than males with bright collars

(Pryke et al. 2001), and are less likely to establish territories (Pryke et al. 2002). From this perspective, male black-throated blue warblers that sing a 'northern' song should have a harder time establishing a territory in the south than males that sing a 'southern' song in the north. Anecdotal evidence suggests that there indeed may be a few individual males each year in New Hampshire that have arrived from further south, as a handful of males there each year sing distinctively 'southern' songs (G. J. Colbeck, personal observation), and a small minority of males each year also have considerable amounts of black plumage (M. S. Webster & T. S. Sillett, unpublished data). The opposite appears to be true in the southern population, as we have yet to identify a typical 'northern' singer in the south, and all males in the south seem to have considerable amounts of black plumage (K. W. Stodola, personal communication). Knowledge of patterns of song and plumage variation throughout intermediate populations will be crucial for any further inferences of asymmetric dispersal.

Conclusions

Our results show strong song differences as well as asymmetric discrimination across two populations of the black-throated blue warbler, and best support a model of asymmetric recognition error. This asymmetric nonlocal recognition may have arisen because of (1) asymmetric exposure during migration, (2) variation across populations in the importance placed on different parts of the song, or (3) asymmetries in female preferences (e.g. a Kaneshiro effect). Regardless of the cause, asymmetric discrimination can have important implications for dispersal, mate choice and gene flow, and is likely to be relevant for many biological systems in which sexual signals vary with geography. The causes of asymmetric discrimination, as well as the consequences for divergence and speciation, will require further attention.

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Supplementary Material

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References

- Arnott, G. & Elwood, R. W. 2009. Assessment of fighting ability in animal contests. *Animal Behaviour*, **77**, 991–1004.
- Baker, M. C. 1982. Vocal dialect recognition and population genetic consequences. *American Zoologist*, **22**, 561–569.
- Balaban, E. 1988. Cultural and genetic variation in swamp sparrows (*Melospiza georgiana*): behavioural salience of geographic song variants. *Behaviour*, **105**, 292–322.
- Ballentine, B. 2006. Morphological adaptation influences the evolution of a mating signal. *Evolution*, **60**, 1936–1944.

