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Male Dark-eyed Juncos (*Junco hyemalis*) Respond Differentially to Playback of Local and Foreign Song

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ABSTRACT.—The songs of oscine birds, given their cultural inheritance, can readily diverge, which can foster assortative mating and ultimately reproductive isolation. The species complex of Dark-eyed Juncos (*Junco hyemalis*) consists of at least six distinct subspecies and represents an ideal system for studying

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divergence in mating signals owing to its recent, rapid radiation throughout North America since the last glaciation (<15,000 yrs). Each subspecies of Dark-eyed Junco is characterized by distinct plumage and morphological characteristics, but divergence among the subspecies in song and the role of song as a potential isolating mechanism remains to be rigorously tested. In this study, I compared territorial responses of male juncos in Virginia to playback of locally recorded songs and songs recorded from a different population and subspecies in California. Males responded aggressively to playbacks from both populations, but they approached more rapidly and closely, and performed more flyovers when responding to local song types. While these results suggest that male juncos can discriminate between songs of different populations, future work should investigate female preferences for local and foreign songs and also address more populations, particularly those located in active hybrid zones. *Received 12 October 2013. Accepted 25 March 2014.*

Key words: aggression, geographic variation, song divergence, speciation, territoriality.

During the breeding season, male passerines rely heavily on songs to both attract and stimulate potential mates (Catchpole and Slater 2008). Consequently, any divergence in song between two populations has the potential to develop into a species-recognition signal and ultimately act as a reproductive isolating mechanism (Coyne and Orr 2004, Price 2008). The factors underlying divergence in birdsong among populations are diverse and can include differences in transmission constraints between habitat types (Slabbekoorn and Smith 2002), changes in morphology associated with local adaptation (Podos 2001), differences in community composition (Grant and Grant 2010), and geographic variation in female mating preferences (Schluter and Price 1993). In addition to these factors, song divergence in oscine songbirds, which culturally inherit their songs, may occur at rapid rates owing to the persistence of copying errors and novel song types between generations (Lachlan and Servedio 2004).

The Dark-eyed Junco (*Junco hyemalis*) represents an ideal model for studying the role of song divergence as an isolating mechanism given that the junco has experienced a recent, rapid radiation across North America since the last glaciation (<15,000 yrs) and may represent a case of incipient speciation (Mila et al. 2007). Currently there are no fewer than six subspecies of Dark-eyed Juncos recognized, and the taxonomy of this group is a topic of both past (Miller 1941) and recent contention (Nolan et al. 2002, Aleixandre et al. 2013). Each junco subspecies is characterized by a distinct plumage, morphology, and geographic range, but in areas where those ranges overlap some interbreeding occurs (Nolan et al. 2002).

The degree of divergence in song among the junco subspecies is less well studied, but all published accounts of junco song structure to date report that the advertising song consists predominantly of a single trill (i.e., one note or syllable repeated in rapid succession; Titus 1998, Nolan et al. 2002, Newman et al. 2008). Published data from the local population used in this study (Mountain Lake Biological Station, Virginia; Titus 1998) and a different foreign population of the Oregon Junco subspecies than the one used in this study (Berkeley, California; Konishi 1964) show a substantial overlap in some song characteristics including song length ($\bar{x}$ ± SE; CA: 1.49 ± 0.2 secs; VA: 1.5 ± 0.2 secs), minimum frequency (CA: 2.98 ± 0.1 kHz, VA: 3.4 ± 0.4 kHz), and maximum frequency (CA: 6.53 ± 0.7 kHz, VA: 6.5 ± 0.6 kHz). Collectively, these data indicate that junco song is fairly homogeneous in general structure across a broad geographic range; however, more rigorous tests of finer scale temporal and spectral differences are lacking.

The song repertoires of male juncos typically consist of 2–8 song types, and sharing of song types between males is very limited such that the majority of song types in all populations studied thus far were sung by only one male (Nolan et al. 2002, Newman et al. 2008, Cardoso and Atwell 2011). The cause of limited song sharing among males can likely be attributed to the high incidence of improvisation, or the creation of novel or heavily modified songs, during junco song development (Marler et al. 1962), which can also result in substantial turnover of song types between seasons and generations. Under these conditions, it is less likely that song types will become fixed, reliable indicators of population origin, which is an essential attribute of signals that serve as isolating mechanisms (Price 2008).

