Female Lincoln's sparrows modulate their behavior in response to variation in male song quality

Samuel P. Caro,[a] **Kendra B. Sewall,**[a] **Katrina G. Salvante,**[a] **and Keith W. Sockman**[a,b]
[a]Department of Biology, University of North Carolina, Chapel Hill, NC, USA and [b]Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599-3280, USA

Sexually reproducing organisms should mate with the highest quality individuals that they can. When female songbirds choose a mate, they are thought to use several aspects of male song that reflect his quality. Under resource-limited environmental conditions, male Lincoln's sparrows (*Melospiza lincolnii*) vary among one another in several aspects of song quality, including song length, song complexity, and trill performance. In a 2-pronged approach, we tested whether variation in song quality of male Lincoln's sparrows influences the behavior of females that are in a reproductive-like state. Over two trials, we exposed females to songs from the high and low ends of the distribution of naturally occurring song quality variation and found a higher level of behavioral activity in females exposed to high-quality songs, especially when they had first been exposed to low-quality songs. We also examined female phonotaxis toward antiphonally played songs with experimentally elevated and reduced trill performance and found that females moved preferentially toward the songs with elevated trill performance. Contrary to most studies investigating the behavioral responses of wild, female songbirds to variation in male song, we obtained our results without administering exogenous estradiol, which can artificially perturb the female's physiology. Our results demonstrate that the behavior of female Lincoln's sparrows is modulated by the quality of male songs to which they are exposed and that trill performance plays a significant role in this behavioral modulation. Furthermore, as the order of song quality presentation matters, it appears that recent song experience also influences female behavior. *Key words:* bird song, mate choice, *Melospiza lincolnii*, phonotaxis, sexual selection, song playback, trill performance, vocal performance. *[Behav Ecol 21:562–569 (2010)]*

Mate choice is a major component of sexual selection. Although mate choice occurs in males and females (Andersson 1994), sexual selection usually acts more strongly on males (Jones and Ratterman 2009). In songbirds, variation among males in their reproductive success is often correlated with variation in their song characteristics (Searcy and Andersson 1986). For song to constitute an evolutionarily stable and honest indicator of an individual's phenotypic or genetic quality (Gil and Gahr 2002), some of its features should be constrained or costly to produce, and therefore, these features should be better performed by high-quality individuals (Grafen 1990; Lambrechts 1996). Variation in the signal could, for example, mirror male age (Forstmeier et al. 2006; Dalziell and Cockburn 2008; Ballentine 2009; Botero et al. 2009; de Kort, Eldermire, Valderrama, et al. 2009), early developmental costs during brain growth and song learning (Nowicki et al. 2002; but see Naguib et al. 2008), or other aspects of male quality (Catchpole and Slater 2008). By choosing the best singer as a mate, a female may derive fitness benefits for herself (direct benefits) or her offspring (indirect benefits) (Jones and Ratterman 2009) and drive the evolution of male song characteristics.

Female choice has been demonstrated to be influenced by the amount of time a male spends singing (Alatalo et al. 1990), the size of his repertoire (Catchpole et al. 1984; Eens 1997; but see Byers and Kroodsma 2009), the dialect of origin (MacDougall-Shackleton et al. 2001), or the length and complexity of the songs (Wasserman and Cigliano 1991; Gentner and Hulse 2000; Nowicki and Searcy 2005). In addition, the recently demonstrated variation among males in the consistency in the reproduction of similar notes (Botero et al. 2009; de Kort, Eldermire, Valderrama, et al. 2009) may well influence female choice too; although to our knowledge, female responses to such variation have not been assessed yet.

Another male song feature that influences female behavior in some species is the trill (Vallet and Kreutzer 1995; Ballentine et al. 2004). A trill is a song or a song section in which a single syllable type is repeated in rapid succession. Podos (1997) hypothesized that performance constraints have limited the evolution of trills, in particular through constraints imposed by the mechanics of sound production. The vocal tract of birds consists of the bill, trachea, and syrinx, and complex sound production requires the precise coordination of the motions of these elements with respiratory activity (Nowicki 1987; Westneat et al. 1993; Podos 1996; Riede et al. 2006). According to this hypothesis, there is a trade-off between how fast a bird can repeat syllables in a trill (trill rate) and how broad a range of frequencies each repeated syllable can encompass (frequency bandwidth); although narrow frequency bandwidths can be produced at all trill rates, large frequency bandwidths cannot be produced at the highest trill rates (Podos 1997). This hypothesis predicts that males performing song features, such as trills that are close to the mechanical limits imposed by their vocal tracts,
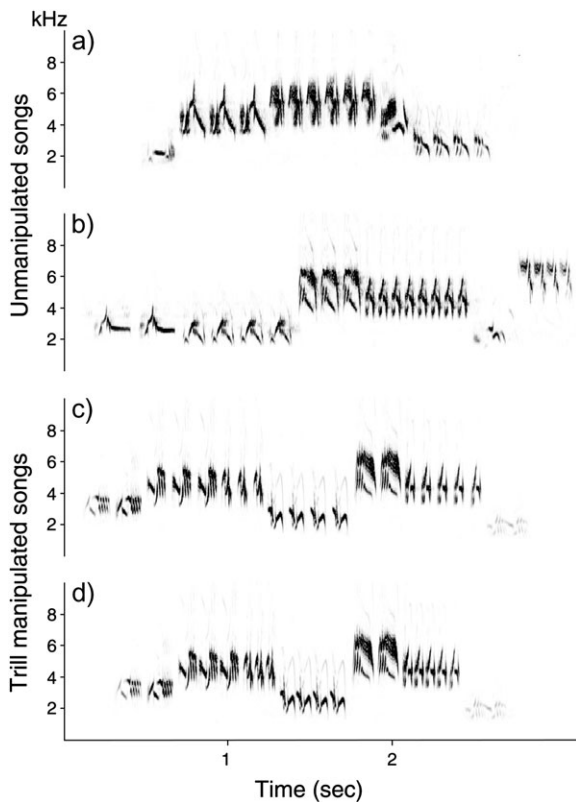
Figure 1
Spectrograms of male Lincoln's sparrow songs. The top 2 songs represent natural songs that were, respectively, classified as (a) low-quality and (b) high-quality songs according to a principal component analysis on song length, syllable count, phrase count, and trill performance. The 2 songs at the bottom represent songs for which the trill rates have, respectively, been experimentally (c) reduced and (d) elevated (see details in methods; we defined a trill as 3 or more consecutive repetitions of a single syllable type).

are more successful in attracting females (Ballentine et al. 2004).

