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Effects of early auditory experience on the development of local song preference in female swamp sparrows

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Abstract A growing number of studies show that learning about male mating signals can shape the way females discriminate among males and may influence the evolution of both female preferences and the male traits under selection. Female songbirds commonly prefer local songs over foreign songs from a different population. In some species, however, the extent of variation among songs sung by different males within a population is as great as the variation observed between populations, raising the question of how females are able to discriminate local from foreign songs. Here, we report that laboratory-reared female swamp sparrows (*Melospiza georgiana*) not only show a preference for the particular song types with which they were tutored as compared with both foreign songs and unfamiliar local songs but also show preference for unfamiliar local songs over unfamiliar foreign songs. An acoustic analysis comparing tutor songs and those presented as unfamiliar local and foreign songs suggests that female swamp sparrows might be attuned to the specifics of local note phonology when assessing the attractiveness of unfamiliar songs. Our results demonstrate that early auditory experience influences response to geographic song variation in female swamp sparrows, and suggest the possibility that female songbirds may generalize what they learn from songs early in life to novel songs heard in adulthood. Additional work is needed to evaluate the contribution of unlearned predispositions for local song.

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Introduction

Understanding the factors that influence how receivers respond to signals is key to understanding how communication signals evolve. Learning is known to influence response to signals in many species (reviewed in ten Cate and Rowe 2007). An individual's response to a particular signal can be affected by what was learned and how it was learned (e.g., sexual imprinting, ten Cate et al. 2006) and also by the degree to which learning influences response to novel signals (Cheng 2002; Ghirlanda and Enquist 2003; ten Cate and Rowe 2007; Verzijden et al. 2007).

As a mating signal, oscine birdsong is particularly enigmatic because the expression of the signal depends so strongly on learning, and thus song can vary across small spatial and temporal scales as a consequence of cultural drift (reviewed in Catchpole and Slater 2008). Female songbirds, then, must themselves acquire a point of reference for discriminating among male songs. One hypothesis is that females acquire this reference through their own learning process (Riebel 2003). Several studies demonstrate the importance of learning for the development of female song preferences in species that show geographic variation in song (Miller 1979; Clayton 1990; Nagle and Kreutzer 1997; Nelson et al. 1997; Riebel et al. 2002; Hernandez et al. 2009), but our understanding of female song learning remains limited. In particular, it is unclear whether females only memorize the specific song types they hear when young, or are instead able to generalize patterns or rules from tutor song models to use in their assessment of unfamiliar songs.

Female songbirds commonly prefer songs from their local population over other "foreign" populations (Baker et al.

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1981, 1987; Balaban 1988a; Clayton 1990; Searcy 1990; Searcy et al. 2002; Anderson 2009). In species with well-defined dialect systems, in which all males in a locale sing the same song type or types (e.g., white-crowned sparrow *Zonotrichia leucophrys*, Baptista 1977; corn bunting *Emberiza calandra*, McGregor 1980), a young female might simply memorize the song type or types sung in her population and discriminate against males that do not sing those particular songs. Such cases suggest a "match or not" comparison; either a song is a song type the female heard when young, or it is not.

In species with large song type repertoires and incomplete sharing of song types among males, however, there is often substantial variation in the songs sung by individuals *within* a single population, with the result that within-population variation can be as great as that observed across different populations (Catchpole and Slater 2008). In such cases, it seems highly unlikely that a female could learn all of the song types sung by males in her population (Lachan and Nowicki 2012). It would be maladaptive, then, for a female to discriminate population differences solely by comparing the large variety of song types sung in her population with subset of song type models she memorized when young, because doing so would exclude as mating partners the males in her population that did not learn from these particular song models. Yet, females in species exhibiting extensive song variation within populations also are known to prefer local songs over foreign songs (e.g., yellowhammers *Emberiza citronella*, Baker et al. 1987; red-winged blackbirds *Agelaius phoeniceus*, Searcy 1990; song sparrows *Melospiza melodia*, Searcy et al. 2002; and swamp sparrows, Balaban 1988a; Anderson 2009). This observation suggests that, rather than a "match or not" heuristic for discriminating local from foreign song, females might instead generalize information they learned about local song from those songs they heard early in life when assessing novel songs they encounter as adults (Lachan and Nowicki 2012).

To test this idea experimentally, we hand-reared female swamp sparrows (*M. georgiana georgiana*) in the laboratory, exposing them early in life to songs from their local (natal) population. When these females reached adulthood, we measured their song preferences using three stimulus contrasts. In a first experiment, we asked whether the females preferred tutor song types over unfamiliar song types from their natal population, as has been shown in other songbird species. Our second experiment then asked the key question of whether the females discriminate in favor of local over foreign song types when all the songs are unfamiliar. In a third experiment, we asked whether preference for familiar tutor song can be modified by adult experience with foreign song types. In an earlier study with adult wild-caught female swamp sparrows, familiarity with foreign song in adulthood did not modify preference for local song (Anderson 2009). However, female songbirds have been shown to modify their preferences to include

songs heard in adulthood, for example, in zebra finches (Clayton 1988), in canaries (Nagle and Kreutzer 1997), and in cowbirds (West et al. 2006), prompting us to ask this question in females with known early song experience.