As an initial test of whether junco song has the potential to reliably signal population of origin, I investigated whether territorial male Dark-eyed Juncos in eastern North America (Virginia) would respond differently to playbacks of song recorded in their local population (*Junco hyemalis carolinensis*) and playbacks of song recorded in a geographically distant population of a different Dark-eyed Junco subspecies (*Junco hyemalis thurberi*) in western

TABLE 1. Loading scores for behaviors measured during song playback. Strongest loadings for each component emphasized in bold type.

Behavior	PC 1 (48.0%)	PC 2 (22.7%)
Closest Approach	**−0.935**	0.025
Latency to 5 m	**−0.590**	**0.597**
Time within 5 m	**0.731**	−0.247
Time within 1 m	**0.780**	−0.041
Flyovers	**0.793**	−0.054
Latency to Song	−0.026	**0.906**
Songs	0.070	**−0.840**

North America (California). I predicted that if the songs of these two populations have diverged enough that males can readily distinguish local from foreign songs, then males would respond more aggressively to locally recorded songs.

METHODS

I conducted song playbacks on the territories of 30 male Dark-eyed Juncos (*Junco hyemalis*) located at Mountain Lake Biological Station and the grounds of Mountain Lake Hotel in Pembroke, Virginia, USA (37° 22′ N, 80° 32′ W). Males received two, 15-min playbacks between the hours of 0745 and 1200 EST presented on consecutive days from 30 May to 15 July 2008. Each playback tape consisted of five unique song types that were either recorded in the local population (hereafter, Virginia) between 8–25 May 2008 (see Reichard et al. 2011 for additional recording methods) or recorded from a distant junco population in the Laguna Mountains of California, USA (hereafter, California; 32° 51′ N, 116° 26′ W), during the 2006 breeding season (see Cardoso et al. 2007 for additional recording methods). All recordings were made using the same type of shotgun microphone (Audio-Technica AT835b) to limit any effect of recording method. Playback order was randomized and counterbalanced between days.

Using Adobe Audition 1.5 (Adobe Systems, San Jose, CA) and following methods previously established by McGlothlin et al. (2007), I created playback tapes in which each song type was repeated nine times at a rate of six songs/min before transitioning to the next song type. Thus, a unique song type was heard every 90 sec for the first 7.5 min of playback, and I then repeated the entire 7.5-min sequence to complete the 15-min playback. I created five unique playback tapes of Virginia songs and six unique tapes of California songs. All tapes were normalized to 90% of the peak amplitude and passed through a high-pass equalizer to remove any low-frequency noise below 250 Hz. When assembling the Virginia tapes, I chose song types for each tape that were recorded in close proximity to one another, which enabled me to choose a tape consisting of song types that had been recorded distantly (>1 km) from each focal male, thereby limiting any ‘Dear Enemy’ effects (Temeles 1994).

Before each playback, I standardized the speaker volume to 85–90 dB sound pressure level measured at 1 m from the speaker via a Radio Shack digital sound level meter (model no. 3-2055). I conducted all playbacks during the incubation stage of nesting, and placed the speaker within 15–20 m of the nest before retreating 10–15 m to start the playback and quantify the male’s response. Speaker placement was shifted slightly between playbacks (<5 m) to limit habituation. I observed each playback with the assistance of a secondary observer that recorded my observations and differed randomly between days. As I was also responsible for choosing and managing the playback tapes during each trial, I was inevitably aware of the treatment being presented. During playback, I noted the focal male’s closest approach to the speaker, latency to approach within 5 m of the speaker, latency to first long-range song bout, flyovers, long-range songs, time spent within 5 m of the speaker, and time spent within 1 m of the speaker. A flyover was defined as any airborne movement directly over the speaker. If a male did not appear for the entire 15-min playback, the trial was discarded and repeated the next day.

I used a principal component analysis to combine the eight behavioral measures into two separate component response scores that explained 70.7% of the total variance in the behavioral data (Table 1). Measures of approach and proximity to the speaker and flyovers loaded most strongly onto PC 1. Vocal behavior and the latency to approach within 5 m of the speaker loaded most strongly onto PC 2. To control for the effects of treatment order and any seasonal decline in aggressive behavior, I analyzed the resulting PC scores with a linear mixed model that included order as a fixed effect, subject as a random effect, and Julian date as a covariate. Although not reported here, the results of a simple paired *t*-test were identical to the outcome of the mixed model analysis.

