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Original Article

Nestling and adult sparrows respond differently to conspecific dialects

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Understanding the causes and consequences of divergence in mate recognition traits has long been a fundamental question in evolutionary biology. In songbirds, songs are culturally transmitted, and cultural divergence can generate discrete geographic variation in song (i.e., dialects). Understanding how responses to within- versus across-species variation in songs changes across life stages may shed light on the functional significance of population divergence in learned traits. Here, we use a novel combination of song playbacks to adult and nestling golden-crowned sparrows to compare responses to local conspecific, foreign conspecific, and heterospecific songs prior to and after song learning. We found that nestlings respond equally little to both foreign conspecific and heterospecific songs. By contrast, the response of adult males to foreign conspecific songs was stronger than their response to heterospecific song, but weaker than their response to local conspecific song. Our study suggests that early local experience may interact with conspecific biases prior to song learning, in a way that has not been previously documented. Our results illustrate the importance of studying behavior at multiple life stages in order to better understand the effect of early experience on cultural and biological evolution.

Key words: **cultural evolution, species recognition, song dialects, Zonotrichia.**

INTRODUCTION

Acoustic communication plays a key role in facilitating both mate choice and interspecies interactions in many species. When communication systems have a learned component, the precise mechanisms by which animals learn can have implications for how these signals, and responses to signals, evolve (Verzijden et al. 2012). Among oscine passerine birds (songbirds), both song production and song recognition can be learned, and the potential implications for song learning on population divergence has been well-documented (Marler 1970; Nelson et al. 1995; Irwin and Price 1999; Servedio et al. 2009; Verzijden et al. 2012; Soha 2017). For example, cognitive mechanisms in young birds to recognize conspecific signals while filtering out other sounds (reviewed in Soha 2017) can allow them to preferentially learn conspecific songs over heterospecific songs, as would be predicted if there are costs to learning errors (Servedio 2001). What is perhaps less clear is how young songbirds should respond to unfamiliar variants of conspecific song—i.e., dialects. While the fitness consequences of dialects have been debated for decades (Baker and Mewaldt 1978; Baptista 1985; Soha et al. 2004; Derryberry 2009), there is little direct evidence for fitness costs of learning a foreign conspecific dialect. Understanding the salience of song variation within *and* across species to young birds may help illuminate the cognitive mechanisms underlying song learning.

One path towards a better understanding of the functional significance of song dialects may be to test responses of birds to song variants at different stages of life. For example, playback experiments can be conducted with birds at early stages of life, either preceding or immediately following the onset of song learning (Dooling and Searcy 1980; Nelson and Marler 1993; Whaling et al. 1997; Nelson 2000; Soha and Marler 2001; Shizuka 2014; McFarlane et al. 2016). In such experiments, one can use behavioral or physiological responses of nestlings or fledglings to determine the salience of particular song types (or song elements; Hudson and Shizuka 2017) during the earliest stages of song learning. The capacity for early song discrimination can set the stage for biases in sensory learning of songs, such that certain songs are preferentially learned over others (Nelson 2000; Wheatcroft and Qvarnström 2017). Thus, dialect recognition during or prior to song learning could have important implications for the evolutionary consequences of geographic song variation in songbirds. For example, the salience of foreign conspecific dialects to juvenile birds at the onset of song learning may influence whether or not song learning is biased towards local dialects of conspecific songs.

Dialect recognition in birds has been tested frequently in other contexts such as territoriality (e.g., Milligan and Verner 1971;

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Derryberry 2011) and mate choice (Chilton et al. 1990; Clayton 1990; Searcy et al. 1997). The results of these experiments have been mixed: there is clear evidence that adults respond differentially to local versus foreign song variants in some species and sexes (Baker et al. 1981; Nelson 1998; Searcy et al. 2002; Brambilla et al. 2008; Uy et al. 2009; Derryberry 2011; De Oliveira Gordinho et al. 2016; Sosa-López et al. 2016), but not others (Matessi et al. 2000; Danner et al. 2011). In some cases, these experiments have shown a level of hierarchical responses to song, in which birds respond most strongly to local song variants, weakest to heterospecific songs (or those of other subspecies), and intermediately to foreign variants of conspecific song (Nelson 2000; Derryberry 2011). This suggests that receivers may sometimes respond in a graded way to songs based on similarity, rather than simply performing binary species recognition (heterospecific vs. conspecific).

Our main goal was to determine how life stage and context affect responses to conspecific variants of songs within a species. We conducted 2 integrated experiments to test for dialect recognition in a songbird at 2 different stages of life—as nestlings and as territorial adults. First, we use golden-crowned sparrows (*Zonotrichia atricapilla*) to test whether nestlings (prior to the earliest stages of song learning) distinguish between local and foreign variants of conspecific songs, as well as sympatric heterospecific (white-crowned sparrow: *Z. leucophrys gambelii*) song. Second, we conduct simulated territory intrusion experiments with the same playback stimuli to elicit responses from territorial adults.