In this study, we investigated whether female Lincoln's sparrows (*Melospiza lincolnii*) show behavioral responsiveness to variation in male song quality. Because the song of male Lincoln's sparrows is relatively complex and contains multiple trills (Figure 1), several components of the song could provide quality indicators. Under limited resource conditions, male Lincoln's sparrows vary among one another in several aspects of their song that are all positively correlated with each other, including song length, the number of syllables per song, song complexity (the number of unique syllables [phrases] per song), and trill performance (Sockman 2009). For convenience, we refer to each of these correlated song traits as an aspect of song quality. We used a 2-pronged approach in which we tested female responses 1) to natural variation in song that was classified using the first-axis factor scores of a principal component analysis (PCA) on each of the song traits listed above (Figure 1) and 2) to songs with experimentally manipulated trill performance (Figure 1).

In most investigations of the behavioral responses of wild, female songbirds to male song variation, females are implanted with 17β-estradiol, a sex steroid that strongly increases the probability that females will display copulation solicitation behavior. Although this method has greatly facilitated our understanding of female behavioral preferences (Searcy 1992), estradiol implants create physiological states that do not necessarily reflect natural conditions (Byers and

Kroodsma 2009), and we chose not to implant the birds used in the present study.

MATERIALS AND METHODS

Animal history

We captured female Lincoln's sparrows in an open-field, sub-alpine meadow (elevation 3250 m) approximately 20 ha in area near Molas Pass, CO (lat 37.74°N, long 107.69°W) (Sockman 2008). We captured female Lincoln's sparrows as adults (4 females caught in 2006) or nestlings (12 females collected from nests in 2006 and 2007 when ca. 8 days of age and subsequently hand reared for 1–2 weeks until they were feeding independently). In July of each year, we transported the birds to animal care facilities at the University of North Carolina at Chapel Hill (USA), where they were placed in outdoor aviaries. Throughout the study, food and water were provided ad libitum. Once they were in captivity, birds were exposed to song playbacks previously recorded from the area where they were captured using 2 short shotgun microphones (Sennheiser ME-66/K6, Wedemark, Germany), each connected to 1 of 2 digital recorders (Marantz PMD 660 and 670, Mahwah, NJ) set to record uncompressed files sampled at 44.1 kHz (Sockman 2009). The playbacks consisted of an iTunes (Apple Inc., Cupertino, CA) playlist made of 1.4 h of recordings and played on an Apple computer connected to a SME-AFS portable field speaker (Saul Mineroff Electronics, Inc, Elmont, NY). Recordings were randomly played for approximately 3 h a day until hand-reared birds were approximately 60 days old. These song files used for tutoring the chicks were different from those used in the present experiments, but all songs were recorded in the same population.

Experiment 1: female behavioral responses to natural variation in song quality

After 6 weeks of photostimulation (16:8 h light:dark), we isolated 8 females (4 adult-captured and 4 hand-reared birds) in each of 8 sound-attenuation chambers (58 × 41 × 36 cm—inside L × D × H; Industrial Acoustics Company, New York, NY) equipped with a light, fresh air supply and exhaust, a speaker (Pioneer TS-G1041R, Tokyo, Japan) connected to an individual amplifier (Audiosource Amp 5.1A, Portland, OR) attached to an 8-channel interface (M-Audio Delta 1010, Irwindale, CA) and a computer running Pro Tools M-Powered software (version 7.1; M-Audio, Irwindale, CA), and a wide-angle surveillance video camera controlled by a computer running Multicam Surveillance software (Ingram Technologies, Price, UT). After 2 h of acclimation in silence, we exposed females to 15 min of song playback. Half of the females heard "high-quality" songs, and the other half heard "low-quality" songs (see description of the songs below; adult-captured and hand-reared females were equally distributed between treatment groups). Playbacks consisted of 3 successive sessions of 3 min of song, separated by 3 min of silence. One song started every 10 s, mimicking a typical singing rate in wild free-living Lincoln's sparrows (Sockman KW, personal observation). The next day, we repeated the same protocol but switched the song quality treatment for each female, so that 4 females heard high-quality songs the first day and then low-quality songs the second day, while the other 4 females heard the reverse order. In this experiment, we used the songs from 29 males. During each trial, each of the 8 females heard 6 different songs recorded from either 2 or 3 males (e.g., 3 songs per male if 2 males) to minimize the risk of habituation to a single song or a single male. Each song and each male's recordings were used for only 1 female in only 1 trial, so each male's recordings were heard by only 1 female. Females heard between 3 and 5 different

Table 1

The mean values and standard errors for measures of songs selected as high- and low-quality stimuli in experiment 1

Song feature	High-quality mean ± SEM	Low-quality mean ± SEM	*t*-test *P* value
Syllable count	19.46 ± 0.32	12.65 ± 0.36	<0.0001
Phrase count	6.69 ± 0.14	4.56 ± 0.14	<0.0001
Song length (ms)	2740.98 ± 35.28	1883.07 ± 40.47	<0.0001
Trill performance[a]	−0.22 ± 0.01	−0.34 ± 0.02	<0.0001

[a] See MATERIALS AND METHODS for a description of trill performance measurement.

song types within each category (high or low quality). There was no significant difference in the number of song types included in the high and low song stimulus sets (independent samples *t*-test, degrees of freedom [df] = 14, $t = 1.000$, $P = 0.334$).

