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Female and male serins (*Serinus serinus*) respond differently to derived song traits

**Gonçalo C. Cardoso · Paulo Gama Mota ·
Violaine Depraz**

Abstract We tested if male or female behavior towards manipulated song indicates intra- or inter-sexual selection of two characteristics of serin song that are extreme and evolutionarily derived in this species: high frequency and fast syllable rate. In a first experiment, we monitored vocal responses and attendance to song playbacks. Female behavior indicated a preference for high-frequency song and suggested an aggressive function for fast syllable rates, as fast songs inhibited vocal response. Males did not show discrimination of frequency or syllable rate with this experimental design. The second experiment used a simple approach/no approach design, and in this experiment, males showed stronger discrimination between stimuli than did females. Therefore, sex differences in discrimination appear not to result from differences in perceptual abilities but from differences in the context of stimulus presentation. The second experiment also supported a role of song frequency in female choice, as the effect of frequency was limited to females: males did not respond differently to song frequency and approached high-frequency songs less than females did. Results of this experiment also supported an aggressive function for fast syllable rates, as the effect of fast songs did extend to male behavior. Taken together, our results indicate that the high frequency and fast syllable rate of serin song cannot result from a single selection process: while high frequency may have evolved by inter-sexual selection, syllable rate provokes a pattern of response that is more consistent with intra-sexual selection.

Keywords Signal evolution · Song frequency · Syllable rate · Intra-sexual selection · Inter-sexual selection

G. C. Cardoso (✉) · P. G. Mota · V. Depraz
Departamento de Antropologia,
Faculdade de Ciências e Tecnologia da Universidade de Coimbra,
3000-056 Coimbra, Portugal
e-mail: goncaloc@ci.uc.pt

Present address:
G. C. Cardoso
Department of Ecology and Evolution,
University of Chicago,
1101 East 57th Street,
Chicago, IL 60637, USA

Introduction

Bird song attracts much attention because of its diversity among species and great elaboration (Catchpole and Slater 1995). Extensive experimental work supports Darwin's assertion that sexual selection is a key agent in song evolution (Searcy and Andersson 1986; Catchpole 1987). The song attribute most thoroughly investigated in the context of sexual selection is repertoire size, as it is often excessively large for the purposes of species identification or individual recognition and, therefore, is a probable sexual ornament (Andersson 1994; Catchpole and Slater 1995). Song usage (e.g., rate and song duration) has also been interpreted as sexually selected (e.g., Wasserman and Cigliano 1991; Vehrencamp 2000; Nolan and Hill 2004).

Phonological and syntactical features of bird song have been less addressed in the context of sexual selection, although there has been ample experimentation on those traits in the context of species recognition (Becker 1982; Nelson 1989). Song differences in phonology and syntax might entail different physiological demands (Suthers et al. 1999), so that these traits can potentially signal aspects of quality and be subject to sexual selection (Gil and Gahr 2002; Vehrencamp 2000). Furthermore, phonology and syntax of song are highly variable between species, and

some exhibit conspicuously exaggerated phenotypes, as usually occurs in sexually selected traits.

This is the case in the serin (*Serinus serinus*), whose songs are remarkable for their high-pitched frequency and fast, continuous delivery of different song elements (Mota and Cardoso 2001). The serin is a small, socially monogamous passerine that lives in open, temperate habitats (Cramp and Perrins 1994), and its song behavior is important for mate guarding and female stimulation (Mota 1999; Mota and Depraz 2004). The mean frequency of maximum intensity in serin song is 6.3 kHz (Mota and Cardoso 2001), which is more than 2 kHz higher than what is expected for its body size (Wallschläger 1980) and the highest in its genus (among 27 *Serinus* spp. studied, Cardoso and Mota 2007). Syllable rates in the serin are also very fast, their songs having the shortest inter-syllable intervals in the genus (Cardoso and Mota 2007).

Song frequency and syllable rate can be clearly identified as evolutionarily derived characteristics in the serin, for two reasons. First, these phenotypes are the most extreme within the genus *Serinus* (see above), suggesting that the high frequency and the fast syllable rate of serin song evolved away from more common phenotypes with lower frequency and slower rates. Second, these extreme phenotypes cannot be the ancestral state of the genus because the speciation of the serin is relatively recent and is located in a speciose lineage, thus far from the root of the phylogenetic tree (Arnaiz-Villena et al. 1999). Because these song traits are so extreme in the serin and because serin songs are among the most complex in their genus (Cardoso and Mota 2007), it is likely that these traits were sexually selected. In this paper, we assume that sexual selection played a role in the evolution of these apomorphies and ask whether intra- or inter-sexual selection is the most likely cause for their evolution.

We tested male and female serins for behavioral discrimination of song frequency and syllable rate with two experimental designs. In a first experiment, we monitored vocal responses and attendance to playbacks, and in a second experiment, we used a simple approach/no approach design. The behaviors assessed in these experiments are not sex-specific and, therefore, can potentially be used to compare the responses of males and females. If the frequency or syllable rate of serin song evolved by inter-sexual selection, we predict that females will respond more to high frequency or fast song, respectively. If these traits evolved due to their intra-sexual function, we predict that males should discriminate between different song frequencies or syllable rates. Song-mediated male–male interactions are mostly agonistic and, in this context, a more effective signal may be one that is more threatening and inhibits response (e.g., Kingston et al. 2003; Illes et al. 2006). Therefore, we evaluated whether response differ-

ences to stimuli types were due to one stimulus type enhancing responses or, alternatively, to the other stimulus directly inhibiting responses.