- Bee, M. A., Perrill, S. A. & Owen, P. C. 2000. Male green frogs lower the pitch of acoustic signals in defense of territories: a possible dishonest signal of size? *Behavioral Ecology*, **11**, 169–177.
- Boul, K. E., Funk, W. C., Darst, C. R., Cannatella, D. C. & Ryan, M. J. 2007. Sexual selection drives speciation in an Amazonian frog. *Proceedings of the Royal Society B*, **274**, 399–406.
- Catchpole, C. K. & Slater, P. J. B. 2008. *Bird Song: Biological Themes and Variations*. Cambridge, Massachusetts: Cambridge University Press.
- Charif, R., Clark, C. & Fristrup, K. 2008. *RAVEN*. Ithaca, New York: Cornell Laboratory of Ornithology.
- Coyne, J. A., Kim, S. Y., Chang, A. S., Lachaise, D. & Elwyn, S. 2002. Sexual isolation between two sibling species with overlapping ranges: *Drosophila santomea* and *Drosophila yakuba*. *Evolution*, **56**, 2424–2434.
- Dabelsteen, T. & Pedersen, S. B. 1992. Song features essential for species discrimination and behaviour assessment by male blackbirds (*Turdus merula*). *Behaviour*, **121**, 259–287.
- Davis, L. A., Roalson, E. H., Cornell, K. L., McClanahan, K. D. & Webster, M. S. 2006. Genetic divergence and migration patterns in a North American passerine bird: implications for evolution and conservation. *Molecular Ecology*, **15**, 2141–2152.
- Derryberry, E. P. 2007. Evolution of bird song affects signal efficacy: an experimental test using historical and current signals. *Evolution*, **61**, 1938–1945.
- Dolman, G. 2008. Evidence for differential assortative female preference in association with refugial isolation for rainbow skinks in Australia's tropical rainforests. *PLoS ONE*, **3**, e3499.
- Ellers, J. & Slabbekoorn, H. 2003. Song divergence and male dispersal among bird populations: a spatially explicit model testing the role of vocal learning. *Animal Behaviour*, **65**, 671–681.
- Endler, J. A. & Houde, A. E. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution*, **49**, 456–468.
- Feulner, P. D., Plath, M., Engelman, J., Kirschbaum, F. & Tiedemann, R. 2009. Electrifying love: electric fish use species-specific discharge for mate recognition. *Biology Letters*, **5**, 225–228.
- Grant, B. R. & Grant, P. R. 2002. Simulating secondary contact in allopatric speciation: an empirical test of premating isolation. *Biological Journal of the Linnean Society*, **76**, 545–556.
- Gray, D. A. 2005. Does courtship behavior contribute to species-level reproductive isolation in field crickets? *Behavioral Ecology*, **16**, 201–206.
- Gray, D. A. & Cade, W. H. 2000. Sexual selection and speciation in field crickets. *Proceedings of the National Academy of Sciences, U.S.A.*, **97**, 14449–14454.
- Grus, W. E., Graves, G. R. & Glen, T. C. 2009. Geographic variation in the mitochondrial control region of the black-throated blue warbler (*Dendroica caerulescens*). *Auk*, **126**, 198–210.
- Guerra, M. A. & Ron, S. R. 2008. Mate choice and courtship signal differentiation promotes speciation in an Amazonian frog. *Behavioral Ecology*, **19**, 1128–1135.
- Holmes, R. T., Rodenhouse, N. L. & Sillett, T. S. 2005. Black-throated blue warbler (*Dendroica caerulescens*). In: *The Birds of North America*, No. 087 (Ed. by A. Poole). Ithaca, New York: Cornell Laboratory of Ornithology.
- Hoskin, C. J., Higgie, M., McDonald, K. R. & Moritz, C. 2005. Reinforcement drives rapid allopatric speciation. *Nature*, **437**, 1353–1356.
- Irwin, D. E., Bensch, S. & Price, T. D. 2001. Speciation in a ring. *Nature*, **409**, 333–337.
- Johannesson, K., Havenhand, J. N., Jonsson, P. R., Lindegardh, M., Sundin, A. & Hollander, J. 2008. Male discrimination of female mucous trails permits assortative mating in a marine snail species. *Evolution*, **62**, 3178–3184.
- Kaneshiro, K. Y. & Boake, C. B. 1987. Sexual selection and speciation: issues raised by Hawaiian *Drosophila*. *Trends in Ecology & Evolution*, **2**, 207–212.
- Nelson, D. A. 1988. Feature weighting in species song recognition by the field sparrow (*Spizella pusilla*). *Behaviour*, **106**, 158–182.
- Nelson, D. A. 1998. Geographic variation in song of Gambel's white-crowned sparrow. *Behaviour*, **135**, 321–342.