Using our greater than 800-song library recorded from the site at which females were captured, we categorized songs as being high quality or low quality based on the first-axis factor scores from a PCA (see Sockman 2009 for first-axis factor loadings and the proportion of variation explained by the first axis) on several measures known to be preferred by females and to likely reflect male quality in other avian systems: number of distinct phrase types, number of syllables, song length, and trill performance (Table 1; see Nowicki and Searcy 2005 for a review). To measure trill performance, we plotted the trill's mean syllable bandwidth in kilo hertz against the trill rate in hertz and calculated an upper-bound regression following Podos (1997) (see Sockman 2009 for more details concerning the song analysis procedure). This upper boundary represents the theoretical limit to this trill performance measure. We then estimated the performance value of each trill by calculating the distance between each trill's location on the bivariate plot and the nearest point on the upper-bound regression (orthogonal distance to the regression line); the greater the distance, the lower the trill performance. In analyses, we only used the trill in each song with the highest performance measure. Note that we used a slightly higher threshold for measuring frequency bandwidth than in other studies (e.g., Podos 1997), but overall, frequency bandwidth values only marginally differ between studies (see Sockman 2009 for more details). Songs assigned as high-quality playback stimuli were selected from the end of the distribution of PCA scores associated with greater song length, syllable count, phrase count, and trill performance; low quality songs were from the end of the distribution associated with shorter song length and lower syllable count, phrase count, and trill performance (Table 1).

Using the video cameras installed in the chambers, we recorded the behaviors of the females for 15 min before the playback period and then during the playback. Females did not perform any clear copulation solicitation display (CSD). Instead, we assessed the general behavioral activity of the females by counting the number of body movements greater than or equal to body size during the preplayback and playback periods (Hauber et al. 2002).

Experiment 2: female phonotaxis toward songs with experimentally manipulated trill performance

After 3.5 weeks of photostimulation (16:8 h light:dark), we used a phonotaxis paradigm to assess the behavioral response to song of the 8 females that were caught and hand-reared in 2007, none of which we used in experiment 1. The 63 × 31 × 31 cm (L × D × H) phonotaxis cage used in the experiment

consisted of a perch on each end and in the middle of the cage. Outside each end of the cage and facing toward the cage interior was an 8.89 × 15.24 cm thin film transistor video display that, during song playback, displayed a short video loop of a perched male Lincoln's sparrow. The size of the image of the perched male corresponded approximately to the normal size of a male Lincoln's sparrow. The same video played on both displays. Partitions that divided the cage in thirds allowed the test female to move easily throughout the cage by passing underneath the partitions but prevented her from seeing the video display unless she was in the third of the cage next to it. Behind the video display on each end of the cage was a speaker that, during a given session, played 1 of 2 song categories, one with experimentally elevated trill performance or the same song with experimentally reduced trill performance. After their transfer to the phonotaxis cage and a 15-min acclimation period in silence, females—one at a time—were exposed to 30 min of song. Songs alternated between the 2 speakers at a rate of 1 song every 10 s. The trill performance levels were balanced such that the high-performance and low-performance songs were played first the same number of times. The order in which each female was tested, as well as the side of the phonotaxis cage (i.e., the speaker) from which high- or low-performance trills played, alternated daily. Thus, if we exposed a female to high-performance song on the left side of the cage and low-performance song on the right side of the cage during trial 1, we exposed her to high-performance song on the right side of the cage and low-performance song on the left side of the cage during the next trial. We conducted 1 trial per female per day and a total of 8 trials per female over a 10-day period. Using video captured on a computer by a camera placed above the phonotaxis cage, the time each female spent in each third of the cage during each trial was quantified using a Matlab (The MathWorks, Inc., Natick, MA) routine that reliably assessed the time each female spent in each third of the phonotaxis cage. Females generally heard multiple songs from both sides of the phonotaxis cage before initiating movement.

We defined a trill as 3 or more consecutive repetitions of a single syllable type. We manipulated trill rates in a manner similar to that in other studies demonstrating female preferences for high-performance trills (Draganoiu et al. 2002). Each female was exposed to 2 digital copies of a single song from 1 of 8 free-living males recorded in the field. Thus, we used 1 song (experimentally manipulated in 2 ways) from each of 8 males and assigned each song uniquely to 1 of 8 female subjects. From 1 copy of each copy pair, we removed 15 ms of silence between each syllable of each trill using bioacoustics software (Raven v 1.2; Cornell Lab of Ornithology, Ithaca, NY). This 15 ms of silence was then inserted between each syllable of each trill in the other copy of the pair (Figure 1). The deletion and insertion of silence between the syllables of a trill, respectively, elevates and reduces trill performance by, respectively, accelerating and decelerating trill rate, 1 of 2 components of a trill that determines trill performance. The experimental trills' measures of rate and performance ranged from 4.42 to 13.52 (mean: 7.91) syllables/s and −0.712 to −0.135 (mean: −0.372), respectively. These values were within the range of natural unmanipulated trills we have measured in this population (rate: 4.03–15.21 syllables/s; performance: −0.724 to 0.121) (Sockman 2009).