Materials and methods

General and playback stimuli

We captured adult serins with mist nets in areas of cultivated fields interspersed with shrubs and trees near Coimbra, Portugal. Birds were kept in one-sex groups of four or five birds, in 75×35×30 cm cages, in an aviary with natural light and ventilation. They were provided seeds and water ad libitum and regular supplements with minerals, proteins, and vitamins. For experiment 1, we captured females from January to June 2002, and experiments took place from March 12th to August 12th (29 birds). Furthermore, for experiment 1, we captured males in January and May 2003, and experiments took place from February 23rd to June 18th (25 birds). For experiment 2, we captured 19 males and 20 females in January and February 2005, and tests were made from February 16th to March 31st. The experiment dates span from early to late breeding in this part of the species range.

In each experiment, we used a different playback set for each bird to avoid pseudoreplication. We digitized recordings with a 22.050-kHz sample rate, applied a high pass filtered at 2 kHz, and then normalized their volume. Loudspeakers' volume was set so that the normalized playbacks sounded realistic in the test room, as perceived by the human ear. All song manipulations were done with the software Avisoft SASLAB v. 4.34 (Avisoft Bioacoustics, Berlin). In both experiments, we tested birds' responses to three pairs of opposed song stimuli: (1) natural serin song vs heterospecific song, (2) high- vs low-frequency serin songs, and (3) fast vs slow serin songs.

(1) Conspecific stimuli were produced from songs of 30 different males, selected for quality of recordings. Heterospecific stimuli were prepared from recordings of 30 birds of 19 congeneric species with good quality recordings available at the National Sound Archive, London. All these species are allopatric to the serin. Stimuli sets for the conspecific vs heterospecific tests consisted of unmodified serin songs and congeneric songs (e.g., Fig. 1a and b). Congeneric songs were truncated at a natural silence to have the same length as the paired serin song. As *Serinus* spp. songs do not generally have predetermined ending syllables (e.g., Güttinger 1985; Mota and Cardoso 2001), this produces syntactically normal songs. Song length of experiment 1 stimuli averaged 5.72 s (±0.99 SD),
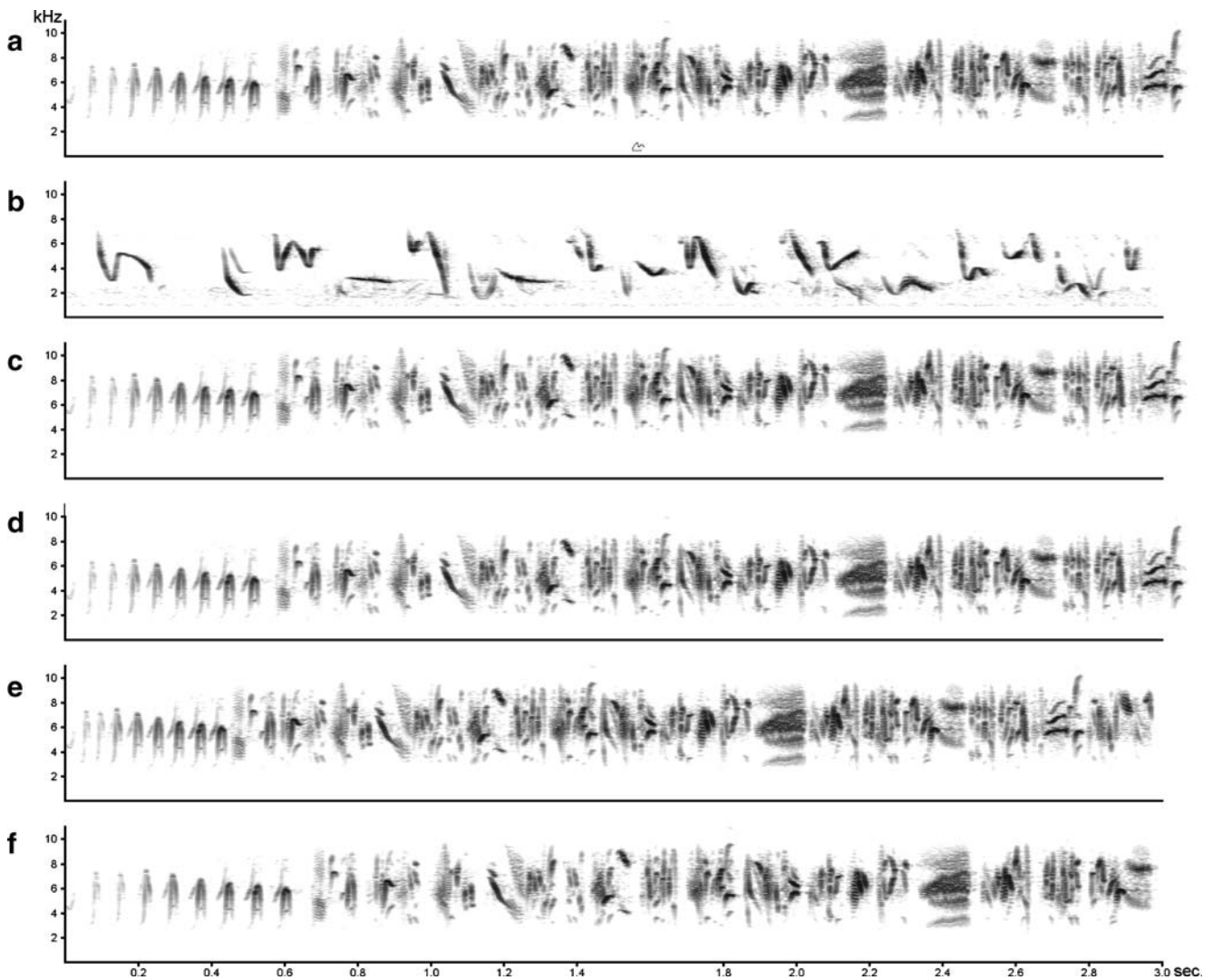
Fig. 1 First 3 s of a set of song stimuli used in the experiments. **a** Natural serin song. **b** Paired heterospecific song, in this case *Serinus leucopygius*. **c** and **d** High- and low-frequency song, produced from **a** by shifting the song 1 kHz higher or lower. **e** and **f** Fast and slow song, produced from **a** by changing inter-syllable intervals

which corresponds to relatively long serin songs (Mota and Cardoso 2001).