Methods

Early song exposure

We collected swamp sparrow nestlings at 2–9 days post hatch from Conneaut Marsh, Crawford County, PA, USA, in June 2007 and reared them in the laboratory following standard methods (Marler and Peters 1988; Searcy et al. 2004). Briefly, we fed birds by hand at half hour intervals from dawn until dusk until 9 days post-hatch, and thereafter at 1 h intervals until the bids were feeding independently, at about 4 weeks of age. We tutored the birds twice daily with Conneaut Marsh songs beginning 1 day after they were brought into the lab (average age=7.5 days; range, 3–10 days) and continuing for 12 weeks; this training spans the sensitive period for song learning in the laboratory previously described for this species (Marler and Peters 1988). During song tutoring we initially housed the birds in a single, large sound isolation room (Acoustic Systems, 1.9×1.8×2.0 m) and reared them under identical conditions on a natural photoperiod. After fledging at around 10 days post-hatch, we housed the fledglings together in large cages (46×30×33 cm), about six birds per cage, within the sound isolation room. At 18 days of age, we moved the birds from the large cages and housed each individually (46×23×25 cm cage) in the sound isolation room. We sexed the birds using DNA (Griffiths et al. 1996); eight females from six nests were used as study subjects in this experiment.

Swamp sparrow males in this population sing an average of three song types (range, 1–4; Ballentine et al. 2004). Each song type is composed of a syllable repeated in a trill, with the repeating syllable comprising one to five note types (Fig. 1). Notes are grouped into six species-universal categories (Marler and Pickert 1984). Syllable types (song types) are defined by note type order. In our study, one observer (SP) categorized note types and syllable types using the criteria set out in Marler and Pickert (1984). The grouping of notes into syllable types by visual assessment of spectrograms has been supported by two computational methods, Clark et al. (1987) and Lachlan et al. (2010).

Tutor song stimuli were 21 songs from 21 different males representing exemplars of 14 structurally distinctive song types from among the more than 60 types we have identified from this population. Each tutoring session included every song exemplar repeated 18 times, once every 10 s in a 3-min bout, each bout followed by 1 min of silence (83 min total). There were two tutoring sessions per day, one beginning at 0800 and one beginning at 1400, during which all 21 tutor
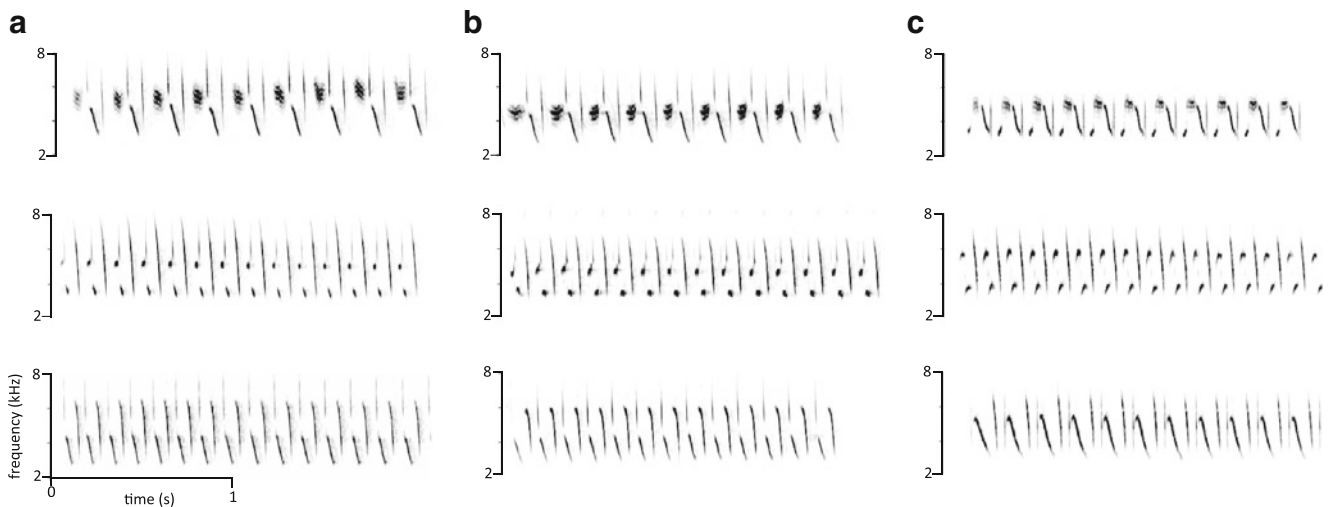
Fig. 1 Examples of swamp sparrow song types used as stimuli. Songs consist of a single syllable repeated in a trill. **a** three song types from the Conneaut Marsh, PA, population; **b** the same song types as in (**a**) but sung by different males, illustrating within-type variation across males; and **c** three different song types recorded from the Millbrook, NY, population

songs were presented using a Marantz PMD 221 recorder and Acoustic Research Powered Partners Speakers. The song bout order was randomized and changed every week. We categorized song types (see Fig. 1 for examples) by visual inspection of spectrograms (44.1 kHz sample, 256 pt FFT, Signal v. 4.0, Engineering Design, Berkeley, CA, USA).

The day after the last day of song tutoring, when the birds were about 13 weeks old, they were moved out of the sound isolation room into a larger animal housing room, where they were maintained on a natural photoperiod and visually and acoustically isolated from males. The females had no further exposure to any swamp sparrow songs until operant preference training began.

Operant song preference training

We tested the females' song preferences at 1 year of age using an operant assay that takes advantage of the reinforcing qualities of song (Riebel and Slater 1998; Riebel 2000) and that has been used successfully with female swamp sparrows (Anderson 2009). After long daylength photoperiod (15L/9D) was reached and 10 days prior to the start of training, we gave each female a subcutaneous implant of 17-β-estradiol hormone, which has been shown to ensure responsiveness to song (Searcy and Marler 1981; Vyas et al. 2009). Throughout training and testing females were housed in cages (46×23×25 cm) inside individual sound isolation chambers (Industrial Acoustics AC-1), with each cage having two fixed perches (23 cm long, placed at a height of 14 cm) and two short "operant perches" (8 cm long and 8 cm high) that were placed 19 cm apart and equidistant from the audio speaker (Realistic 40-1298C). Landing on an operant perch triggered playback of one song, amplitude 78±2 dB SPL (measured at 1 m using a RadioShack 33-2055 sound level meter, Fast Response, A-weighting). A PC computer running Sound Analysis Pro software (http://ofer.sci.ccny.cuny.edu/sound_analysis_pro) automated the operant training by logging perch hops and playing song stimuli.