Statistical analyses

Our data set consisted of a hierarchically nested combination of fixed and random effects, each of which could differ from the others in its correlation structure. For example, in experiment 2, the time observed on the side of the cage corresponding to each song category was nested within phonotaxis trial,
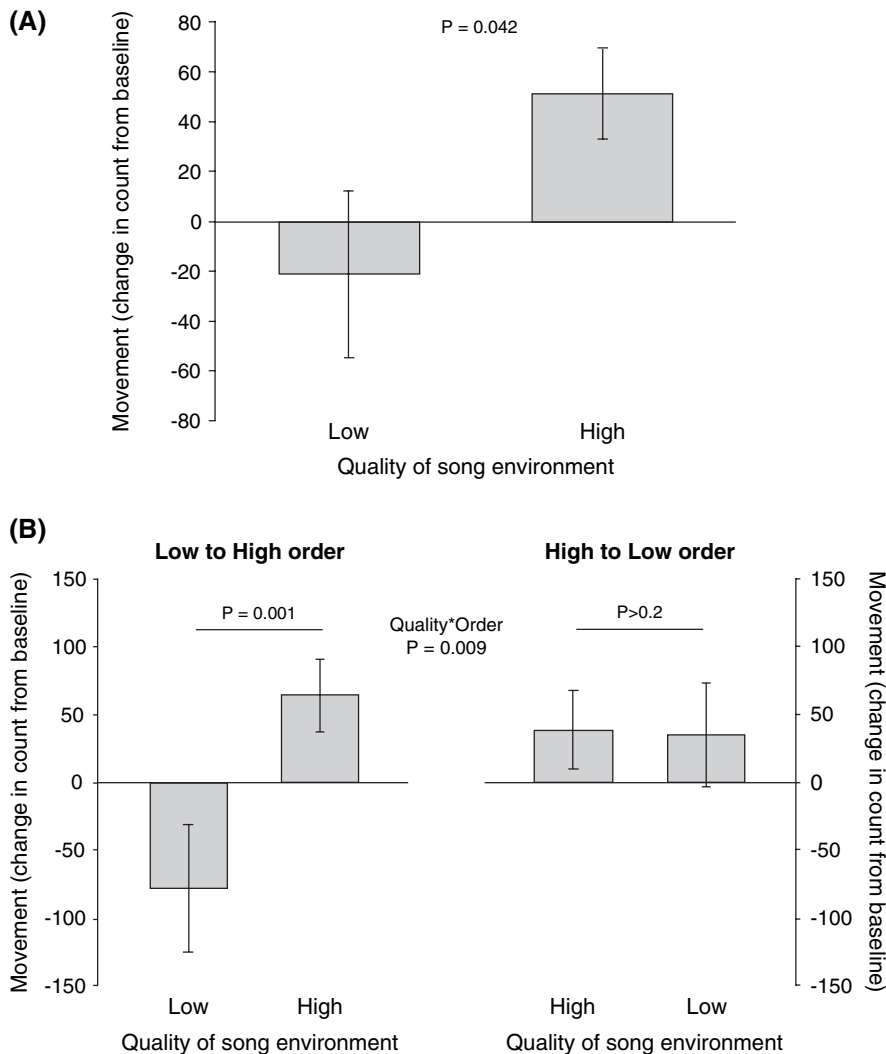
Figure 2
Behavioral activity of female Lincoln's sparrows ($N = 8$ females) exposed to playbacks of low- and high-quality song. We used songs from the low and high ends of the natural variation in song quality, determined by the first-axis factor scores of a principal components analysis on song length, syllable count, phrase count, and trill performance. The baseline movements recorded before the first play-back session were subtracted from the number of significant movements ($\geq$body size) observed during playback. Results from the 2 consecutive song playback exposures were (A) pooled, irrespective of the order of song quality presentation and (B) separated, to show the influence of recent song experience on female response to song playback. Bars represent mean $\pm$ SEM. In (B), the horizontal lines and their associated P values reflect post hoc comparisons.

which itself was nested within individual females. Therefore, for our analyses, we mostly used a mixed-model framework (using Stata IC 10.0 for the Macintosh; Stata Corporation, College Station, Texas), which is readily amenable to complex data sets with hierarchical structure (Burton et al. 1998; Goldstein et al. 2002; Rabe-Hesketh and Skrondal 2005). This mixed-modeling approach has the added advantage of estimating parameters with maximum likelihood procedures (we used restricted maximum likelihood), which are often more accurate and more powerful than the traditional least squares estimates used in analyses of variance and other linear models (e.g., Goldschmidt and Timm 2003; Whitman 2003; Orton and Lark 2007). We coded the categorical variables song quality and trill performance as 0 (low quality and low performance) and 1 (high quality and high performance). For more information on applying this mixed multilevel modeling approach to hierarchically structured measures of animal performance, see Sockman et al. (2008).

RESULTS

Experiment 1: female behavioral responses to natural variation in song quality

To determine whether female behavioral activity differed between exposure to naturally high- or low-quality songs, we used a mixed-effects linear regression with song quality as a predictor and trial ($n = 16$) nested within female ($n = 8$) as a random intercept. Thus, for this initial analysis, the 2 trials were pooled. To account for individual variability in baseline behavioral activity, we subtracted the number of body movements recorded during the 15 min that preceded the playback period of the first trial from the number of movements recorded during each trial's playback period. Overall, females were more active when exposed to playback of naturally high-quality songs compared with playback of naturally low-quality songs (song quality coefficient = 72.375 ± 35.642 [standard error of the mean], $z = 2.03$, $P = 0.042$) (Figure 2A). In separate analyses, we examined the change in activity from the preplayback period (coded 0 in statistical analyses) to the playback period (coded 1). We did not find a reliable difference between these periods when the playback was low-quality song (activity-type coefficient = -21.000 ± 31.844, $z = -0.66$, $P > 0.2$). Activity during high-quality song-playback was higher than preplayback activity (activity-type coefficient = 51.375 ± 18.816, $z = 2.73$, $P = 0.006$). Additionally, there was large variation in behavior within each song quality exposure. We asked whether this variation could be accounted for by the order in which the song categories were presented (low-quality songs first or high-quality songs first) and, therefore, whether recent song experience could have affected behavioral activity. Again, to account for
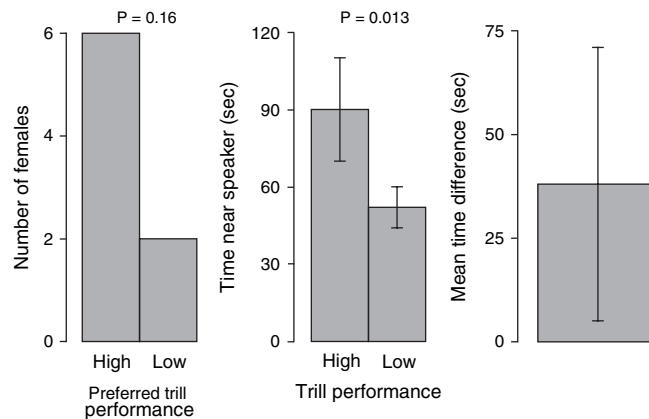
Figure 3
Song preference based on phonotaxis in Lincoln's sparrows ($N = 8$ females) for songs that were artificially manipulated for their trill rates and therefore trill performance. Bars indicate the number of females moving toward 1 song type more than the other (left panel), the time (mean ± SEM) moving toward each song type (middle panel), and the individual time (mean ± 95% CI) moving toward songs with elevated trill performance (high) minus time moving toward songs with reduced trill performance (low) (right panel).