Based on the previous studies in this system and other bird species, we predict the following 4 scenarios to be most likely. First, subjects could respond equally to all conspecific song types (both foreign and local) but not to heterospecific song, suggesting that there are species-level responses to songs, and geographic variation in song does not induce differential responses (e.g., Matessi et al. 2000; Parra et al. 2017). Second, subjects could respond strongly to all locally common songs, responding equally strongly to local conspecific and (local) heterospecific song, while ignoring foreign conspecific song. While this has never been tested directly to our knowledge, many species eavesdrop on heterospecific signals (Morse 1977; Magrath et al. 2015), even those that do not vocalize themselves (Griffin et al. 2005), showing that heterospecific signals are sometimes equally or more salient than those of conspecifics. Third, birds may respond only to local conspecific song, and show equally little response to heterospecific and foreign conspecific songs (Grant and Grant 2002). This result would suggest dialect recognition, and that signals produced by local conspecifics are most salient. Finally, birds may respond to all conspecific song, but more strongly to local than to foreign song (Searcy et al. 1985, 2002; Nelson 2000; Derryberry 2007). This type of hierarchical responses suggest that local song variants act as the most salient signal, but that foreign conspecifics nevertheless elicit some response. By determining which pattern prevails in playback responses by nestlings and territorial adults, we can better understand how song recognition develops across life stages and contexts.

Study system background:

The current study expands upon a long history of ecological and behavioral studies of song variation in *Zonotrichia* sparrows. The golden-crowned sparrow is sister species to the well-studied white-crowned sparrow (Klicka et al. 2014; Tuttle et al. 2016), and the 2 species breed in the same tree-line habitat throughout large portions of their breeding ranges. Golden-crowned sparrows also exhibit discrete variation in songs across their breeding range in Western Canada and Alaska, with the vast majority of individuals (>90%) singing 1 of 5 main song types (Shizuka et al. 2016). These "dialects" contain clear differences in song phrase composition and frequency intervals between notes. White-crowned sparrows also have well-characterized regional dialects; songs within-species, and even within subspecies, show discrete song characteristics across their range (Marler and Tamura 1962, 1964; Baker and Thompson 1985; Harbison et al. 1999; Soha et al. 2004). In both species, songs begin with what appear to be distinct, species-universal notes—flat introductory whistle in white-crowned sparrows (Soha and Marler 2000) and descending introductory whistle in golden-crowned sparrows (Shizuka et al. 2016)—followed by elements that vary across populations. In white-crowned sparrows, adults of both sexes have been shown to discriminate between these dialects, showing a stronger response to the local song type than a distant conspecific dialect or different subspecies (e.g., Baker 1982; Lampe and Baker 1994; Nelson and Soha 2004; Derryberry 2011; Lipshutz et al. 2017; though see Chilton et al. 1990 for a counterexample). In experiments that include local conspecific dialect, foreign conspecific dialect and songs from another subspecies song treatments, responses to heterospecific songs are weakest, and responses to foreign conspecific dialects are intermediate (Derryberry 2011). Thus, there seems to be some evidence that white-crowned sparrow adults show hierarchical responses to song variation within and between species.

In the white-crowned sparrow (Marler 1970) as well as in many other songbirds that have been studied, there is evidence that song memorization peaks sometime after fledging from the nest (Marler 1970; Slater 1983; Marler and Peters 1987, 1988). Assuming that the closely related golden-crowned sparrows follow a similar learning program, testing for song discrimination at the nestling stage can shed light on the salience of song variation prior to or at the onset of song learning, as the salience of songs to nestlings can influence the ability to filter information during song memorization. Previous experiments in both golden-crowned and white-crowned sparrows showed that young fledgling birds between 7 and 25 days after hatching will preferentially chirp in response to conspecific songs over heterospecific songs (Nelson and Marler 1993; Whaling et al. 1997; Nelson 2000; Soha and Marler 2001; Shizuka 2014; Hudson and Shizuka 2017). In white-crowned sparrows, recently fledged juveniles will respond more to conspecific phrases than heterospecific phrases, while there were no differences to their responses to different conspecific phrases (Whaling et al. 1997; Soha and Marler 2001). In golden-crowned sparrows, nestlings also respond more to conspecific songs than heterospecific (white-crowned sparrow) song, and the first phrase (introductory whistle) is sufficient to elicit this differential response (Shizuka 2014; Hudson and Shizuka 2017).