We first trained the females to hop on operant perches to trigger song playback. Training sessions occurred each morning from 0600 to 1100 h, during which time the operant perches were activated; landing on one of the perches triggered playback of a single tutor song (one song exemplar played once), while landing on the other perch (the "silent perch") had no effect. The silent perch was switched from one side to the other of the cage each day to prevent the association of song with a particular perch on one side. Throughout training and testing, the operant perches triggered playback of only a single song as long as the females remained perched on them; a female had to leave a perch and hop onto it again to trigger another song playback.

The females were trained in the operant setup using four tutor song types chosen randomly from among the set of 14 tutor song types. All females heard the same four tutor songs during training but heard them presented in different orders, with a different song presented each day. The four tutor song types used as training stimuli were not used as test stimuli for any of the birds. To enter the test phase, a female had to show a preference for the perch that triggered tutor song over the silent perch at a ratio of 60:40 or greater for two consecutive days. Seven females reached this criterion at 6 days, and the remaining female reached it after two additional days.

Testing song preference

After training, we tested the females' responses to pairs of songs in three experiments. Each experiment used two operant perches; one perch played a single exemplar of one song category (e.g., tutor song) and the second perch played a single exemplar of the other song category (e.g., unfamiliar local song). We paired stimuli based on similar vocal performance, a measure of how physically challenging a song is to produce (Podos 1997) and a song feature known to affect female preference in this species (Ballentine et al. 2004). In swamp sparrow songs, an operational definition of performance, "vocal deviation," has been developed that measures the distance (i.e. deviation) of syllable repetition rate and frequency bandwidth from the upper bound regressions of those parameters calculated for the whole population (Podos 2001): low vocal deviation scores represent high performance songs. Stimulus songs ranged in vocal deviation (a unit-less measure) from −5.7 to 18.45 with a mean difference between song pairs of 0.9. Vocal performance did not differ overall between stimulus sets for any of the three experiments (Mann–Whitney U tests, $p > 0.4$ for all tests).

In Experiment I, we tested the females' preference for songs heard early in life compared with novel songs from their natal population. We presented six of the Conneaut Marsh song types used as tutor stimuli (tutor local songs) paired with six unfamiliar song types from the same population (unfamiliar local songs). The six tutor local songs were chosen from among the 14 song types used as tutor stimuli, excluding the four types that had been used in operant training. In Experiment II, we asked if females would prefer local over foreign songs when both sets of songs were novel. We used six pairs of completely unfamiliar songs: six unfamiliar local song types (different from the unfamiliar local song types used in Experiment I) and six song types recorded from a population of the same subspecies (M. georgiana georgiana) over 500 km distant near Millbrook, NY (unfamiliar foreign songs). In Experiment III, we asked whether preference for familiar tutor song is modified after experience with foreign song types. Here we used four song pairs: four tutor local song types (excluding those heard during operant training and during Experiment I) paired with four of the foreign song types the females heard during Experiment II. As the females had heard these four foreign song types in the previous experiment, they were no longer unfamiliar (familiar foreign songs). The four song types were chosen without knowledge about how the females had responded to these song types in Experiment II so as to avoid intentionally choosing stimulus songs that were particularly unattractive.

Operant preference test sessions ran each day for 5 h from 0600 to 1100 hours. We presented a given pair of stimulus songs on two consecutive days and averaged the number of hops on each perch for each stimulus across the 2 days. In each experiment, all females heard the same pairs of song stimuli, but the pairs were presented in different orders and we balanced assignment of stimulus category pairs between the two perches for each subject. Experiments I, II and III ran for 12 days, 12 days, and 8 days respectively for a total of 32 test days. We allowed 48 h to pass between each of the three experiments. The females remained in their cages within acoustic isolation chambers throughout the study.

All tutor local and unfamiliar local songs were recorded from a population of males in the Conneaut Marsh, Crawford County, PA, USA, using a Sony TCM-5000 EV or Sony TCD-5M recorder, a Shure SM-57 microphone, and a Sony Parabolic Reflector-330 or a Saul Mineroff SME Parabolic Reflector-1000. All foreign songs were recorded from eight birds wild-caught from a population in Millbrook, NY. Recordings were made in an anechoic room using a realistic 33-100 microphone, Shure FP 11 pre-amplifier and a Marantz PMD 221 cassette recorder. We were careful to choose unfamiliar local song stimuli that were of comparable recording quality to tutor local songs, and that were not particularly rare or atypical song types in our population.