differences in baseline activity, we first subtracted the number of body movements recorded during the 15 min that preceded the playback period of the first trial from the number of movements recorded during each trial's playback period. We used a mixed-effects linear regression with song quality, order of song category presentation (coded 0 for high to low and 1 for low to high), and their interaction as predictors and trial nested within female as a random intercept. In this analysis, we found no main effect of song quality on female activity (song quality coefficient = 3.500 ± 37.187, $z = 0.09$, $P > 0.2$). However, the order in which song qualities were presented mattered (order coefficient = -112.500 ± 51.012, $z = -2.21$, $P = 0.027$), and the interaction between song quality and order of presentation was highly reliable (interaction coefficient = 137.750 ± 52.591, $z = 2.62$, $P = 0.009$) (Figure 2B). Post hoc comparisons of activity revealed a reliable difference between the low-quality and high-quality song exposure for individuals that were first exposed to low-quality song (song quality coefficient = 141.25 ± 44.169, $z = 3.20$, $P = 0.001$) but not for individuals that were first exposed to high-quality song (song quality coefficient = 3.500 ± 28.547, $z = 0.12$, $P > 0.2$). There was also a difference, although not particularly reliable, between individuals that were first exposed to low-quality song and those first exposed to high-quality song in their responses to low-quality song (order coefficient = -112.500 ± 60.487, $z = -1.86$, $P = 0.063$) but not in their responses to high-quality song (order coefficient = 25.250 ± 39.318, $z = 0.64$, $P > 0.2$). In separate analyses, we did not find reliable differences between preplayback activity and activity during either song quality exposure, regardless of order (all comparisons: $|z| \le 1.64$, $P > 0.1$).

Experiment 2: female phonotaxis toward songs with experimentally manipulated trill performance

In experiment 1, we assessed female behavior in response to songs that differed naturally in their quality, meaning that the songs differed in length, syllable count, phrase count, trill performance, and probably in other ways that we did not assess. In experiment 2, we examined the effects of trill performance per se not on female behavior in general but specifically on female phonotaxis behavior. Six of 8 females ($\chi^2 = 2.0$, df = 1, $P = 0.16$) spent more time moving toward song with experimentally elevated trill performance than toward the same song but with experimentally reduced trill performance (Figure 3, left

panel). To more precisely determine whether females exhibited song preferences related to trill performance, we used a mixed-effects linear regression with trill performance as a factor, observation ($n = 128$) nested within trial ($n = 64$) as a random coefficient on trill performance, and trial nested within female ($n = 8$) as a random intercept. Models using observations over the entire 30-min playback period, over the first 15 min of each playback period, and over the first 10 min of each playback period did not converge on a solution, perhaps because females quickly learn that no amount of movement got them any closer to the putative male producing the song. However, over the first 5 min of each playback period, females moved toward songs with elevated trill performance almost twice as much as they did toward songs with reduced trill performance (trill performance coefficient = 38.0 ± 15.2, $z = 2.50$, $P = 0.013$) (Figure 3, middle panel). Also, despite this statistical difference, there was still substantial variation in time spent moving toward one or the other song type. However, much of this variation is due to differences between individual females in the time they spent moving toward song at all (i.e., the time not spent in the neutral center third of the cage). We controlled for this difference in total phonotaxis time by calculating each female's mean difference between time moving toward one or the other song stimulus. On average, females spent approximately 38.0 ± 33.0 s (95% confidence interval [CI]) longer moving toward songs with elevated trill performance than toward the other recordings over the first 5 min of each playback period (Figure 3, right panel).

DISCUSSION

Variation in the quality of male Lincoln's sparrow songs affects female behavior, and this effect was measurable even in the absence of administering exogenous estradiol to females, as is often used for assessing wild, female songbird responses to male song. Females exposed to naturally high-quality songs showed a higher level of behavioral activity compared with females exposed to naturally low-quality songs (Figure 2A). Similarly, females spent more time near speakers playing songs with experimentally elevated trill performance than they did near speakers playing songs with experimentally reduced trill performance (Figure 3). The types of behavioral activities we measured may not necessarily reflect a mate choice preference (Searcy 1992). Nonetheless, phonotaxis in general

appears to be a relatively robust measure of preference in female birds (Holveck and Riebel 2007; Woolley and Doupe 2008; Anderson 2009), so it is not unreasonable to hypothesize that these behavioral differences reflect some component of female mate preference. Moreover, the phonotaxis data presented here are consistent with the hypothesis that female Lincoln's sparrows use male trill performance to make mate choice decisions.