A series of experiments to both adult male and juvenile white-crowned sparrows (*Zonotrichia leucophrys oriantha*) showed that birds in both stages show 1) strongest responses to their own subspecies song, 2) intermediate response to different subspecies song, and 3) weakest response to heterospecific songs (Nelson and Marler 1993; Nelson 2000). Specifically, Nelson (2000) tested captive juvenile (fledgling) white-crowned sparrows just after fledging and determined that they responded more to their own subspecies songs than those of another subspecies. Here, we present results of a similar experiment with golden-crowned sparrows in the field at a slightly earlier developmental stage. In addition, we contrast these results with responses of adult golden-crowned sparrows to conspecific variation in songs (which consists of about 5 main dialects: Shizuka et al. 2016), which had never been tested.

METHODS

Data were collected at Hatcher Pass Management Area outside Palmer, Alaska in June and July of 2013, 2015, and 2017. At this site, white-crowned and golden-crowned sparrows breed syntopically at treeline, with overlapping territories. However, across all years, adult golden-crowned sparrows were far more abundant than white-crowned sparrows. Nests were found by following females during nest building, incubation, or feeding.

Playback stimuli

For each song treatment, 6 stimulus files were prepared, each based on a recording from a different individual. Local conspecific songs were produced by males singing the local Alaskan song type, but were recorded several years earlier at sites at least 100 km away, and were thus unlikely to be individuals familiar to the subject. Foreign conspecific songs were recorded in the Yukon or British Columbia (3 individuals each; see all song stimuli in Figure 1). Heterospecific white-crowned sparrow songs were recorded in Alaska, and thus were the local dialect to the study site, but were likewise recorded at least 100 km away. All stimuli .wav files were sampled at 48 khz and 16 bits per sample, and were standardized for root mean squared amplitude. Stimulus files were prepared in Raven Pro 1.4 (Cornell Lab of Ornithology 2011). For each file, a single song was repeated every 10 s for 2 min, and 1 min of white noise was added before