Because the Millbrook songs (foreign) were recorded from captive birds in the laboratory using different equipment than the Conneaut Marsh songs (tutor, local unfamiliar) recorded in the field, we confirmed that the foreign and local song exemplars did not differ in sound quality. First, we took care to select song type exemplars from both recording sets that were of extremely high quality. In addition, we compared measurements of signal-to-noise ratio, reverberation and frequency bandwidth between the foreign and local song stimuli using the nonparametric Mann–Whitney U test (with exact p values) performed in SPSS v19 (IBM corp., USA). We calculated the signal-to-noise ratio for each song file using the averaged maximum amplitudes from the loudest note in each syllable (signal), and the averaged maximum amplitudes of each intersyllable interval (noise). We made these measurements from sections of equal duration for each file (mean=33 ms). We calculated signal-to-noise ratio using the formula $SNR_{dB} = 10\log_{10}(Amp_{signal}/Amp_{noise})^2$. Signal-to-noise ratios did not differ significantly between local stimuli and foreign stimuli, although there was a nonsignificant trend for larger signal-to-noise ratios in foreign stimuli in both experiments (Experiment II: SNR local vs. foreign, median (±interquartile range)=17.8 (±4.1) vs. 26.6 (±7.4) dB, Mann–Whitney U test: $n_1=n_2=6$, $U=28$, $p=0.09$; Experiment III: SNR tutor vs. foreign, median (±interquartile range)=20.2 (±1.1) vs. 26.6 (±9.8) dB, $n_1=n_2=4$, $U=4$, $p=0.34$). To estimate the contribution of reverberation to background noise, we compared the noise amplitude measured between syllables (as above) to the amplitude of a segment of equal duration sampled immediately before the onset of song (i.e., the background noise level without reverberation), and applied the same SNR ratio formula above. Again, local songs

did not differ significantly in reverberation as compared with foreign songs (Experiment II: reverberation local vs. foreign, median (±interquartile range)=18.9 (±9.5) vs. 13.2 (±11.8) dB, Mann–Whitney U test: $n_1=n_2=6$, $U=11$, $p=0.31$; Experiment III: reverberation tutor vs. foreign, median (±interquartile range)=15.2 (±12.0) vs. 14.6 (±16.1) dB, $n_1=n_2=4$, $U=7$, $p=0.89$). Finally, we compared frequency bandwidth between local and foreign songs used in Experiments II and III and found no significant differences (Experiment II: bandwidth local vs. foreign, median (±interquartile range)=7,592 (±908) Hz, foreign=7,993 (±1,056) Hz, Mann–Whitney U test $n_1=n_2=6$, $U=12$, $p=0.39$; Experiment III: bandwidth tutor vs. foreign, median (±interquartile range)=8,032 (±943) vs. 7,993 (±981) Hz, $n_1=n_2=4$, $U=7$, $p=0.89$).

Comparison of test stimuli to tutor songs

We conducted an acoustic analysis to ask whether female responses to a given pair of local and foreign songs (Experiments II and III) were related to the specific acoustic features of the tutor songs the females heard early in life. We asked this question by comparing acoustic features among tutor local, unfamiliar local and foreign song stimuli using two approaches: (1) comparing note type sequence similarity, and (2) comparing overall acoustic *dissimilarities* among song syllables and among note types as measured by a dynamic time-warping (DTW) algorithm (Lachlan et al. 2010).

Pennsylvania (Conneaut Marsh) and New York (Millbrook) swamp sparrow song syllables are composed of notes that can be categorized into a limited number of categories (Marler and Pickert 1984; Lachlan et al. 2010). When choosing pairs of songs to be used as stimuli, we choose different song types that were clearly distinct. However, as two different song types can share the same note type category sequence, we compared this level of syntax among all tutor, unfamiliar local and foreign songs. One of us (SP) first assigned each note, by visual comparison, to a note type category following the criteria established by Marler and Pickert (1984). SP then determined the note type category sequence of all syllables (visually using spectrograms) to assess syntactical similarities among tutor local, unfamiliar local and foreign syllables. In a second analysis we asked if the foreign songs were more dissimilar to the tutor local songs than were the unfamiliar local songs. Here we compared each local and foreign syllable to all tutor local syllables and found the best match. We then computed an average *dissimilarity* score (see below) between unfamiliar local syllables and their best-matched tutor local syllables and between foreign syllables and their best-matched tutor local syllables. Similarly, we compared each local and foreign note to all tutor local notes, found the best match, and then computed an average dissimilarity score between unfamiliar local notes and their best-

matched tutor local notes, and between foreign notes and their best-matched tutor local notes.

We computed syllable and note dissimilarity using an implementation of the dynamic time-warping (DTW) algorithm (Luscinia acoustic analysis software, Lachlan et al. 2010, http://luscinia.sourceforge.net). This method finds the optimal alignment of two time series allowing measurement of differences in vectors of acoustic parameters that vary throughout the signals. For example, it allows stretching and compression along the time dimension of the series to find the best match, and the amount of deviation along the time dimension is reflected, in a linear manner, in the final score. DTW analysis produces a measure of dissimilarity between two signals with scores ranging 0–1, with 0 indicating the signals are identical. In addition to the DTW analysis of syllable and note dissimilarities, we used Luscinia to measure the duration, maximum frequency, minimum frequency, and inter-syllable and inter-note intervals for tutor, unfamiliar local and foreign song stimuli. We took acoustic measurements from spectrograms with a 5.8 ms time resolution and 0.5 ms window overlap, dynamic range set at 40 dB with a Hamming windowing function.

Statistical analysis

Because there is often large variation in individual performance in operant assays, the percentage of responses for a given song treatment divided by the total number of hops on both perches (referred to as "preference level" herein), rather than the number of hops for each treatment, is tested for deviation against a chance rate of 0.5 (Collins 1999; Riebel 2000; Riebel et al. 2002; Leitao et al. 2006). We tested whether preference levels deviated from 0.5 using one-sample Wilcoxon tests performed in R (R Project for Statistical Computing, http://www.r-project.org/). We tested the relationship between female perch hop activity and mean preference level for all three experiments using Spearman correlations performed in JMP v.10 Statistical Software (SAS Institute Inc). We compared mean preference levels in favor of tutor or local song across the three experiments using Friedman tests performed in JMP. We tested whether dissimilarity to a tutor song differed between unfamiliar local and unfamiliar foreign songs using Mann–Whitney U tests performed in JMP.