The number of studies on behavioral responses of female songbirds to variation in male songs has dramatically increased since the discovery that administration of exogenous estradiol greatly facilitates the quantification of their behaviors, mainly through the expression of CSDs (Searcy 1992). By nature, the display is closely tied to the act of copulation and therefore should convincingly reflect a female mate preference (Searcy 1992; Anderson 2009). A recent study indeed demonstrated in swamp sparrows (*M. georgiana*) that preference to male song assessed by the CSD technique closely mirrored the results obtained using an operant conditioning procedure where the females had to actively induce song playback (Anderson 2009). With the exception of a few species (e.g., brown-headed cowbird—*Molothrus ater*, King and West 1977), wild, female songbirds kept in captivity do not generally perform CSDs in the absence of estradiol implants or injections of gonadotropin-releasing hormone (Maney et al. 1997, 2008). On the other hand, estradiol-implanted females often spontaneously solicit (i.e., in the absence of relevant cues) (Moore 1983; Searcy 1992), whereas other estradiol-implanted individuals never solicit, thus complicating the interpretation of results from these studies (Maney et al. 2003) and challenging their biological relevance (Byers and Kroodsma 2009). Because hand-rearing chicks facilitates the subsequent expression of natural reproductive behaviors in captive females (Baptista and Petrinovich 1986; but see Campbell and Hauber 2010 for the possible adverse effects of lab rearing on song discrimination), we might have expected that the Lincoln's sparrow females used in this study would show some CSD in response to several weeks of photostimulation and in response to male song playbacks, even with no exogenous estradiol. This was not the case, however. Although we do not know whether or not hand-rearing birds specifically facilitates CSD responses, in the wild, CSD is not normally associated with mate choice but is instead performed just prior to copulation, well after formation of the social pair bond and the settlement of the territory (Byers and Kroodsma 2009). Therefore, not observing CSD when (unimplanted) females have only been briefly exposed to male songs is not surprising. Even in the absence of the most widely used (and a potentially controversial) proxy for female songbird behavioral preference, our study demonstrates that it is still possible to measure female responses to sexual signals, at least in some species.

There are several potential ways to measure differential behavioral responses to male songs without administering exogenous hormones to females. For example, female canaries (*Serinus canaria*) exposed to attractive male songs have been shown to accelerate nest building (Kroodsma 1976) or to lay larger eggs (Leitner et al. 2006). Although these behavioral observations can hardly be made in wild bird females kept in captivity, other techniques such as phonotaxis or operant conditioning are promising (e.g., Campbell et al. 2009; Riebel et al. 2009; this study) and have been shown to mirror the results obtained using the classical copulation solicitation assay (Anderson 2009) or using association tests with live males (Holveck and Riebel 2007).

Among the traits that characterize high-quality songs, trill performance alone seems to elicit substantial changes in female behavior. Female Lincoln's sparrows modulate their behavior in response to natural variation in songs (see

DISCUSSION above), and our results also demonstrate that altering trill rate only is sufficient to improve the attractiveness of male songs. Male (Illes et al. 2006; Cramer and Price 2007; Schmidt et al. 2008; DuBois et al. 2009; de Kort, Eldermire, Cramer, and Vehrencamp 2009) and female (Vallet and Kreutzer 1995; Draganoiu et al. 2002; Ballentine et al. 2004; Christensen et al. 2006) behavioral responses to variation in trill performance have recently been a major focus of song communication studies, mainly because there is a clear and identified mechanism limiting the performance of trills, which may reflect developmental or physiological constraints and perhaps the phenotypic or genetic quality of males. In swamp sparrows, females produce more CSDs in response to a high trill performance version of a song type than to a low performance version of the same song type but sung by a different male (Ballentine et al. 2004). Although in that study the different versions of the song types originated from different males whose songs may have differed in other ways as well, the study is consistent with the hypothesis that females use trill performance as a proxy for male quality in a mate choice context. Similar conclusions were drawn when female canaries more strongly responded to rapid than to slower trills (Vallet and Kreutzer 1995) and preferred trills that were beyond the natural range of vocal performance (Draganoiu et al. 2002). Other arguments supporting the idea that trill performance reflects male quality come from the observations that trill performance increases with age (and therefore experience), body size, social status, and reproductive success (Ballentine 2009; Botero et al. 2009; de Kort, Eldermire, Cramer, and Vehrencamp 2009).

Finally, our results also suggest that females use their recent acoustic experience to modulate their behavioral response to male song. If females are first exposed to low-quality songs, their behavioral activity strongly increases when subsequently exposed to high-quality songs (Figure 2B). Other studies have demonstrated that recent acoustic experience could modulate either the behavioral or the neural response bias for preferred songs or calls (Nagle and Kreutzer 1997; Freeberg et al. 1999; MacDougall-Shackleton et al. 2001; Sockman et al. 2002; Woolley and Doupe 2008; Freed-Brown and White 2009; Sewall and Hahn 2009). In starlings (*Sturnus vulgaris*) for example, recent experience with long-bout songs, which are preferred by females in a mate choice context (Gentner and Hulse 2000), establishes neural selectivity for high-song quality (Sockman et al. 2002). But this selectivity is greatly reduced if females first experience short-bout songs. In the present study, although we were interested in the behavioral rather than the neural response to song quality and although female Lincoln's sparrows were not exposed to several days of song as was the case in study by Sockman et al. (2002), the results suggest a different control mechanism at the behavioral level. Female Lincoln's sparrows seem to establish selectivity for high-quality songs too but only when they previously experienced low-quality songs. In other words, Lincoln's sparrows modulate their behavioral activity when the song quality they hear is higher than a threshold previously set, whereas female starlings modulate their neural response when the song quality is lower than the previously set threshold. Whichever the mechanism involved, these studies suggest that female birds may rapidly adjust their physiological and behavioral responses to changes in the signaling environment to which they are exposed (Badyaev 2009). This should be ecologically relevant as females arriving on their breeding grounds in early spring may set their song preference levels in agreement with the prevailing quality of the local song environment (Sockman 2007). The mean quality of the song environment shows annual variation in Lincoln's sparrows (Sockman 2009). That is, under more limited resource conditions, the

mean song quality is lower than under less limited resource conditions. Thus the female's behavioral plasticity we have shown here would seem adaptive and possibly allow them to modulate an important reproductive decision, that of mate choice, according to variation in the distribution of male secondary sexual characteristics.