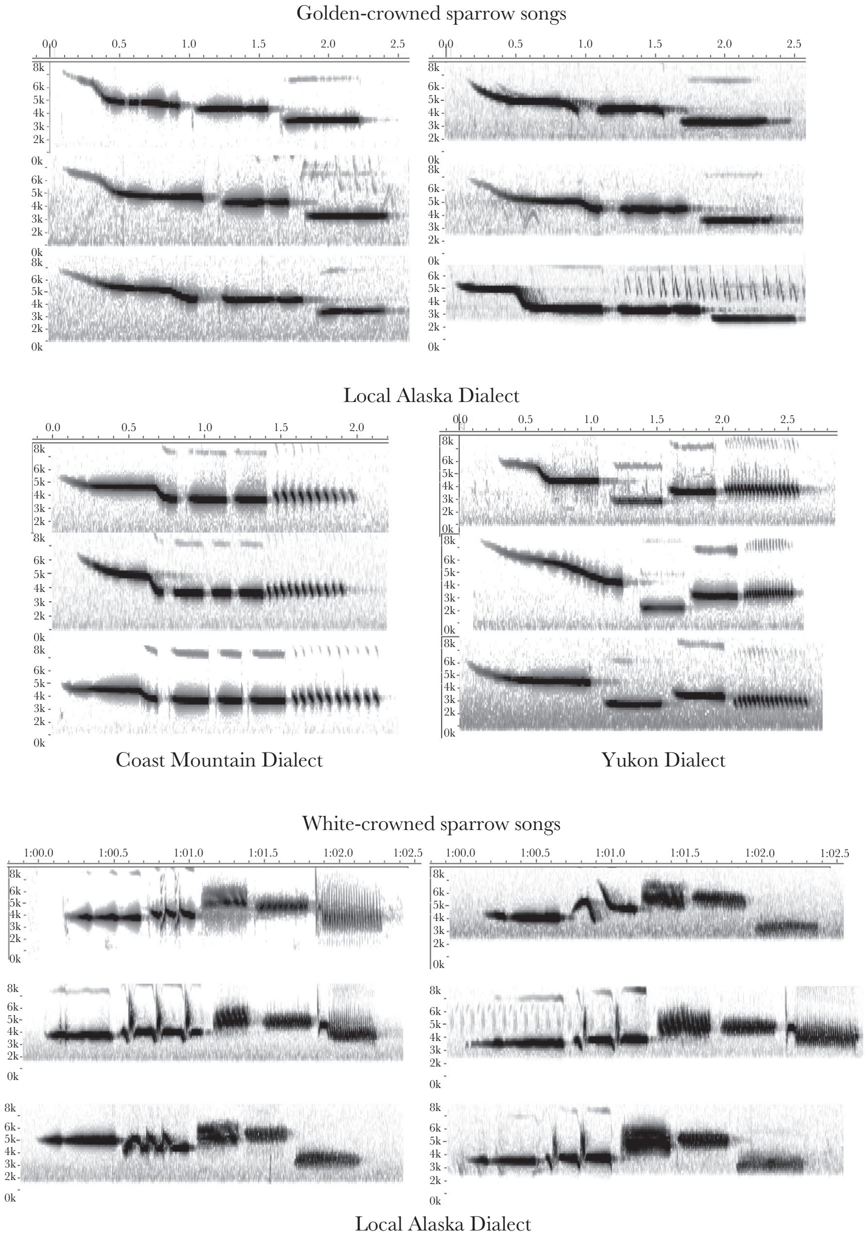

Figure 1

Song stimuli used for each treatment condition. (A) Local golden-crowned sparrow dialect song recorded in south-central Alaska. (B) Foreign golden-crowned sparrow songs from 2 dialects, recorded in Yukon and British Columbia. (C) Heterospecific song from the sympatric subspecies of white-crowned sparrow (*Z. l. gambelii*).

and after the 2 min of songs. White noise was added to better standardize the pretrial acoustic environment for each chick by blocking out background noise.

Nestling playbacks

Based on prior experiments at this site (Shizuka 2014), nestlings were judged to be ready for playback on the first day when most of the birds in a nest showed at least 6 mm of exposed primary feather (though occasionally 1 day later due to logistical constraints such as adverse weather). A prior study with known-aged nestlings at this study site showed that golden-crowned sparrows attain this primary feather length at approximately 8 days after hatching (Shizuka 2014), although exact age could not always be determined in this study (e.g., when nests were found after hatching). All nestlings were tested prior to fledging, and thus prior to the putative onset of song learning.

Prior to playback, all chicks were removed from the nest simultaneously and held in an insulated cloth lunchbox. Chicks were individually banded, measured, and then systematically assigned to one treatment: local conspecific (n = 34), foreign conspecific (n = 27), or heterospecific (n = 27), in a random order within the nest. Each of the 6 local conspecific song stimuli was used between 5 and 7 times; the 6 foreign conspecific song types were used between 5 and 6 times; each of the 6 heterospecific (white-crowned sparrow) song stimulus was used between 4 and 6 times. Nestlings tested in 2017 were part of a separate study that did not include a foreign conspecific treatment, but received identical local conspecific (n = 43) and heterospecific (n = 40) stimuli following the same protocol, and were included in this analysis to increase sample size. During playback, each chick was placed in a portable soft-sided pet carrier (26 × 27 × 48 cm). Following (Shizuka 2014; Hudson and Shizuka 2017) we measured response as the number of chirps in response to playbacks. If a chick was chirping prior to the start of the trial, we waited until the chick was silent to begin playback. Any additional chirps produced during the initial 1-min period of white noise were categorized as prestimulus response. An iHome model IM60 or IM70 speaker (SDI Technologies, Inc., Rahway, NJ) was placed immediately outside the pet carrier in a standardized position to broadcast playback stimuli from an iPod Nano mp3 player (Apple). Number of chirps produced during the 1-min prestimulus period, 2-min song playback, and 1-min postplayback period were recorded. If a chick received a heterospecific or foreign treatment and did not chirp during the entire 4-min trial, it was returned to the lunchbox for 5 min, then given a local conspecific playback trial following the above experimental protocol, as a positive control. Otherwise, each chick was only tested once, with a single treatment. A subsample of 12 trials were rescored on video by an individual not present during fieldwork, and the number of chirps counted was very similar to the original values obtained in the field (r = 0.997).

Adult playbacks

Adult playbacks (n = 26) were conducted before noon at the same site as nestling playbacks, in June and July of 2013 and 2015. For focal males banded during the current season, we waited at least 24 h after banding to conduct the first playback trial. Males were tested using the same stimuli as the nestling trials, but each male received all 3 treatments in a randomized order. Playbacks were separated by at least 1 h (median hours between playbacks: 2.07), and no playbacks were separated by more than 2 days. Response was measured as mean distance of approach to the speaker; lower values represent a closer approach and stronger response. Mean distance was calculated by recording the amount of time a male spent in each of 5 distance categories during the playback, as in (Nelson 1998). Additionally, the number of vocal rattles produced and number of fly-by's (instances where the focal male flew within 1 m of the speaker without landing) were recorded.

Molecular sexing

We extracted DNA from blood samples collected on filter paper using the Qiagen DNEasy kit. We determined the sex of individual nestlings using a standard DNA-based sexing protocol (Griffiths et al. 1998), which has been validated for this species (Chaine et al. 2011).