Results

As in previous operant conditioning studies testing the song preferences of female songbirds (Riebel and Slater 1998; Collins 1999; Riebel 2000), we observed large variation in individual operant responses (Table 1). There was no correlation between female perch hop activity and mean preference

level for Conneaut Marsh songs in any of the three experiments (Spearman correlations, all $p > 0.10$).

In Experiment I, females showed a significant preference for tutor local songs compared to unfamiliar local songs at a level greater than chance (one-sample Wilcoxon test: $V = 34$, $p = 0.012$, $n = 8$; Fig. 2), with a median preference level of 0.55 (range, 0.47–0.62). Seven of the eight females preferred the tutor local songs over the unfamiliar local songs at a mean preference level greater than 0.50 (Table 1). In Experiment II, when presented with pairs of songs that they had never heard previously, the females preferred unfamiliar local over unfamiliar foreign songs (one-sample Wilcoxon test: $V = 36$, $p = 0.004$, $n = 8$), with a median preference level of 0.56 (range, 0.50–0.69). Seven of eight females preferred unfamiliar local songs in this second experiment at a mean preference level greater than 0.50. When presented in Experiment III with pairs of tutor local songs vs. familiar foreign songs, the females preferred tutor local songs at a median preference level of 0.63 (range, 0.52–0.84), also significantly greater than chance (one-sample Wilcoxon test: $V = 36$, $p = 0.004$, $n = 8$). All eight females preferred tutor local over familiar foreign songs at a preference level greater than 0.50.

The females' preference levels for local song did not differ significantly across the three experiments (Friedman test $\chi^2 = 4.75$, $df = 2$, $p = 0.093$, $n = 8$). However, because Experiment III ran for eight test days while Experiments I and II ran for 12 days, we repeated the above analysis including data for only the first eight test days of all three experiments. The preference levels when considering only the first 8 days compared to all 12 days were similar for Experiments I (8-day median = 0.54 vs. 12-day median = 0.55) and II (8-day median = 0.54 vs. 12-day median = 0.56). Considering only the data from the first 8 days, we again found no significant difference in preference level across the three experiments (Friedman test $\chi^2 = 3.81$, $df = 2$, $p = 0.149$, $n = 8$).

Because all females heard the same pairs of song stimuli in each experiment—six song pairs in Experiments I and II, four pairs in Experiment III—we tested the possibility that one or more tutor or local songs were highly attractive and tipped the balance in favor of the tutor or local song treatment. For each experiment, we ran a Friedman test comparing responses across the six song stimulus pairs. For Experiment I the mean preferences expressed for each of the six tutor local songs ranged from 0.44 to 0.61, and the Friedman result approached significance ($\chi^2 = 10.81$, $df = 5$, $p = 0.060$). One tutor local song in particular (mean preference level, 0.44) was not preferred by six of eight females relative to the unfamiliar local song with which it was paired, reducing the overall mean preference level for tutor local songs in Experiment I. Thus, we must remain circumspect about the influence of particular song exemplars on the results in Experiment I. For Experiment II mean preference levels in favor of the six unfamiliar local songs ranged from 0.56 to 0.61 (Friedman $\chi^2 = 3.83$, $df = 5$, $p = 0.573$). In Experiment III preferences for the four unfamiliar local songs ranged from 0.62 to 0.68 (Friedman $\chi^2 = 1.35$, $df = 3$, $p = 0.722$). For these experiments, there is no evidence that particular song exemplars used as stimuli contributed significantly to the overall preferences expressed by the females.

Comparison of test stimuli to tutor songs

We found that females preferred local songs over foreign songs, even when the local songs were unfamiliar. This led us to ask if the females' responses were influenced by a given unfamiliar song's similarity to one of the tutor songs. First, we examined note type sequence similarity. We found that most (87 %) of the note sequences in the unfamiliar songs were unique, and that only two sequences were shared among five unfamiliar local and foreign songs. As no unfamiliar local songs shared a note type category sequence with tutor songs,

Table 1 Female perch hop activity and preferences expressed during the three experiments

Female	Experiment I tutor local vs. unfamiliar local			Experiment II unfamiliar local vs. unfamiliar foreign			Experiment III tutor local vs. familiar foreign		
	Hops	SD	Preference level	Hops	SD	Preference level	Hops	SD	Preference level
1	282	±236	0.51	169	±229	0.69	361	339	0.84
2	1,088	±665	0.54	1,279	±597	0.68	1,303	287	0.74
3	1,565	±292	0.60	962	±1,135	0.54	277	27	0.70
4	261	±251	0.62	81	±36	0.53	219	245	0.65
5	204	±88	0.54	90	±49	0.57	167	82	0.52
6	105	±49	0.47	82	±52	0.58	63	18	0.60
7	226	±111	0.59	406	±481	0.53	245	121	0.55
8	69	±76	0.56	47	±20	0.50	58	28	0.59
Median±interquartile range	244±531		0.55±0.08	130±603		0.56±0.13	232±204		0.63±0.17

Values for each female are the mean±SD hops per day across 12 test days for Experiments I and II, and eight test days for Experiment III, and the mean preference levels (e.g., hops for tutor local/total hops) in favor of tutor local (Experiments I and III) and novel local (Experiment II) stimuli