(2) Each stimuli set for the frequency experiment consisted of a serin song with the frequency shifted 1 kHz higher and the same song with the frequency shifted 1 kHz lower. This frequency manipulation uses a fast Fourier transformation and is additive, so that the frequency bandwidth of the two manipulated songs is identical (Fig. 1c and d). As serin songs have a wide frequency bandwidth (about 8 kHz wide, Mota and Cardoso 2001), manipulated songs have dominant frequencies well inside the species frequency range.

(3) We produced stimuli for the syllable rate experiment by manipulating the lengths of inter-syllable intervals within their natural range of variation. The modal length of inter-syllable intervals in serin song is about 20 ms, and the shorter inter-syllable intervals are approximately 10 ms long (Mota and Cardoso 2001). We made fast stimuli by reducing inter-syllable intervals to 10 ms (Fig. 1e). This is the largest manipulation possible that keeps within the natural spacing of syllables in serin song. Paired with each fast stimulus, a slow song was produced by adding 10 ms of silence to the inter-syllable intervals of the same original serin song and then truncating the song at a natural silence so that its length approximates that of the fast one (Fig. 1f). This alters interval duration by approximately the same degree as in the fast stimuli. We did not set a fixed interval length for slow stimuli (i.e., circa 30 ms) because some natural intervals are longer than this (Mota and Cardoso

2001). Average duration of songs in these sets was 5.33 s (±0.92 SD).

Experiment 1

Each bird was used once in each of the three tests (conspecific vs heterospecific song, high- vs low-frequency song, and fast vs slow song), and tests with the same bird were at least 5 days apart. The order of tests changed from bird to bird in a balanced way.

Tests took place in a large indoor aviary (Fig. 2a) with a sand floor and artificial light closely matching natural

Fig. 2 **a** Diagram of top view of experimental room for experiment 1. *Solid lines* are walls, and the *clear line* is a glass wall separating the larger compartment from the two others. Height of the room is 220 cm. *MP* Perches with a mounted male at 150 cm high, *P* perches with infrared sensors at 150 cm high, *LS* 5-W loudspeakers at 220 cm high, *WS* platform with water and seed containers at 88 cm high. Size of perches and platform drawn to scale. **b** Diagram of top view of experimental cage for experiment 2. Position of the three perches and the water and seed containers are shown. Perches are at the height of 14 cm from the cage floor and the height of the cage is 35 cm

daylight time. A glass wall separated a larger compartment from two adjacent smaller compartments. There was a mounted serin male on a perch facing the glass in each of the smaller compartments when testing females, but not males, because the visual presence of a male is necessary for females of some species to express their song preferences (Tchernichovski et al. 1998). The two male mounts had typical male yellow coloration and were in upright perching posture. Possible effects of mount differences on female behavior were controlled for both by presenting playback types in one or the other side of the testing room in a balanced way across tests and also by including side of playback as a factor in the statistical analysis (see below). Above the glass, there was a 5-W loudspeaker (Sony SRS-A57) in front of each male mount and facing the large compartment. Inside the large compartment, there were two wood perches equipped with infrared sensors, one in front of each loudspeaker. The sensors were connected to a computer outside the room that monitored attendance to the perches and that controlled playbacks. There were seeds and water on a platform near the wall opposite to the perches and the loudspeakers (Fig. 2a).

We introduced each experimental bird to the large compartment the night before testing, to allow for acclimation, and ran tests the following morning, starting within 2 h after room lights were illuminated. In the first part of the test, which lasted 27 min, birds were presented song playbacks, and their vocal responses were tape-recorded. A playback stimulus was repeated ten times through one speaker, at 15-s onset-to-onset intervals, thus forming a block of 150 s. After a silent interval of 30 s, the opposite stimulus, with the same temporal arrangement, was played through the other speaker. A total of nine stimuli blocks were alternatively presented, so that the stimulus type that began each test also ended it, to account for a possible decrease in responsiveness due to habituation. The order and side of presentation of stimuli types was changed between birds in a balanced way.

The second part of the test started 3 min after the end of the first and lasted until 1900 hours. During this period, the loudspeakers were silent unless the bird moved onto a perch, which caused the speaker in front of it to play the stimulus it had played in the first part of the experiment. This stimulus was then repeated every 10 s while the bird remained perched. The computer maintained a record of visits and time spent on perches.