Statistics

All statistics were conducted using R ver. 3.3.3 (R Core Team 2018). For nestlings, playback responses were analyzed using the R package lme4 (Bates et al. 2015). Because nestling response data were overdispersed, we used a generalized linear mixed model with quasi-Poisson error distribution to test for treatment effects. This test was carried out using the glmmPQL function (GLMM with penalized quasi-likelihood) in the "MASS" package (Venables 2002).

The number of chirps during the trial was the response variable, while song treatment (local conspecific, foreign conspecific, or heterospecific), feather length and number of pretrial chirps were treated as fixed effects. Nest of origin was included as a random effect. Using the package "multcomp" (Hothorn et al. 2008), we conducted Tukey's honestly significant difference post-hoc tests for pairwise differences in response between each of the 3 treatments.

Because penalized quasi-likelihood models cannot estimate the overall effect of treatment, nor pairwise effect sizes, we also conducted likelihood ratio tests on models with and without the song treatment as a fixed effect to determine the overall effect of treatment type on chirp response. Effect sizes (Cohen's D) for nestling responses were estimated using the package "effsize" (Torchiano 2015).

We tested for an effect of sex on nestling response by comparing models with and without sex as a fixed effect using a likelihood ratio test, as described above. We also tested models with and without a treatment-by-sex interaction term.

Adult playback responses were based on multiple different behaviors, in contrast to the single response variable (chirps) in the nestling playbacks. However, a principal components analysis loaded approach distance very highly on the first principal component, indicating that this variable captures most of the variation in response (see Supplementary Table 1). Therefore, we use mean approach distance as the adult response variable in all discussion below.

Pairwise post-hoc tests for adult results were conducted using Tukey's honestly significant difference post-hoc tests. To compare effect sizes between adult and nestling experiments, an approximation of Cohen's d for mixed-effects models was calculated for each adult comparison following Nakagawa and Cuthill (2007).

RESULTS

Song treatment had a strong effect on nestling response (n = 171, likelihood ratio: $P < 0.001$, Figure 2). As seen in post-hoc Tukey contrast comparisons (Table 1), nestlings respond more strongly to the local dialect than to foreign dialects of conspecific song

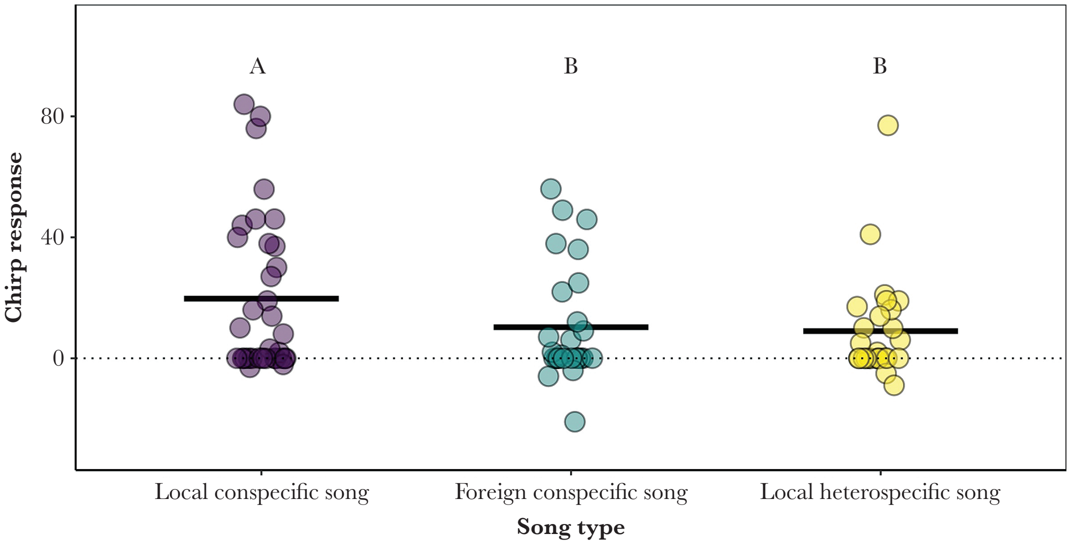

Figure 2
Nestling golden-crowned sparrow response to local conspecific, foreign conspecific, and heterospecific (white-crowned sparrow) song. The *y* axis represents the difference in chirp rate; thus, negative chirp values represent individuals that chirped at a higher rate before the stimulus than during the playback. Each circle represents the change in chirp rate for an individual nestling, and the bold horizontal line represents the mean response.