Fig. 2 Song preference shown by
females during each of the three
experiments: Experiment I,
preference for tutor local songs
over unfamiliar local songs;
Experiment II, preference for
unfamiliar local songs over
unfamiliar foreign songs; and
Experiment III, preference for
tutor local songs over familiar
foreign songs). *Box boundaries*
indicate the 25th and 75th
percentiles, the *lines* within the
boxes mark the medians, and the
whiskers above and below the
boxes indicate the maximum and
minimum data values. The
dashed line at 0.5 indicates equal
hops for stimulus categories (no
preference)
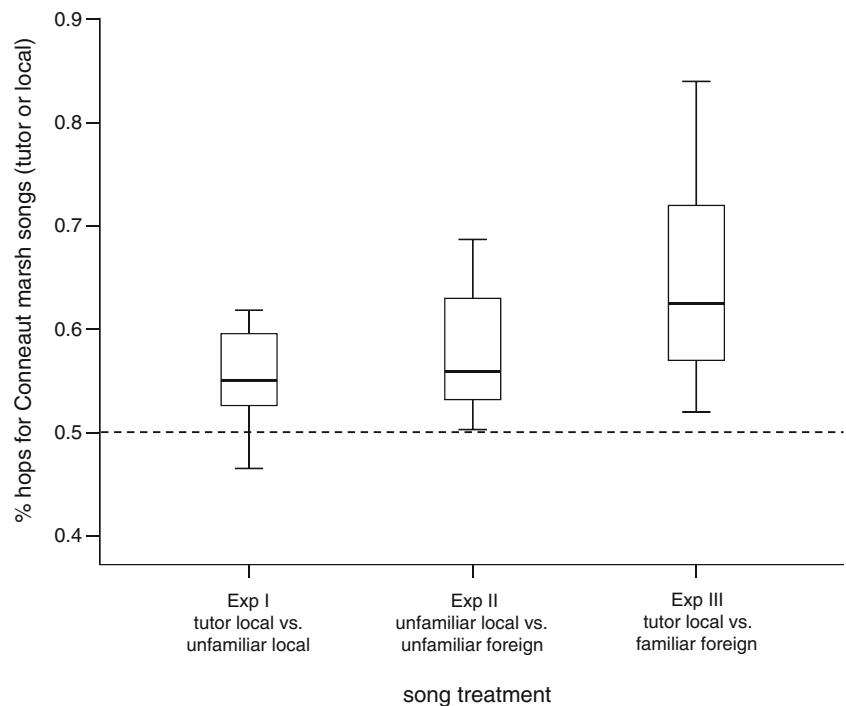
this level of syntax similarity is unlikely to explain the preference for unfamiliar local over unfamiliar foreign songs.

We also examined syllable and note dissimilarity between stimulus sets using dynamic time-warping to ask whether females could have learned the specifics of syllable or note structure and used this information to judge among song exemplars in the operant preference assay. We found that the local syllables ($n=12$) and foreign syllables ($n=6$) did not differ in similarity when compared to their best match among the tutor syllables ($n=21$; Mann–Whitney U test, $n_1=12$, $n_2=6$, $z=-1.26$, $p=0.21$; Fig. 3a). However, foreign notes ($n=22$) were significantly more dissimilar to their best match among the tutor notes ($n=78$) than were local notes ($n=39$; Mann–Whitney U test, $n_1=39$, $n_2=22$, $z=3.25$, $p=0.001$; Fig. 3b). This result is supported by differences in acoustic features between local and foreign songs compared with their best-matched tutor songs (Table 2). Foreign syllables and unfamiliar local syllables did not differ from each other when compared to their best-matched tutor syllables. Foreign notes, however, were more dissimilar from tutor notes than were unfamiliar local notes in duration, maximum frequency, and inter-note interval (Table 2). Foreign notes had on average shorter duration, lower maximum frequency, and longer inter-note interval compared to their most similar tutor notes.

Discussion

Female swamp sparrows exposed as nestlings to songs from their natal population preferred these specific songs as adults.

Tutor song types were preferred over unfamiliar types from the natal population (Experiment I), and were strongly preferred over foreign song types (Experiment III). These results agree with previous studies showing that early auditory experience influences the song preferences of adult females (reviewed in Riebel 2003), including the preference for local song (Baker et al. 1981; Clayton 1990; Hernandez et al. 2009).

What is different about the results we present here is that the preferences expressed in Experiment II cannot be explained by the memorization of tutor song types; females showed a preference for local over foreign song even when tested with song types in both categories that they had never heard before. This result suggests the possibility that female swamp sparrows generalize information they learn about features of song and apply that information when discriminating among novel songs. In support of this interpretation, recent work demonstrates that female swamp sparrows give more sexual displays in response to song type exemplars that are prototypical of population norms compared to exemplars that are atypical (Lachlan et al., under review). This result suggests that females must develop some concept of the structure of song type categories by learning from multiple versions of the same type, and then apply this concept to novel songs.