Behavioral analysis

In the first part of the tests, we measured (1) the number of vocalizations given per block of playback and (2) the average number of syllables per contact call, which
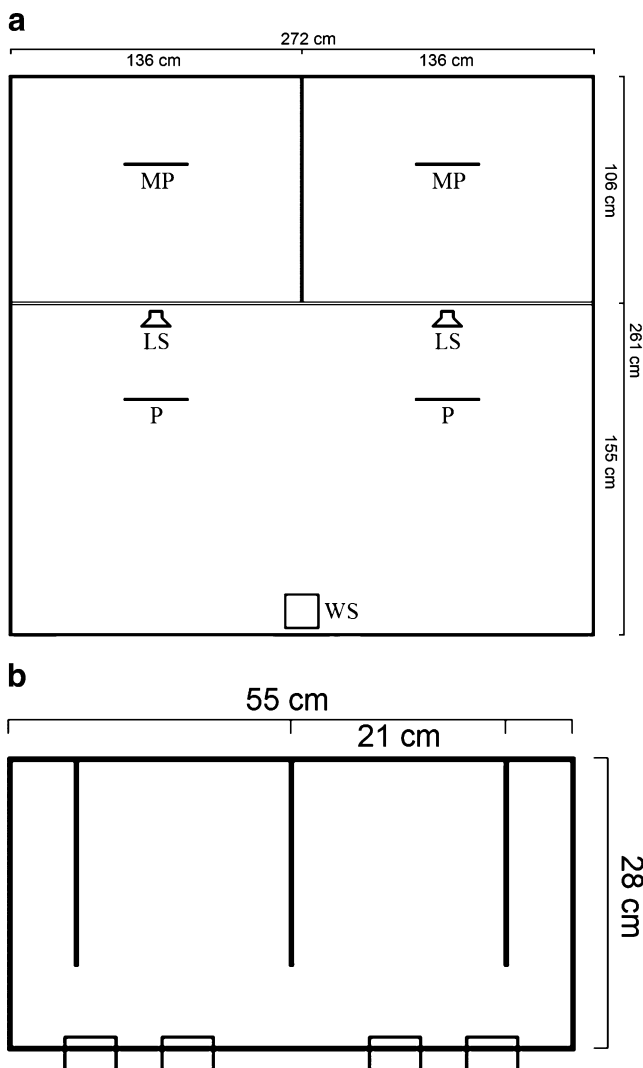
indicates the intensity of each call. Contact calls are the most common type of serin vocalizations in the wild and also in this experiment (94% of vocalizations in the female tests were contact calls). They consist of a single trill (Cramp and Perrins 1994), always separated from other calls by an interval several orders of magnitude longer than the inter-syllable intervals, and the number of syllable repetitions within the trill varies in different renditions (Pacheco 2002). When testing males, we took two additional vocal measures: (3) the number and (4) average duration of songs sung per block of playback.

In the second part of the experiment, we recorded (1) the number of visits to each perch and (2) the average visit duration at each perch. Discrimination of stimuli types by the number of perch visits implies that the birds learn to associate each perch with the song it triggers (e.g., Riebel and Slater 1998). The duration of perch visits can be regarded as an immediate reaction to the playback and should be an effective response independently of whether there was previous learning or not.

In each test, the behavioral variables not fitting a normal distribution (D'Agostino and Pearson test, in Zar 1996) were normalized with a $\log(x+1)$ transformation. Responses to opposed stimulus types in each experiment were then compared with a two-tailed repeated measures analysis of variance (ANOVA). Order of playback and side of playback enter the model as between-subjects factors to control their effect statistically. Tests with zero responses to both stimulus types were eliminated, as these birds did not show evidence of reacting to the experiment at all (sample sizes in Table 1). As some birds only responded vocally or by visiting the perches, sample sizes differ for different behaviors.

Distinguishing stimulation from inhibition

It is generally assumed that differences of response are due to increased responsiveness to the stimulus type with the highest score, which, in the case of females, indicates a preference. When basal levels of response are low, this is the simplest interpretation and should be correct in most cases. However, it is possible that different responses result from inhibition by the other stimulus (e.g., Kingston et al. 2003; Illes et al. 2006). These two situations can be distinguished by analyzing behavioral responses with graded levels of intensity. In a preference situation, response differentials should be statistically dependent on the high intensity responses, as the subjects will respond at above average levels to preferred stimuli. On the contrary, in an inhibition situation, the differential should be caused by the lowest intensity responses, as the subjects will be constrained to sub-average response when faced with inhibiting stimuli.

We assume that the number of syllables per contact call is a graded intensity response: contact calls with the fewest syllable repetitions can be considered the lowest intensity responses and vice-versa. Contact calls with very few syllable repetitions (often just one syllable) were common in the birds' responses to this experiment (one-syllable contact calls averaged 10.8% of contact calls across all female tests), while in nature, these consist almost always of a trill, with a variable number of syllable repetitions (Pacheco 2002). We assessed the influence of these lowest-intensity calls in the response differentials by repeating the analysis of number of syllables per call after eliminating the one-syllable contact calls. If test statistics remain unchanged or are strengthened, then the original difference can be viewed as driven by medium- and high-intensity vocalizations, which is compatible with the increased response of a preference. On the contrary, if the difference becomes weaker and loses significance, then the original difference can be regarded as having been caused by the low-intensity vocalizations, indicating inhibition by the stimulus that evoked the lesser response.

Table 1 Statistics of two-tailed repeated measures ANOVAs for the behavioral responses of females in experiment 1

Test	Vocalizations	Syllables per call	Visits to perch	Average time on perch	Syllables per call (excluding one-syllable contact calls)
Conspecific vs heterospecific song	N=27	27	24	24	24
	F=3.29	6.96	0.02	1.33	6.52
	P=0.08	0.01	0.88	0.26	0.02
Song frequency	24	24	22	22	19
	1.35	2.82	0.10	10.16	3.26
	0.26	0.11	0.76	0.005	0.09
Syllable rate	26	26	23	23	20
	5.85	5.72	0.64	0.00	0.13
	0.02	0.03	0.43	0.99	0.72

In all cases, the remainder degrees of freedom is $N-4$.

Experiment 2

Tests were made in a birdcage (Fig. 2b) placed in the center of an empty, symmetrical compartment (large compartment of Fig. 2a), at a height of 75 cm above the ground. On the front of the cage, there were water and seed containers, and on the back, there were three perches equipped with infrared sensors. Containers and perches were placed symmetrically (Fig. 2b). At a distance of 100 cm from each side of the cage, there was a 5-W loudspeaker (Sony SRS-A57). Sensors and loudspeakers were connected to a computer outside the room that monitored bird movements and controlled playbacks. Each bird was tested once; males and females were tested on alternating days. A bird was placed in the experimental cage on the night before the testing day and was removed the following night. Tests began and ended simultaneously with the lighting of the room, which turned on approximately 15 min before sunrise and off approximately 15 min after sunset.