REFERENCES

Alatalo RV, Glynn C, Lundberg A. 1990. Singing rate and female attraction in the pied flycatcher—an experiment. Anim Behav. 39:601–603.

Anderson RC. 2009. Operant conditioning and copulation solicitation display assays reveal a stable preference for local song by female swamp sparrows *Melospiza georgiana*. Behav Ecol Sociobiol. 64:215–223.

Andersson M. 1994. Sexual selection. Princeton (NJ): University Press.

Badyaev AV. 2009. Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the Baldwin effect. Philos Trans R Soc B Biol Sci. 364:1125–1141.

Ballentine B. 2009. The ability to perform physically challenging songs predicts age and size in male swamp sparrows, *Melospiza georgiana*. Anim Behav. 77:973–978.

Ballentine B, Hyman J, Nowicki S. 2004. Vocal performance influences female response to male bird song: an experimental test. Behav Ecol. 15:163–168.

Baptista LF, Petrinovich L. 1986. Egg production in hand-raised white-crowned sparrows. Condor. 88:379–380.

Botero CA, Rossman RJ, Caro LM, Stenzler LM, Lovette IJ, de Kort SR, Vehrencamp SL. 2009. Syllable type consistency is related to age, social status and reproductive success in the tropical mockingbird. Anim Behav. 77:701–706.

Burton P, Gurrin L, Sly P. 1998. Extending the simple linear regression model to account for correlated responses: an introduction to generalized estimating equations and multi-level mixed modelling. Stat Med. 17:1261–1291.

Byers BE, Kroodsma DE. 2009. Female mate choice and songbird song repertoires. Anim Behav. 77:13–22.

Campbell DLM, Hauber ME. 2010. Conspecific-only experience during development reduces the strength of heterospecific song discrimination in zebra finches (*Taeniopygia guttata*): a test of the optimal acceptance threshold hypothesis. J Ornithol doi: 10.1007/s10336-009-0466-3.

Campbell DLM, Weiner SA, Starks PT, Hauber ME. 2009. Context and control: behavioural ecology experiments in the laboratory. Ann Zool Fenn. 46:112–123.

Catchpole CK, Dittami J, Leisler B. 1984. Differential responses to male song repertoires in female songbirds implanted with estradiol. Nature. 312:563–564.

Catchpole CK, Slater PJB. 2008. Bird song: biological themes and variations. 2nd ed. Cambridge (MA): Cambridge University Press.

Christensen R, Kleindorfer S, Robertson J. 2006. Song is a reliable signal of bill morphology in Darwin's small tree finch *Camarhynchus parvulus*, and vocal performance predicts male pairing success. J Avian Biol. 37:617–624.

Cramer ERA, Price JJ. 2007. Red-winged blackbirds *Agelaius phoeniceus* respond differently to song types with different performance levels. J Avian Biol. 38:122–127.

Dalziell AH, Cockburn A. 2008. Dawn song in superb fairy-wrens: a bird that seeks extrapair copulations during the dawn chorus. Anim Behav. 75:489–500.

Draganoiu TI, Nagle L, Kreutzer M. 2002. Directional female preference for an exaggerated male trait in canary (*Serinus canaria*) song. Proc R Soc Lond B Biol Sci. 269:2525–2531.

DuBois AL, Nowicki S, Searcy WA. 2009. Swamp sparrows modulate vocal performance in an aggressive context. Biol Lett. 5:163–165.

Eens M. 1997. Understanding the complex song of the European starling: an integrated ethological approach. Adv Stud Behav. 26:355–434.

Forstmeier W, Hasselquist D, Bensch S, Leisler B. 2006. Does song reflect age and viability? A comparison between two populations of the great reed warbler *Acrocephalus arundinaceus*. Behav Ecol Sociobiol. 59:634–643.

Freeberg TM, Duncan SD, Kast TL, Enstrom DA. 1999. Cultural influences on female mate choice: an experimental test in cowbirds, *Molothrus ater*. Anim Behav. 57:421–426.

Freed-Brown G, White DJ. 2009. Acoustic mate copying: female cowbirds attend to other females' vocalizations to modify their song preferences. Proc R Soc B Biol Sci . 276:3319–3325.

Gentner TQ, Hulse SH. 2000. Female European starling preference and choice for variation in conspecific male song. Anim Behav. 59:443–458.

Gil D, Gahr M. 2002. The honesty of bird song: multiple constraints for multiple traits. Trends Ecol Evol. 17:133–141.

Goldschmidt L, Timm NH. 2003. A comparison of noniterative generalized least squares and iterative maximum likelihood estimators when testing hypotheses in random coefficient growth curve models. Commun Stat Simulat. 32:297–318.

Goldstein H, Browne W, Rasbash J. 2002. Partitioning variation in multilevel models. Underst Stat. 1:223–231.

Grafen A. 1990. Sexual selection unhandicapped by the Fisher process. J Theor Biol. 144:473–516.

Hauber ME, Pearson HE, Reh A, Merges A. 2002. Discrimination between host songs by brood parasitic brown-headed cowbirds (*Molothrus ater*). Anim Cogn. 5:129–137.

Holveck M-J, Riebel K. 2007. Preferred songs predict preferred males: consistency and repeatability of zebra finch females across three test contexts. Anim Behav. 74:297–309.

Illes AE, Hall ML, Vehrencamp SL. 2006. Vocal performance influences male receiver response in the banded wren. Proc R Soc B Biol Sci. 273:1907–1912.

Jones AG, Ratterman NL. 2009. Mate choice and sexual selection: what have we learned since Darwin? Proc Natl Acad Sci U S A. 106:10001–10008.

King AP, West MJ. 1977. Species identification in North-American cowbird—appropriate responses to abnormal song. Science. 195: 1002–1004.

de Kort SR, Eldermire ERB, Cramer ERA, Vehrencamp SL. 2009. The deterrent effect of bird song in territory defense. Behav Ecol. 20:200–206.

de Kort SR, Eldermire ERB, Valderrama S, Botero CA, Vehrencamp SL. 2009. Trill consistency is an age-related assessment signal in banded wrens. Proc R Soc B Biol Sci. 276:2315–2321.