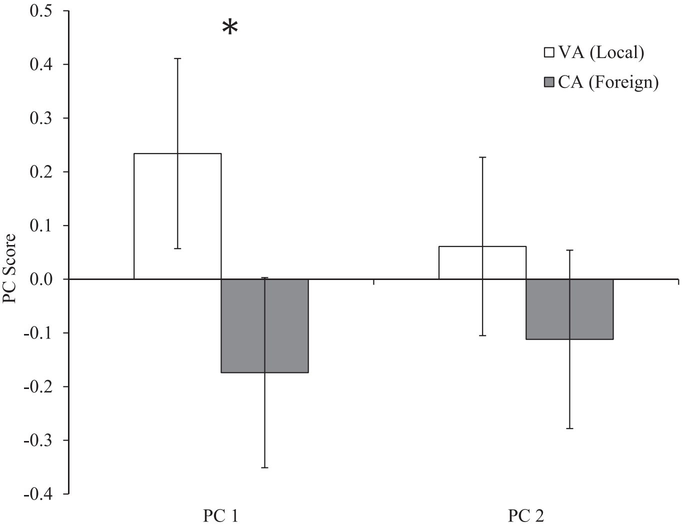

FIG. 1. Estimated marginal means (± 1 SEM) of PC scores for male response to playback of Virginia (local) songs and California (foreign) songs. Significant effect of song treatment on PC 1 score ($P = 0.022$) such that males receiving Virginia song had faster, closer approaches to the speaker, more total time spent in close proximity to the speaker, and more flyovers, $n = 30$.

RESULTS

A mixed model controlling for subject, order, and Julian date revealed a significant effect of song treatment on PC 1 score ($F_{1,28.0} = 5.866$, $P = 0.022$). Males responding to VA song had significantly higher PC 1 scores than males responding to CA song (Fig. 1). A higher PC 1 score was indicative of a rapid, close approach to speaker, more total time spent in close proximity to the speaker, and more flyovers (Table 1). There was no detectable effect of treatment order (Fig. 2; $F_{1,29.2} = 0.204$, $P = 0.66$) or the interaction of treatment and order ($F_{1,27.0} = 2.071$, $P = 0.16$). Additionally, I found no detectable effect of Julian date on PC 1 score ($F_{1,27.3} = 2.057$, $P = 0.16$).

A second mixed model found no effect of song treatment on PC 2 score (Fig. 1; $F_{1,28.0} = 0.550$, $P = 0.47$), which was most indicative of vocal behavior and latency to approach within 5 m of the speaker (Table 1). There was a significant effect of playback order on PC 2 score ($F_{1,28.3} = 12.017$, $P = 0.002$), such that PC 2 scores were lower when males were receiving their second playback regardless of the playback's population origin (Fig. 2). Lower PC 2 scores are indicative of a lower latency to song production, more songs, and a faster approach to within 5 m. I found no detectable treatment by order interaction ($F_{1,27.0} = 2.598$, $P = 0.12$) or effect of Julian date ($F_{1,27.1} = 1.035$, $P = 0.32$).

DISCUSSION

Male Dark-eyed Juncos responded more aggressively to playbacks of songs recorded in their local population than to playbacks of songs recorded in a foreign population consisting of a different junco subspecies. This result is somewhat surprising given the apparent similarities in the general structure of junco song across a wide geographic range, and it suggests that song has the potential to reliably indicate population of origin in juncos. However, testing the ability of male juncos to discriminate between the songs of two geographically distant and allopatric populations (>3,000 km of separation) may be a weak indicator of song's ability to signal population or subspecies origin in circumstances where dispersal between populations can occur and the acoustic environments of the two populations are nearly identical. Future work should investigate whether males continue to discriminate between songs of populations separated by smaller distances and particularly in hybrid zones where selection favoring subspecies recognition should be highest (Price 2008).