Empirical work across many taxa has established generalization of this type as a fundamental phenomenon in auditory perception, as well as perception in other sensory modalities, suggesting that stimulus generalization is a fundamental property of learning mechanisms (Purtle 1973; Cheng 2002; Ghirlanda and Enquist 2003). Further, there is accumulating evidence for generalization from learned songs to novel songs
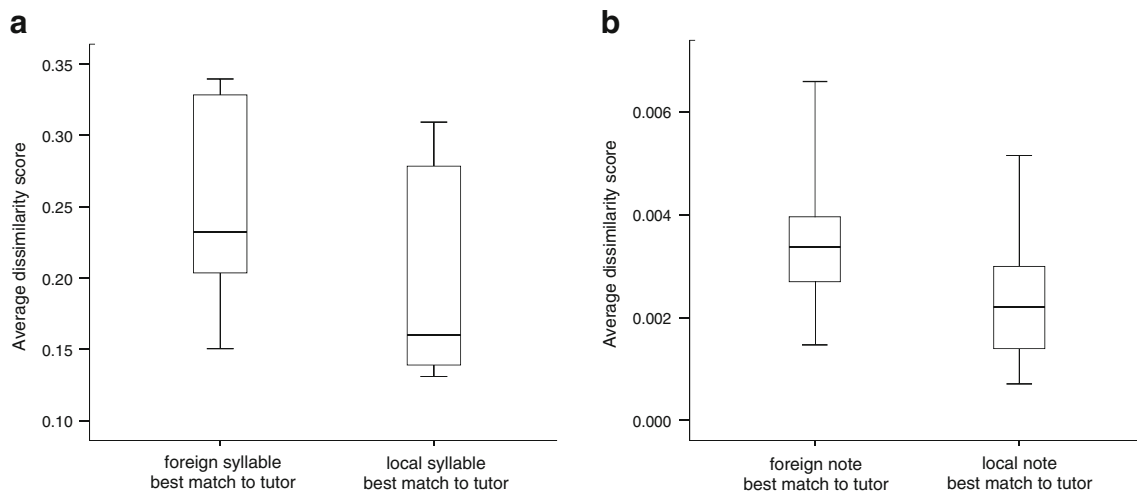
Fig. 3 **a** Syllable dissimilarity and **b** note dissimilarity between unfamiliar test songs and their closest match among tutor songs, compared between local and foreign song stimuli. *Larger values* indicate greater dissimilarity between notes and syllables. Dissimilarity was assessed using a dynamic time-warping algorithm in Luscinia. *Box boundaries* indicate the 25th and 75th percentiles, the *lines* within the *boxes* mark the medians, and the *whiskers* above and below the *boxes* indicate the maximum and minimum data values

in birds. For example, Clayton (1990) showed that female zebra finches generalize a learned preference for tutor songs to novel songs of the same subspecies (but see Riebel and Smallegange 2003 where females' learned preference for fathers' songs did not generalize to the similar songs of sons). Verzijden et al. (2007) demonstrated that zebra finches trained to discriminate between categories of song stimuli can generalize their responses to novel songs, and show generalization patterns similar to those observed in experiments using simple stimuli such as pure tones. Both starlings (Gentner et al. 2006) and zebra finches (van Heijningen et al. 2009) are able to generalize from training songs to novel songs that contained the same element types as those used in training, demonstrating that birds are capable of generalizing from familiar to novel stimuli using phonetic characteristics. The results of the note similarity analysis presented here suggest a similar mechanism in laboratory-reared swamp sparrows: females may have learned the fine details of note structure in the songs used as tutor stimuli and used the greater note similarity between tutor songs and unfamiliar local songs to judge these songs as more attractive than the foreign songs. Future work with songbirds is needed to determine the perceptual

Table 2 Differences between foreign and unfamiliar local songs in their acoustic dissimilarity to their best-matched tutor songs

	Duration (ms)	Max frequency	Min frequency	Inter-syllable interval	Inter-note interval
Difference between foreign syllables ($n=6$) and best matched tutor syllables ($n=21$)					
Median	18.8	58.9	−134.6	−2.4	
Interquartile range	46.4	1,840.5	341	6.4	
Difference between local unfamiliar syllables ($n=12$) and best matched tutor syllables ($n=21$)					
Median	−8.9	−179.8	−9.1	−3.2	
Interquartile range	44.8	1,427.8	589.4	11	
Comparison of differences	$z=1.55$ ($p=0.12$)	$z=0.33$ ($p=0.74$)	$z=-0.61$ ($p=0.54$)	$z=0.33$ ($p=0.74$)	
Difference between foreign notes ($n=22$) and best matched tutor notes ($n=78$)					
Median	−2.6	−527.9	−138.9		1.8
Interquartile range	18.21	1,198.59	1,423.8		5.7
Difference between local unfamiliar notes ($n=39$) and best matched tutor notes ($n=78$)					
Median	0.3	87.9	−31.9		−0.9
Interquartile range	8.13	939.44	609.09		5.55
Comparison of differences	$z=-2.04$ ($p=0.04$)	$z=-2.01$ ($p=0.04$)	$z=-0.40$ ($p=0.69$)		$z=2.23$ ($p=0.03$)

Comparisons of differences were made using non-parametric Mann–Whitney U tests

processes involved in generalizing from learned song models to novel songs, and how patterns of generalization may differ when song categories are learned outside of an operant training and reward scheme.

An alternative explanation for our results is that female swamp sparrows possess an unlearned predisposition for their natal population's song. The control for this possibility requires rearing a group of female nestlings with tutor songs from the foreign Millbrook population, which we did not do in the present study because of the limited number of female nestlings that were available. We suggest that this possibility is unlikely, however, based on the weight of the experimental evidence to date demonstrating the influence of early auditory experience, rather than unlearned predispositions, in determining preference for local over foreign song in female songbirds. Nelson (2000) provided evidence of a genetic bias for own-subspecies song in the white-crowned sparrow (*Z. leucophrys oriantha* and *Z. leucophrys nuttalli*), but to our knowledge, an unlearned bias for local song has not been demonstrated for populations of the same subspecies, such as the Pennsylvania and New York swamp sparrow populations studied here. In fact, Nelson (2000) found that, before tutoring, young *oriantha* and *nuttali* birds did not discriminate between different dialects within each subspecies. He states, "Apparently the representation of song naive birds possess [sic] is sufficiently detailed to allow them to recognize conspecific songs and, possibly, to recognize con-subspecific songs but does not encode the details of particular dialects" (Nelson 2000, p. 13352).