Kroodsma DE. 1976. Reproductive development in a female songbird: differential stimulation by quality of male song. Science. 192: 574–575.

Lambrechts MM. 1996. Organization of birdsong and constraints on performance. In: Kroodsma DE, Miller EH, editors. Ecology and evolution of acoustic communication in birds. Ithaca (NY): Cornell University Press. p. 305–320.

Leitner S, Marshall RC, Leisler B, Catchpole CK. 2006. Male song quality, egg size and offspring sex in captive canaries (*Serinus canaria*). Ethology. 112:554–563.

MacDougall-Shackleton SA, MacDougall-Shackleton EA, Hahn TP. 2001. Physiological and behavioural responses of female mountain white-crowned sparrows to natal- and foreign-dialect songs. Can J Zool. 79:325–333.

Maney DL, Goode CT, Lange HS, Sanford SE, Solomon BL. 2008. Estradiol modulates neural responses to song in a seasonal songbird. J Comp Neurol. 511:173–186.

Maney DL, MacDougall-Shackleton EA, MacDougall-Shackleton SA, Ball GF, Hahn TP. 2003. Immediate early gene response to hearing song correlates with receptive behavior and depends on dialect in a female songbird. J Comp Physiol A. 189:667–674.

Maney DL, Richardson RD, Wingfield JC. 1997. Central administration of chicken gonadotropin-releasing hormone-II enhances courtship behavior in a female sparrow. Horm Behav. 32:11–18.

Moore MC. 1983. Effect of female displays on the endocrine physiology and behaviour of male White-crowned sparrows, *Zonotrichia leucophrys*. J Zool Lond A. 199:137–148.

Nagle L, Kreutzer ML. 1997. Adult female domesticated canaries can modify their song preferences. Can J Zool. 75:1346–1350.

Naguib M, Heim C, Gil D. 2008. Early developmental conditions and male attractiveness in zebra finches. Ethology. 114:255–261.

Nowicki S. 1987. Vocal-tract resonances in oscine bird sound production—evidence from birdsongs in a helium atmosphere. Nature. 325:53–55.

Nowicki S, Searcy WA. 2005. Song and mate choice in birds: how the development of behavior helps us understand function. Auk. 122:1–14.

Nowicki S, Searcy WA, Peters S. 2002. Brain development, song learning and mate choice in birds: a review and experimental test of the "nutritional stress hypothesis. J Comp Physiol A. 188:1003–1014.

Orton TG, Lark RM. 2007. Estimating the local mean for Bayesian maximum entropy by generalized least squares and maximum likelihood, and an application to the spatial analysis of a censored soil variable. Eur J Soil Sci. 58:60–73.

Podos J. 1996. Motor constraints on vocal development in a songbird. Anim Behav. 51:1061–1070.

Podos J. 1997. A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). Evolution. 51:537–551.

Rabe-Hesketh S, Skrondal A. 2005. Multilevel and longitudinal modeling using Stata. College Station (TX): Stata Press.

Riebel K, Naguib M, Gil D. 2009. Experimental manipulation of the rearing environment influences adult female zebra finch song preferences. Anim Behav. 78:1397–1404.

Riede T, Suthers RA, Fletcher NH, Blevins WE. 2006. Songbirds tune their vocal tract to the fundamental frequency of their song. Proc Natl Acad Sci U S A. 103:5543–5548.

Schmidt R, Kunc HP, Amrhein V, Naguib M. 2008. Aggressive responses to broadband trills are related to subsequent pairing success in nightingales. Behav Ecol. 19:635–641.

Searcy WA. 1992. Measuring responses of female birds to male song. In: McGregor PK, editor. Playback and studies of animal communication. New York: Plenum Press. p. 175–189.

Searcy WA, Andersson M. 1986. Sexual selection and the evolution of song. Ann Rev Ecol System. 17:507–533.

Sewall KB, Hahn TP. 2009. Social experience modifies behavioural responsiveness to a preferred vocal signal in red crossbills, *Loxia curvirostra*. Anim Behav. 77:123–128.

Sockman KW. 2007. Neural orchestration of mate-choice plasticity in songbirds. J Ornithol. 148:S225–S230.

Sockman KW. 2008. Ovulation order mediates a trade-off between pre-hatching and post-hatching viability in an altricial bird. PLoS ONE. 3:e1785.

Sockman KW. 2009. Annual variation in vocal performance and its relationship with bill morphology in Lincoln's sparrows, *Melospiza lincolnii*. Anim Behav. 77:663–671.

Sockman KW, Gentner TQ, Ball GF. 2002. Recent experience modulates forebrain gene-expression in response to mate-choice cues in European starlings. Proc R Soc Lond B Biol Sci. 269:2479–2485.

Sockman KW, Weiss J, Webster MS, Talbott V, Schwabl H. 2008. Sex-specific effects of yolk-androgens on growth of nestling American kestrels. Behav Ecol Sociobiol. 62:617–625.

Vallet E, Kreutzer M. 1995. Female canaries are sexually responsive to special song phrases. Anim Behav. 49:1603–1610.

Wasserman FE, Cigliano JA. 1991. Song output and stimulation of the female in white-throated sparrows. Behav Ecol Sociobiol. 29:55–59.

Westneat MW, Long JH, Hoese W, Nowicki S. 1993. Kinematics of birdsong: functional correlation of cranial movements and acoustic features in song. J Exp Biol. 182:147–171.

Whitman CS. 2003. Accelerated life test calculations using the method of maximum likelihood: an improvement over least squares. Microelectron Reliab. 43:859–864.

Woolley SC, Doupe AJ. 2008. Social context—induced song variation affects female behavior and gene expression. PLoS Biol. 6:525–537.