- Nelson, D. A. & Soha, J. A. 2004. Male and female white-crowned sparrows respond differently to geographic variation in song. *Behaviour*, **141**, 53–69.
- O'Connell-Rodwell, C. E., Wood, J. D., Kinzley, C., Rodwell, T. C., Poole, J. H. & Puria, S. 2007. Wild African elephants (*Loxodonta africana*) discriminate between familiar and unfamiliar conspecific seismic alarm calls. *Journal of the Acoustical Society of America*, **122**, 823–830.
- Podos, J. 2007. Discrimination of geographical song variants by Darwin's finches. *Animal Behaviour*, **73**, 833–844.
- Podos, J., Huber, S. K. & Taft, B. 2004. Bird song: the interface of evolution and mechanism. *Annual Review of Ecology, Evolution and Systematics*, **35**, 55–87.
- Podos, J. & Warren, P. S. 2007. The evolution of geographic variation in birdsong. *Advances in the Study of Behavior*, **37**, 403–458.
- Price, T. 1998. Sexual selection and natural selection in bird speciation. *Philosophical Transactions of the Royal Society of London, Series B*, **353**, 251–260.
- Price, J. J., Earnshaw, S. M. & Webster, M. S. 2006. Montezuma oropendolas modify a component of song constrained by body size during vocal contests. *Animal Behaviour*, **71**, 799–807.
- Pryke, S. R., Lawes, M. J. & Andersson, S. 2001. Agonistic carotenoid signalling in male red-collared widowbirds: aggression related to the colour signal of both the territory owner and model intruder. *Animal Behaviour*, **62**, 695–704.
- Pryke, S. R., Andersson, S., Lawes, M. J. & Piper, S. E. 2002. Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of size and color. *Behavioral Ecology*, **13**, 622–631.
- Reynolds, R. G. & Fitzpatrick, B. M. 2007. Assortative mating in poison-dart frogs based on an ecologically important trait. *Evolution*, **61**, 2253–2259.
- Riesch, R., Ford, J. K. B. & Thomsen, F. 2006. Stability and group specificity of stereotyped whistles in resident killer whales, *Orcinus orca*, off British Columbia. *Animal Behaviour*, **71**, 79–91.
- Ritchie, M. G. 2007. Sexual selection and speciation. *Annual Review of Ecology, Evolution and Systematics*, **38**, 79–102.
- Routto, J., Mazzi, D., Van Der Linde, K., Mirol, P., Butlin, R. K. & Hoikakala, A. 2007. The extent of variation in male song, wing and genital characters among allopatric *Drosophila montana* populations. *Journal of Evolutionary Biology*, **4**, 1591–1601.
- Rubenstein, D. R., Chamberlain, C. P., Holmes, R. T., Ayres, M. P., Waldbauer, J. R., Graves, G. R. & Tuross, N. C. 2002. Linking breeding and wintering ranges of a migratory songbird using stable isotopes. *Science*, **295**, 1062–1065.
- Ryan, M. J. & Rand, A. S. 1993. Species recognition and sexual selection as a unitary problem in animal communication. *Evolution*, **47**, 647–657.
- Searcy, W. A. & Beecher, M. D. 2009. Song as an aggressive signal in songbirds. *Animal Behaviour*, **78**, 1281–1292.
- Searcy, W. A., Nowicki, S. & Hughes, M. 1997. The response of male and female song sparrows to geographic variation in song. *Condor*, **99**, 651–657.
- Sillett, T. S. & Holmes, R. T. 2002. Variation in survivorship of a migratory songbird throughout its annual cycle. *Journal of Animal Ecology*, **71**, 296–308.
- Stodola, K. W., Linder, E. T., Buehler, D. A., Franzreb, K. E. & Cooper, R. J. 2009. Parental care in the multi-brooded black-throated blue warbler. *Condor*, **111**, 497–502.
- Tchernichovski, O., Nottebohm, F., Ho, C. E., Pesaran, B. & Mitra, P. P. 2000. A procedure for an automated measurement of song similarity. *Animal Behaviour*, **59**, 1167–1176.
- Tinghitella, R. M. & Zuk, M. 2009. Asymmetric mating preferences accommodated the rapid evolutionary loss of a sexual signal. *Evolution*, **63**, 2087–2098.
- Uy, J. C., Moyle, R. G. & Filardi, C. E. 2009. Plumage and song differences mediate species recognition between incipient flycatcher species of the Solomon Islands. *Evolution*, **63**, 153–164.
- Vannoni, E. & McElligott, A. G. 2008. Low frequency groans indicate larger and more dominant fallow deer (*Dama dama*) males. *PLoS ONE*, **3**, e3113.
- Wright, T. F. & Dorin, M. 2001. Pair duets in the yellow-naped amazon (*Psittaciformes: Amazona auropalliata*): responses to playbacks of different dialects. *Ethology*, **107**, 111–124.