Table 1
Nestling response Quasi-Poisson Multiple Comparisons of Means (Tukey Contrasts); values from 2013 and 2015 data only in parentheses

Treatment comparison	*Z*	*P*
Local – Foreign	3.19 (2.76)	**0.003 (0.015)**
Local – Heterospecific	5.79 (2.48)	**<0.001 (0.015)**
Foreign – Heterospecific	0.6 (0.12)	0.55 (0.91)

Bold values indicate significance at $P < 0.05$.

Table 2
Effect sizes (Cohen's *d*; for adults, estimated as in Nakagawa and Cuthill (2007))

Treatment comparison	Adult, mean approach distance	Nestling, Chirp number
Local – Foreign	0.5	0.36
Local – Heterospecific	1.43	0.48
Foreign – Heterospecific	0.79	0.13

(n = 104, P = 0.003). Indeed, nestlings did not respond any more to foreign dialect of conspecific song than to heterospecific song (n = 94, P = 0.55). As expected, nestlings responded more strongly to local conspecific song than to heterospecific song (n = 144, P < 0.001), as shown in Shizuka (2014) and Hudson and Shizuka (2017). Effect sizes are shown in Table 2. Excluding the nestlings from 2017 (season when only local conspecific and heterospecific trials were conducted) produces a qualitatively similar pattern. Excluding chicks that failed to respond to positive controls from the foreign and heterospecific treatment (n = 24) likewise did not significantly change the result. Therefore we will only present data from all 3 years, with all nestlings included, for clarity. Removing year as a random effect from the model produced no significant difference (likelihood ratio: P = 1).

Because nestling trials included both male (n = 74) and female (n = 90) subjects, we tested for an effect of sex on nestling responses, as well as an interaction between sex and treatment type. We found no significant effect of sex (P = 0.67), nor was there a significant interaction between sex and treatment (P = 0.8), as determined by likelihood ratio tests (Supplementary Figure 1).

In contrast to nestlings, adult male golden-crowned sparrows (n = 26) responded intermediately to foreign song, differentiating between heterospecific and conspecific foreign songs in their response (Figure 3). Likelihood ratio tests showed an overall effect of song treatment type (P < 0.001). Pairwise Tukey's honestly significant difference post-hoc tests (summarized in Table 3) revealed differences between local and heterospecific response (P < 0.001), foreign and heterospecific response (P = 0.02), and local versus foreign song (P = 0.047).

DISCUSSION

We found that nestling golden-crowned sparrows distinguish local conspecific from foreign conspecific song, prior to fledging and the putative beginning of the song memorization phase. Interestingly, while nestling response to foreign song was as low as their response to heterospecific song, we found that adult males' territorial response to foreign song was intermediate between local and heterospecific response. In other words, both nestlings and adult males are able to reliably distinguish between local conspecific songs and local heterospecific (white-crowned sparrow) songs. Finally, adult males, but not nestlings, respond more to foreign conspecific songs compared with heterospecific songs.

There are 2 potential explanations for the contrasting patterns we found in nestlings and adults. First, the difference in adult versus nestling responses to dialects could be due to the difference in ecological contexts of song responses—i.e., nestling responses may incur different benefits and costs than adult territorial responses. Alternatively, differences in song responses between life stages may represent changes due to acoustic and social experience that birds