When the bird perched on the central perch and stayed there for 2 s, the computer played once one of six stimuli: a conspecific or heterospecific song, a high- or low-frequency song, a fast or slow song. The stimulus and the loudspeaker it was played through were randomly assigned each time the bird perched, so that no associations with side or sequence could be established. Because in this experiment the side of presentation of stimuli was randomized from playback to playback, we did not use male mounts. During 1 min from the beginning of the playback, the computer registered if the bird approached the playback (i.e., if the bird flew to the perch near the active loudspeaker). During the next 2 min, the system was paused, not producing any playback. After this time, if the bird was already on the central perch, the system waited for it to leave and only then became ready for new playbacks. With this experimental design, the conditions in which the birds listened and responded to stimuli were standardized and ideal (i.e., the bird was equidistant from the two loudspeakers). It also has the advantages that (1) the experimenter does not intervene, and (2) simple approach/no approach responses can be obtained without previously conditioning the birds (Falls 1992).

We used 30 different sets of song stimuli. Each fast, slow, high-, and low-frequency stimulus was produced from the conspecific song in the same set. Each bird was tested with a different set. After we tested the 15th female or the 15th male, we used again the stimuli set of the same-sex test that gave the fewest responses so far (i.e., with less playbacks elicited) and then discarded the test with the fewest responses. This way, we obtained 15 female tests and 15 male tests, all using different stimuli sets.

Analysis

For each stimuli type, we calculated the proportion of times the bird approached the playback. In this calculation, we discarded events in which the bird flew from the central perch but did not land on any perch, as we could not determine whether these movements were approaches or not. Several birds flew to the right or left perch much more often than 50%, denoting a bias for one side of the cage. To control for this, in each bird, we regressed the proportions of approach to each of the six stimuli types on the proportions of times that these stimuli were played on one side. We then used the residual proportions of approach instead of the original ones.

All residual proportions of approach to opposed stimuli fitted a normal distribution (D'Agostino and Pearson tests). Comparisons between opposed stimuli types were made with two-tailed repeated measures ANOVAs. Opposed stimuli types are the two levels of the main factor, individual birds are subjects, and sex is a between-subjects factor. A similar analysis was also made for each sex separately (i.e., by paired samples t tests). Comparisons between non-opposed stimuli were not attempted because song manipulations alter aspects of song that are not controlled for in cross comparisons: frequency manipulations modify the natural harmonic relations (octaves, etc.) and therefore should not be compared with the others; furthermore, rate manipulations alter the total duration of the song and therefore should not be compared with the others.

Results

Experiment 1

Females vocalized with more syllables per contact call in response to conspecific than to heterospecific playbacks ($P=0.01$, Table 1, Fig. 3a). Females also vocalized more often in response to conspecific playbacks, but this was not significant ($P=0.08$). Differences in perching behavior were not significant in this test (Table 1).

In the song frequency test, females spent, on average, more than twice as long perched during visits to high-frequency perches than to low-frequency perches ($P=0.005$, Table 1, Fig. 3b). This bias in perching duration withstands correction for multiple comparisons (Bonferroni adjusted critical alpha is 0.012). Both vocal variables also scored higher to high-frequency song, but the differences were not significant (both $P>0.1$, Table 1, Fig. 3b). The difference in number of visits to perches was also not significant ($P=0.76$, Table 1).

Fig. 3 Mean response untransformed scores of females per block of playbacks in experiment 1 and standard errors of the mean. **a** Test of conspecific vs heterospecific song. **b** Test of high-frequency vs low-frequency serin songs. **c** Test of fast vs slow serin songs. *Asterisk* Significant differences by a two-tailed repeated measures ANOVA (Table 1)
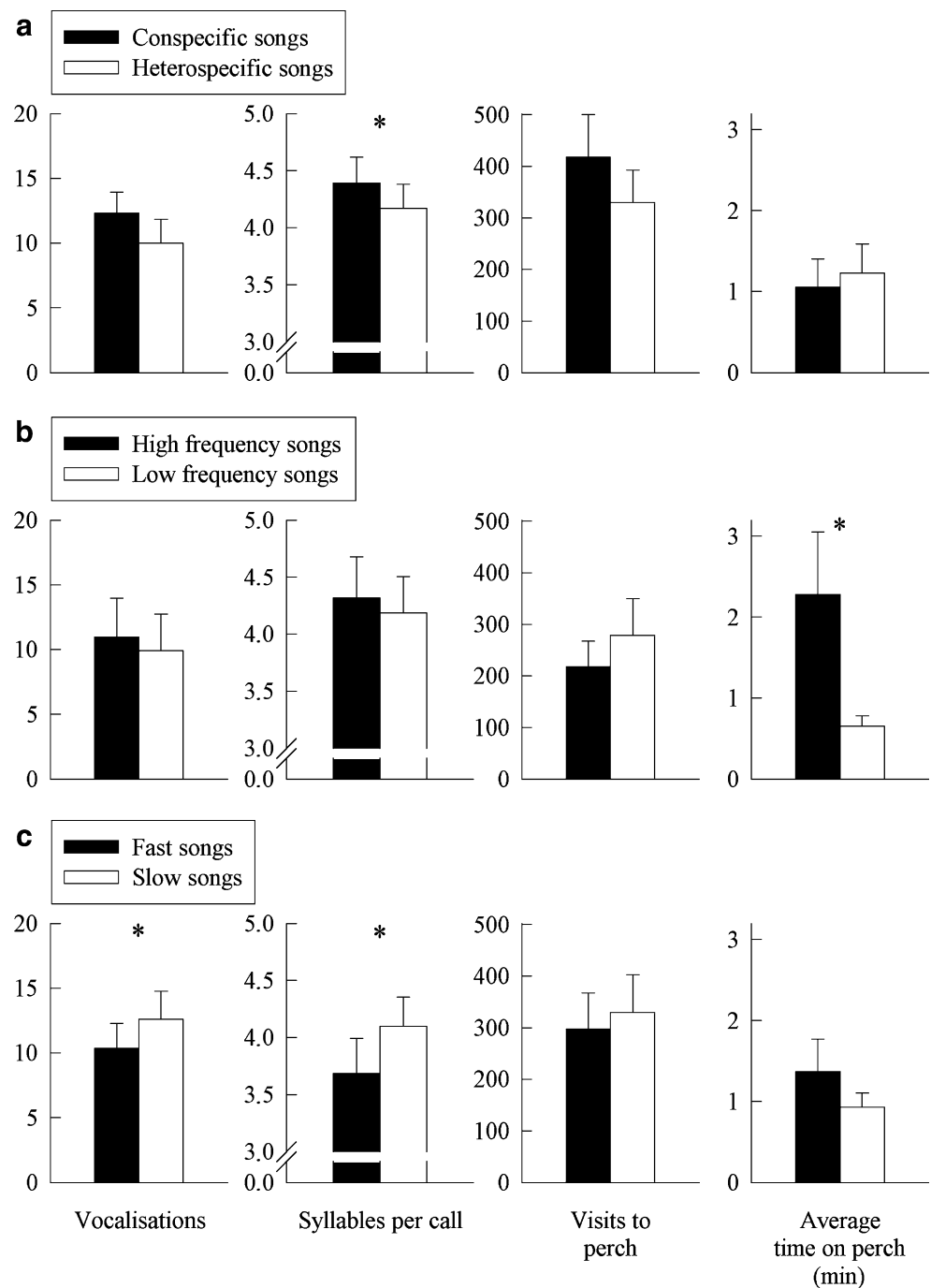
In the syllable rate test, the number of vocalizations was significantly higher in response to slow songs ($P=0.02$, Table 1, Fig. 3c), and the average number of syllables per contact call was also higher ($P=0.03$). The differences in perching behavior were not significant in this test (both $P>0.43$).