Further, several experiments that have controlled for genetic bias using balanced cross-tutoring designs have shown that females preferred songs they were tutored with when young over songs from their genetic population. For example, studies with female cowbirds (*Molothrus ater ater*) have demonstrated that preference for subspecies song variants is determined by the subspecies of the rearing parents and not by the genetics of the biological parents (Freeberg 1996, 1998; Freeberg et al. 2001). Clayton (1990) cross-fostered two subspecies of the zebra finch (*Taeniopygia guttata guttata* and *T. guttata castanotis*) and found that adult females gave more copulation solicitation displays in response to unfamiliar songs of their foster subspecies than to their genetic subspecies, demonstrating that early exposure to song can override genetic predispositions for own subspecies song. Finally, in a study of a close relative of the swamp sparrow, the song sparrow, Hernandez et al. (2009) tutored females from two populations in Eastern and Western Ontario with songs from their own or from the foreign population and found that the females preferred the tutor population songs in adulthood. Females reared in acoustic isolation did not discriminate between population variants.

Although we cannot rule out the possibility that non-learned predispositions for song qualities in female songbirds played some role, we suggest that a bias for local song

variants, in populations within a subspecies, and in a species with such a large degree of within-population variation, is unlikely to account for the preferences measured in this study, nor is it in line with the bulk of previous research that demonstrates the influence of learning on female preference for local song. Rather, our data suggest that early experience with local songs influences preference for those songs in adulthood in female swamp sparrows, and also suggests the hypothesis that a learned preference can generalize to novel song types in this species. Future work will be necessary to explicitly test the generalization hypothesis against the alternative possibility of an unlearned bias for natal population song.

Our song analysis (Fig. 3) shows that unfamiliar local notes had a better match among tutor local notes than did unfamiliar foreign notes. While it may appear obvious that the tutor songs would be acoustically more similar to a set of songs from the same population than to songs from a foreign population, this was not necessarily expected based on previous work. Consistent acoustic differences in note structure have been demonstrated for *different subspecies* of the swamp sparrow at both the syllable and note level (Balaban 1988a, b), however, no study to our knowledge has identified any systematic acoustic differences in note phonology that could serve as population markers *within subspecies* of the swamp sparrow, nor between the particular populations we studied here. The acoustic analyses we performed were not exhaustive. Rather, our analysis demonstrates that among the unfamiliar song stimuli used here, the unfamiliar local notes had greater similarity to tutor notes than did foreign notes. In particular, foreign notes differed more than local notes when compared to tutor notes in duration, maximum frequency, and inter-note interval. This result suggests that female swamp sparrows might be attuned to the specifics of local note phonology when assessing the attractiveness of novel songs.

Phonology has been shown to serve as a population marker to female songbirds, for example in the yellowhammer *Emberiza citronella* (Wonke and Wallschläger 2009). Earlier work by Marler and Pickert (1984) and Clark et al. (1987) suggested that swamp sparrow songs across all populations are comprised of only six note type categories (i.e., a "species-universal" phonology), making discrimination based on phonological markers unlikely. More recent work has shown, however, that the structure and number of distinct note type categories differs among populations of the same subspecies (Lachlan et al. 2010; RF Lachlan, unpublished data). Further work is needed to test whether these note category differences are perceptible and salient to female swamp sparrows in the context of mate choice.

Another factor that could have influenced the song preferences expressed by the females in these experiments is that females were trained to associate perch hopping with a song reward using a subset of the local tutor songs. As studies with other species have demonstrated that tutor songs are preferred

to unfamiliar songs (e.g., Clayton 1990; Riebel 2000), we reasoned that tutor songs would be more effective operant training stimuli than unfamiliar songs. It is possible, however, that exposure to these local tutor songs during training primed the females to respond more strongly to the other tutor songs used during Experiment I, and to the unfamiliar local songs used in Experiment II. That is, in addition to learning during early exposure to song, the preferences expressed by the females in our study may also have been influenced during training by re-exposure to tutor songs, and by recent familiarization with local songs. It is well-established that the quantity and qualities of training stimuli presented during discrimination learning lead to biases that shape responses to novel stimuli (reviewed in Ghirlanda and Enquist 2003; ten Cate and Rowe 2007), and such effects have been shown for songbirds when learning about sexual signals such as beak coloration (ten Cate et al. 2006) and song (Verzijden et al. 2007). Future work is needed, however, to better understand how stimuli experienced during early learning may interact with recent song exposure to influence female song preferences.

As our local and foreign song stimuli were recorded under different conditions and using different equipment, we compared SNR, frequency bandwidth and estimates of reverberation between the stimulus sets. Although we found no significant differences between local and foreign songs, our statistical power to detect such differences was limited by small sample sizes. In particular, the non-significant trend for greater SNR in foreign stimuli in Experiment II may hint at a systematic difference in recording quality between the local and foreign songs. It is possible that this difference could have influenced our results because the local songs used as tutor, training and test stimuli all came from the same set of recordings. If the females attended to SNR in the song learning and operant training phases of our experiment, their preferences for local song during operant testing may have been influenced by the SNR of the stimuli. Although we deem this an unlikely explanation for our results overall, we acknowledge the possibility that female songbirds may imprint on subtle acoustic features of tutor songs that can influence their adult song preferences (reviewed in Riebel 2003; Hernandez et al. 2008).

Understanding how female songbirds acquire the ability to discriminate differences among the songs of potential mates contributes to a broader understanding of how learning processes can influence both the evolution of female mate preferences, and the male traits under selection (Riebel 2003; Lynn et al. 2005; Lynn 2006; ten Cate and Rowe 2007; Verzijden et al. 2007; Tramm and Servedio 2008; Servedio et al. 2009). Identifying the features of song that female songbirds use to assess potential mates, and how females learn about these features, is important for understanding how song can serve as a reliable assessment signal. For example,

females have been shown to express preference for songs that are well learned as compared with poorly learned (Nowicki et al 2002), yet it remains unclear how females acquire their own point of reference about the qualities of well-learned songs. The work presented here is a first step toward answering these kinds of questions by showing that the development of female song preferences involves learning both the specific features of tutor song models, and the general rules or patterns that characterize song as attractive.

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