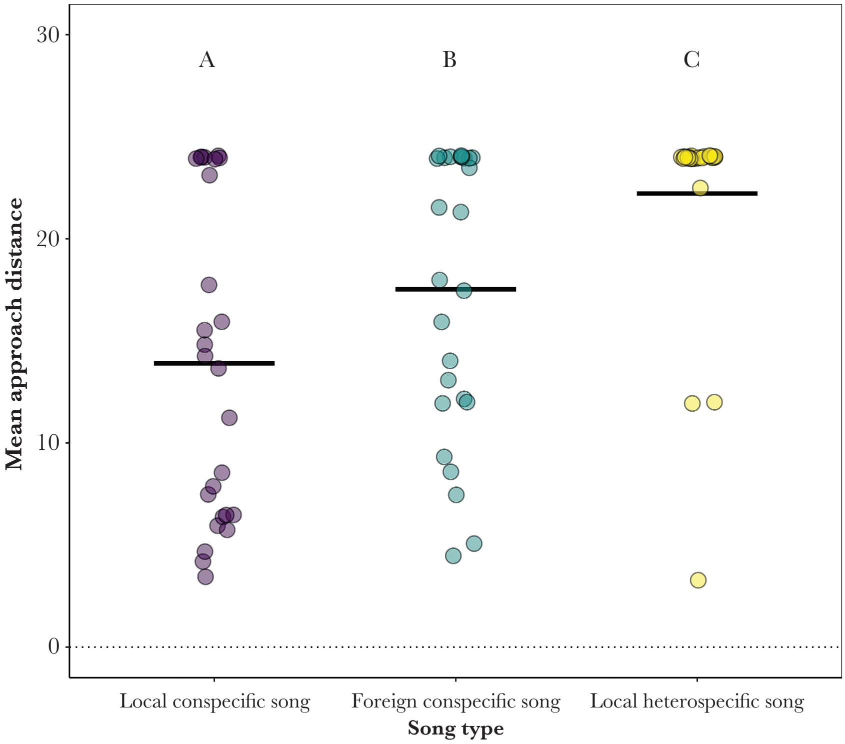

Figure 3

Adult male golden-crowned sparrow response to local conspecific, foreign conspecific and heterospecific (white-crowned sparrow) song. Response is measured as mean approach distance to the playback speaker during a 2-min playback period, where a smaller distance to the speaker indicates a stronger aggressive response. Bold horizontal lines represent the mean approach distance.

Table 3

Adult response multiple comparisons of means (Tukey Contrasts)

Treatment comparison	*Z*	*P*
Local – Foreign	1.987	0.047
Local – Heterospecific	4.56	<0.001
Foreign – Heterospecific	2.57	0.02

accumulate between the nestling and adult stages, as discussed below. What we can confidently infer from these results is that nestlings are capable of differentiating between local conspecific songs and foreign conspecific songs at the onset of song learning.

The evolutionary implications of the salience of conspecific song variation at this early life stage may depend on how song recognition becomes modified across life stages. Nestling birds in our population would never have been exposed to foreign dialects of conspecifics, whereas adult males may have had the opportunity to interact with conspecifics that sing foreign dialects and learn to recognize those songs as conspecific song. If golden-crowned sparrow nestlings are basing their responses on conspecific songs they hear early in life, this suggests they may begin with a narrower template of "conspecific song," which becomes generalized to encompass foreign dialects of conspecifics—a process hypothesized by Irwin and Price (1999). The duration of the sensitive period for song learning in males is a flexible trait that seems to be under selection in white-crowned sparrow subspecies (Nelson et al. 1995; Nelson 1999); perhaps the period in which recognition is learned may continue into adulthood. This is consistent with the finding that adult female white-crowned sparrows' initial preference for natal dialect songs is weakened after exposure to foreign dialect songs as adults (MacDougall-Shackleton et al. 2001). How likely adult golden-crowned sparrows in this population are to hear other dialects during their lifetime is unclear; migratory connectivity between dialect regions has been little studied (but see Cormier et al. 2016). Since golden-crowned sparrows sing outside the breeding season (Norment et al. 1998), there are potentially ample opportunities for exposure to foreign dialects as different populations come in contact during the winter and on migration; more study is warranted to confirm how extensive such opportunities really are.

We found that nestling sex did not have a significant effect on response, suggesting that male and female chicks were equally capable of discriminating between local, foreign, and heterospecific songs. Earlier work suggests that female nestlings undergo a similar sensitive period for learning song as males, with perhaps similar recognition mechanisms in place prior to learning. For example, female song sparrows prefer local song (Patten et al. 2004) and female white-crowned sparrows can be induced to sing natal song elements if injected with testosterone (Baptista and Morton 1982), indicating that females attend to local song when young. Moreover, females may generalize the characteristics of the songs they are exposed to while young, preferring unfamiliar songs that conform in some way to the song type they learned (Clayton 1990; Anderson et al. 2014). Female birds are also often (but not always) more discriminating as adults between variations of male song (reviewed in Ratcliffe and Otter 1996).When, and whether, females develop preferences for local song types is an important question for understanding the maintenance of geographical dialects and patterns of gene flow between population and subspecies of birds, and has been the subject of a great deal of empirical work in adult birds, discussed below. Future work should test for sex differences in early recognition to help untangle this longstanding question.

The results of this study need to be reconciled with the previous finding that golden-crowned sparrow nestlings are able to discriminate between golden-crowned and white-crowned song based on the introductory whistle alone (Hudson and Shizuka 2017). Descending introductory whistles appear to be a universal feature across all golden-crowned sparrow dialects (Shizuka et al. 2016), including the foreign song stimuli used in this experiment. Thus, if nestlings exclusively use introductory whistle as the song recognition cue, we should have seen no difference in response between local and foreign conspecific songs, with nestlings responding more to both of these song types compared with heterospecific song. However, we found that nestlings respond little to foreign conspecific dialect, with no difference in response to foreign conspecific dialect and heterospecific song. What makes them discriminate against foreign conspecific songs, which also feature a whistle? We present 2 possible explanations below.