To determine if some of these differences in female response were caused by an inhibitory effect, we analyzed the average number of syllables per contact call after excluding the lowest-intensity vocalizations. There were only very small changes in the results of the conspecific vs heterospecific test and the frequency test (Table 1). On the contrary, in the rate experiment, results changed markedly (F decreased to near zero, Table 1), indicating that the behavioral discrimination of syllable rate was specifically due to a reduced intensity of responses to fast songs.

In tests with males, many individuals did not respond (25 males tested; birds that responded varied between 8 and 18 for different behaviors). Using this experimental design,

males did not show discrimination between any pairs of song stimuli with any of the six behavioral responses (all $F < 2.30$, all $P > 0.16$).

Experiment 2

The average number of playbacks per test in this experiment, induced when birds perched on the middle perch, was 116.5 (males 123.7, females 109.3; difference between the sexes not significant: t test, $t = 0.98$, $P = 0.34$, $N = 30$). The average proportion of approach to all playbacks was 0.41, and males had a non-significant tendency to approach more often than females (males 0.43, females 0.38; t test, $t = 1.74$, $P = 0.09$, $N = 30$).

The proportion of approaches was greater to conspecific than heterospecific song in males (paired samples t test, $t = 2.43$, $P = 0.03$, $N = 15$) but not in females (paired samples t test, $t = 0.89$, $P = 0.39$, $N = 15$, Fig. 4a). Analyzing both sexes together, the approaches to conspecific and to heterospecific songs did not differ significantly (repeated measures ANOVA, $F_{1,28} = 0.77$, $P = 0.39$). The interaction with the sex (whether the sexes differ in their tendency to approach more one or the other stimulus) was significant ($F_{1,28} = 5.02$, $P = 0.03$), and the inter-subjects effect of sex (whether overall one sex approaches more than the other) was not significant ($F_{1,28} = 1.23$, $P = 0.28$).

The proportion of approaches to high- and low-frequency songs did not differ significantly when considering both sexes together (repeated measures ANOVA, $F_{1,28} = 0.02$, $P = 0.89$). The interaction with sex was not significant ($F_{1,28} = 3.21$, $P = 0.08$), but the inter-subjects effect of sex was significant ($F_{1,28} = 4.52$, $P = 0.04$). The inter-subjects effect was due to females approaching high-frequency songs more than males (Fig. 4b; t test, $t = 3.55$, $P = 0.001$, $N = 30$; note that this difference is not a methodological artifact, as experiment-wide female average approach to playbacks was actually lower than male's, see above). The sexes did not differ in their approach to low-frequency songs (Fig. 4b; t test, $t = 0.11$, $P = 0.92$, $N = 30$).

The proportion of approaches to fast songs was lower than to slow songs when considering both sexes together (repeated measures ANOVA, $F_{1,28} = 5.04$, $P = 0.03$). The