In the absence of additional data from juncos, previous research in Song Sparrows (*Melospiza melodia*) that has tested the ability of both males and females to discriminate between local and foreign songs from varying distances may further

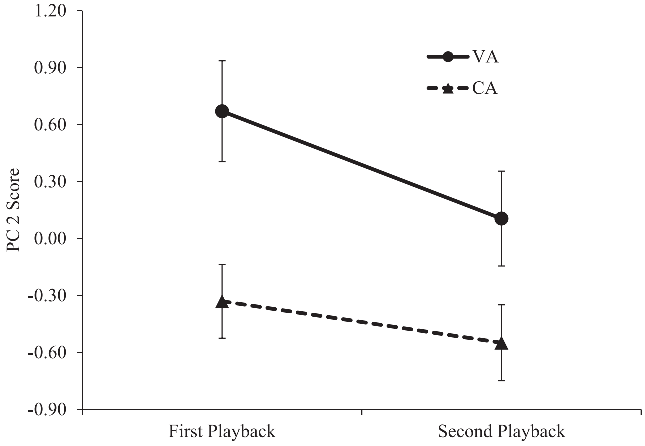

FIG. 2. Mean (± 1 SEM) of PC 2 scores for male response to Virginia and California songs when receiving songs as the first or second playback. Significant effect of order on PC 2 score ($P = 0.002$) such that PC 2 scores were lower during the second playback, $n = 30$.

inform these results. Male Song Sparrows sing relatively small repertoires of 5–16 song types, and in eastern sparrow populations there is relatively low song sharing among males (Arcese et al. 2002), similar to what is observed in Dark-eyed Juncos. In a test of song discrimination in an eastern population of Song Sparrows, males only differentiated between local and foreign songs that were recorded at least 540 km away, while females, in contrast, were able to differentiate between local and foreign songs recorded as close as 34 km away (Searcy et al. 2002). Female songbirds are often more discriminatory than males (Podos and Warren 2007), and these results suggest that if male juncos are capable of discriminating between the songs of distant populations, then females may be more likely to discriminate between the songs of populations separated by much smaller distances.

The results of this study also raise the question of which song attributes allowed males to distinguish between the playback types. Unfortunately, direct comparisons of the temporal and spectral characteristics between the two junco populations used in this study are currently lacking and represent an important topic for future research. As an initial test, I compared the song length, syllable length, and trill rate of the song types used in the playback tapes of this experiment, and I found that songs recorded in the Virginia population were significantly longer ($\bar{x}$ ± SE; VA: 1.73 ± 0.06 sec, CA: 1.45 ± 0.04 sec; $P < 0.001$) and included significantly longer syllables ($\bar{x}$ ± SE; VA: 0.098 ± 0.005 sec, CA: 0.074 ± 0.004 sec; $P < 0.001$) that were produced at a significantly slower trill rate (VA: 9.03 ± 0.40 syllable/sec, CA: 11.5 ± 0.62 syllables/sec; $P = 0.001$) than those recorded in California. Thus, despite the fact that the basic structure of junco song (i.e., a simple trill) is conserved across the two populations studied here, there do appear to be differences in song characteristics that may reliably indicate population origin.

Previous work in a different junco population found that males sing significantly longer songs when they are singing in response to another male's song or a playback tape, which suggests that males may use song length as a signal of motivation (Cardoso et al. 2009). Thus, an alternative explanation for these results could be that males responded more strongly to local songs because they were longer rather than because they were recognized as local types. However, whether male juncos respond differently to songs of varying lengths remains to be empirically tested, and in this study, I found that syllable structure also differed significantly between the local and foreign songs. Evidence from Swamp Sparrows (*Melospiza georgiana*), which also sing a trilled song similar to juncos, suggests that even minute differences in song syntax, specifically the ordering of notes within a syllable, can contribute to the ability of males and females to discriminate between the songs of different populations (Balaban 1988, Liu et al. 2008). Ultimately, more research is needed to fully understand both the signal value of song length in juncos and to identify those song attributes that might serve as important recognition cues.

In summary, Dark-eyed Juncos represent an ideal model for studies of signal divergence and speciation, and here I note the first evidence that male juncos may discriminate between local songs and songs from a foreign population. It is also worth noting that in addition to the high amplitude, long-range song studied here, male juncos also produce a structurally distinct, low-amplitude song during directed courtship that represents another potential vocal isolating mechanism within this species (Reichard et al. 2011, Reichard et al. 2013). Future research should investigate the structural divergence in both of these song classes among additional populations that encompass the different subspecies of Dark-eyed Juncos as well as the responses of both males and females to local and foreign songs. Collectively, these data will enhance our understanding of how acoustic mating signals can contribute to diversification during rapid radiation events.

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