One possibility is that nestlings possess an innately-encoded neural template of the local dialect of song. This innate template may specify a song without trilled notes, which in this population are found only in heterospecific song (that of white-crowned sparrows). Therefore, the presence of trilled notes in the foreign dialect stimuli may suppress nestling response. Whether this innate template varies geographically to match other regional dialects needs to be investigated further, since other golden-crowned sparrow dialects outside of Alaska do contain trilled final notes (Shizuka et al. 2016). However, a genetically-determined local template would require little to no gene flow between dialect regions, and there is no evidence to date that golden-crowned populations show any degree of systematic genetic differentiation (Norment et al. 1998). Clarifying the genetic relationships between different dialect populations would help determine the likelihood that genetically-based local song preferences could arise.

An alternative explanation is that nestling behavior may be affected by early acoustic experience with the local dialect, contrary to the prevailing view that learning does not begin until fledging (Marler 1970). Under this explanation, nestlings would respond most strongly to the songs that they hear most frequently prior to the playback experiment—i.e., while still in the nest. Because golden-crowned sparrows are the most abundant *Zonotrichia* species at our study site, and all golden-crowned sparrows sing the local, south-central Alaska dialect, this song type elicits the strongest response, while unfamiliar foreign dialects are ignored. However, since white-crowned sparrows are present (albeit less common) at this site, we might expect some response to their song if experience determines response. This could be tested by measuring the degree of white-crowned sparrow song exposure at each nest and determining whether this factor explains the occasional response to white-crowned song in golden-crowned sparrow nestlings. Although song memorization is not thought to have begun at the nestling stage in this species, social learning prior to fledging has been demonstrated in other birds (Colombelli-Négrel et al. 2012; Colombelli-Negrel et al. 2014; Villain et al. 2015) and merits further investigation in this system.

Ultimately, the functional role of song divergence in the buildup of genetic differentiation between populations depends on the extent to which adults recognize and respond differently to foreign dialects. This question has been tested in white-crowned sparrows at varying spatial scales, with mixed results. For example, within the *Z. l. oriantha* subspecies, song dialect patterns correspond only weakly to genetic structure (MacDougall-Shackleton et al. 2001) and females at the *gambelii-oriantha* subspecies contact zone show inconsistent song type preferences (Chilton et al. 1990; Chilton and Lein 1996). Although no strong evidence has been found to date of within-subspecies song barriers in white-crowned sparrows, at the between-subspecies level, song may act as a barrier; for example, song elements are a better predictor of subspecies (genetic) identity than geography in the *nutalli* subspecies where it forms a contact zone with *pugetensis* (Lipshutz et al. 2017). In white-crowned sparrows and other passerines, then, dialects learned at natal sites may potentially promote premating isolation, but only under limited circumstances. The golden-crowned sparrow, as a closely related but reproductively isolated congener, represents a promising system to expand on the insights gained from white-crowned sparrow research.

Decades of work have sought to determine if song dialect boundaries coincide with boundaries to gene flow, and whether song divergence is a cause of genetic divergence between populations (Baker 1982; MacDougall-Shackleton et al. 2001; Wilkins et al. 2013). Cultural divergence in song may affect gene flow when birds of different populations exhibit differential response to their own versus foreign variants of song—i.e., “dialect recognition.” When do culturally divergent songs contribute to the evolutionary divergence between populations? Clearly, divergence in signals per se is not enough to promote premating isolation in all cases (reviewed in Hudson and Price (2014)). Rather, signal and genetic differences may arise concurrently during allopatry (Sosa-López et al. 2016; Wilkins et al. 2016), but by what process, and which pattern precedes the other, remains to be understood. In birds, intrinsic postmating incompatibilities are thought to evolve slowly (Price and Bouvier 2002), implicating extrinsic factors and/or premating isolation in promoting speciation. Signal discrimination, as a prerequisite for assortative mating, has thus long been of interest due to its potential role in promoting premating (e.g., Immelmann 1975; Verzijden et al. 2012). Across diverse taxa, the processes by which recognition signals are learned (Servedio et al. 2009; Svensson et al. 2010) and perceived (Seddon and Tobias 2010; Amézquita et al. 2011; Pasch et al. 2017) have been investigated as a mechanism for promoting divergence in signals and between populations. The parameters of the learning process—e.g., imprinting on one parent versus learning obliquely (Verzijden et al. 2005)—potentially have large and varying effects on the likelihood of speciation. In addition, the timing of the acquisition of signal recognition, as well as the process of modification of recognition templates, will affect how signal divergence relates to assortative mating (Irwin and Price 1999). We suggest that further work using field behavioral assays to compare responses to songs across different life stages could illuminate how these processes play out in the wild and could contribute to our understanding of the interplay between learning and evolution.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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