Fig. 4 Mean residuals of approach to playbacks in experiment 2 (side biases controlled for, see text) and standard errors of the mean. Sample sizes are 15 males and 15 females. **a** Approaches to conspecific vs heterospecific song. **b** Approaches to high-frequency vs low-frequency songs. **c** Approaches to fast vs slow songs. *Asterisk* Significant difference of approach between stimuli in one sex (paired samples t test, $P < 0.05$, see text), *dagger* significant difference of approach between stimuli in entire sample (repeated measures ANOVA, $P < 0.05$, see text), *double dagger* interaction with sex (**a**) or between-subjects effect of sex (**b**) significant (repeated measures ANOVA, $P < 0.05$; see text for details)
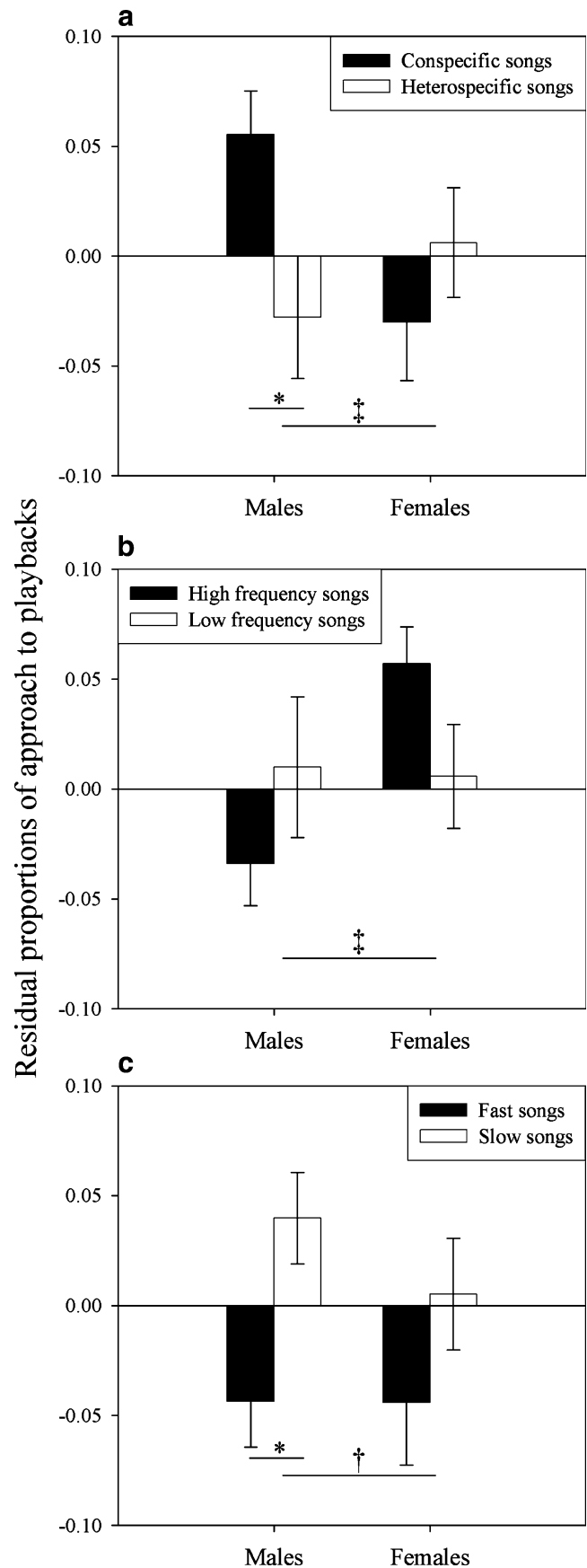
interaction with sex and the inter-subjects effect of sex were not significant ($F_{1,28}=0.33$, $P=0.57$, and $F_{1,28}=1.06$, $P=0.31$, respectively). Considering each sex separately, the difference remained detectable in males (Fig. 4c; paired samples t test, $t=2.34$, $P=0.04$, $N=15$) but not in females ($t=1.05$, $P=0.31$, $N=15$).

Discussion

Female serins showed discrimination of conspecific vs heterospecific song in experiment 1, and they also showed discrimination based on song frequency and syllable rate. Males did not show any discrimination in experiment 1, but in experiment 2, they actually showed stronger discrimination than females. This may be due to sex differences in context-specific motivation or decision rules that make them more responsive with one or the other design. For example, the simplicity of the behavioral task in experiment 2 may be more favorable for males to show discrimination in this laboratory setting, while the presence of male mounts in experiment 1 may have contributed for female responsiveness (e.g., Tchernichovski et al. 1998). Overall, we find no evidence that males differ from females in discrimination abilities, as both showed discrimination of fine song traits in one of the experiments.

In other species, the expression of song discrimination also depends on testing context. For example, females are typically more discriminating in laboratory sexual responses compared with male territorial responses (reviewed in Ratcliffe and Otter 1996), but Nelson and Soha (2004) found that female white-crowned sparrows (*Zonotrychia leucophrys*) tested in field conditions actually show less discrimination than males. Studies that attempted to test both sexes with the same method either did not find sex differences in discrimination (Zimmer 1982; Diehl and Helb 1986; Clayton 1988; Johnsrude et al. 1994; Ikebuchi and Okanoya 2000; Riebel et al. 2002) or attributed differences in discrimination to motivational aspects (Cynx and Nottebohm 1992; Ikebuchi et al. 2003; Nelson and Soha 2004). Our results agree with these studies in suggesting that differences between the sexes in expressed discrimination result more likely from different decision rules or motivation rather than different discrimination abilities per se.

On the assumption that sexual selection played a role in the evolution of song frequency and syllable rate in the serin, the type of behavioral discrimination obtained and the differences found between males and females may provide insight into whether intra- or inter-sexual selection was the most relevant (e.g., Leitão and Riebel 2003; Anderson et al. 2007). The result of the first experiment suggested that females prefer high-frequency songs. It was unexpected that females would use different behaviors to discriminate

song frequency and to discriminate conspecific from heterospecific song, but it is possible that species recognition and within species song preferences trigger different behaviors. Furthermore, the second experiment corroborated a uniquely inter-sexual function of song frequency, as males did not behave differently towards high- and low-frequency songs and differed significantly from females in their response to high-frequency song. The result of the first experiment also suggested that fast syllable rate is an aggressive signal, as this was the only stimulus type that inhibited female vocal responses. Aggressive signals are more consistent with evolution by intra-sexual selection, which predicts that the effect of syllable rate should extend to males. This was also confirmed with the second experiment.

Song frequency

Responses of male and female birds to variation in absolute frequency of song were studied in two other species of birds: blackbirds (*Turdus merula*; males, Dabelsteen and Pedersen 1985; females, Dabelsteen and Pedersen 1993) and white-throated sparrows (*Zonotrichia albicollis*; males, Hurly et al. 1992; females, S.B. Meek, L. Ratcliffe and R. Weisman, unpublished data, in Ratcliffe and Otter 1996). In both species, females, but not males, respond more to high- than low-frequency songs, like in the serin. Although the female responses measured were sexual, the authors interpreted their results only in the context of species recognition. Dabelsteen and Pedersen (1993) explained the difference between the sexes as an adaptation of females to sound degradation, as female blackbirds occupy a microhabitat of denser vegetation than males. This explanation is only applicable to species where males and females preferentially occupy different microhabitats, which is not the case in the serin. On the contrary, a female preference explanation is applicable to the serin, and it is also suggested by the inter-sexual function of its song (Mota 1999; Mota and Depraz 2004) and by its extreme high frequency within the genus. A female preference for high frequency song is also suggested by the recent finding that male chestnut-sided warblers (Dendroica pensylvanica) with high frequency songs have greater extra-pair reproductive success (Byers 2007).

A female preference for high-frequency song in passerines would contrast with the preferences that are often found in non-avian taxa for low-pitched vocalizations (e.g., Brown et al. 1996; Howard and Young 1998; Collins 2000; review in Andersson 1994). These non-avian preferences are thought to originate because low frequencies are size dependent (e.g., Davies and Halliday 1978; Pfefferle and Fischer 2006) and can thus signal quality or competitive advantage of males (e.g., Davies and Halliday 1978; Ladich 1998) and explain adaptive female preferences (Berglund et al. 1996). This may not apply to birds that interact mostly

in the air, where smaller sizes can have a competitive advantage due to greater flight agility (Andersson and Norberg 1981). Agility is particularly important to many birds that, like the serin, fight and display in the air (Hedenström and Møller 1992; Mota 1999), and there is evidence from non-passerine birds that females prefer smaller and more agile males (Hakkarainen et al. 1996; Grønstøl 1996; Blomqvist et al. 1997; Figuerola 1999).

Furthermore, singing at high frequencies may be costly because it presumably requires increased muscular contraction (Suthers et al. 1999). We may hypothesize that this makes high song frequency an indicator of male quality. For example, the physiological cost per se of producing high vocal frequencies may be a general handicap related to male condition. It is also possible that, if singing under strong muscular contraction is difficult, other song traits could be affected (e.g., temporal accuracy, loudness), and high frequency could thus be an amplifier handicap of male vocal ability. In support of this view, there is evidence that singing higher-frequency song types disturbs bout patterns in the great tit (*Parus major*, Lambrechts 1997) and that shifting to higher song frequencies decreases note loudness and length in black-capped chickadees (*Poecile atricapillus*, Christie et al. 2004). Such indicator mechanisms can function equally in mammals and other vertebrates, but in those taxa, it would be opposed by the possibly stronger signal value of the size-related low-frequency vocalizations.

Alternatively, it is possible that the female preference is unrelated to any intrinsic signal value of high-frequency song. Sensory bias seems unlikely, as the auditory system of small birds is most sensitive to frequencies lower than the ones in serin song (Okanoya and Dooling 1987; Okanoya et al. 1990). However, the preference might, for example, be an ontogenetic (Guilford and Dawkins 1991) or evolutionary (Enquist and Arak 1993) consequence of species recognition, as serin song frequency is higher than that of most sympatric species. Studies of female responses to song frequency in more bird species and tests of the signal value of song frequency are needed to evaluate these hypotheses. Our result, however, suggests that sexual selection can intervene in unexplored ways in the evolution of this very basic property of bird song.

Syllable rate

We expected females to respond more strongly to fast songs because this is the derived condition in the serin and also because female preferences for fast syllable rates are known in other species, one of which is a congeneric of the serin: the canary (*Serinus canaria*; Vallet and Kreutzer 1995; Vallet et al. 1998; Drăgănoiu et al. 2002) and the swamp sparrow (*Melospiza georgiana*; Ballentine et al. 2004).

Most of the work with canaries and swamp sparrows tests the effect of syllable rate in conjunction with frequency bandwidth, as maximizing both may indicate higher vocal performance (Podos 1997), but Drăgănoiu et al. (2002) manipulated rate and bandwidth separately and still obtained significant effects of rate alone. Furthermore, female dusky warblers (*Phylloscopus fuscatus*) have more extra-pair offspring from males with greater proportion of sound within songs (Forstmeier et al. 2002). Like the fast songs in our experiments, this trait has a smaller ratio of interval to syllable duration, which implies greater demands on the song production physiology (Suthers et al. 1999). Therefore, faster syllable rates with shorter intervals in between could indicate male quality much in the same way as song rate can (Vehrencamp 2000).

Female vocal responses were, however, lower to fast than to slow songs. Female vocal responses in the closely related canary tend to replicate the same pattern of sexual responses (Nagle et al. 2002). Therefore, syllable rate does not seem to serve an inter-sexual function in the serin; if anything, it seems to hinder it. Fast songs were the only stimulus type that inhibited vocal responses, which suggests an aggressive and mostly intra-sexual role for syllable rate. This was also supported by experiment 2 where, as predicted, males approached fast song less often. These results agree with the recent finding of Illes et al. (2006) that territorial banded wren males (*Thryotorus pleurostictus*) perceive fast song as a more threatening signal and are repelled by it.

Because bird song serves both intra- and inter-sexual functions, it is possible that a song trait adaptive to one function trades off with the other. For example, chaffinch song (*Fringilla coelebs*) contains a trilled and a non-trilled portion, and songs with a longer relative length of trills are more efficient as male–male signals but are less attractive for females (Leitão and Riebel 2003; Riebel and Slater 1998). A similar phenomenon could explain the responses of male and female serins to syllable rate, whereby fast songs seem to be adaptive in male–male interactions but are also less attractive to females. Whether syllable rate is generally adaptive for its dual intra- and inter-sexual function, as indicated by work with other species (males, Illes et al. 2006; females, Drăgănoiu et al. 2002; Forstmeier et al. 2002; Ballentine et al. 2004), or whether one function may prevail over the other, as suggested by our experiments, remains to be determined.

Taken together, our results indicate that the apomorphies of serin song cannot be explained as the outcome of a single evolutionary process. While female preference is compatible with the evolution of high-frequency song in this species, syllable rate provokes a distinct pattern of response and seems more likely to be selected in an intra-sexual context.

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