



Female European starling preference and choice for variation in conspecific male song

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Data from several field studies support the hypothesis that female European starlings, *Sturnus vulgaris*, attend to variation among the songs of conspecific males when making mate-choice decisions. However, for a variety of methodological reasons, direct evidence for female preferences based on song in starlings has been lacking. This study presents a novel technique for assaying directly female preference and choice in European starlings by using the presentation of conspecific male song as an operant reinforcer in a controlled environment. Using an apparatus in which the playback of songs from different nestboxes is under the operant control of the subject, we demonstrate how the reinforcing properties of conspecific song can be used to measure female preference and choice. The results of the study suggest three conclusions. First, female starlings prefer naturally ordered conspecific male songs over reversed songs. Second, female starlings display robust preferences for longer compared with shorter male song bouts. Behaviour in the operant apparatus varied directly with male song bout length. Third, preferences based on song bout length are sex specific. Male starlings failed to respond differentially to the same stimuli for which females showed strong preferences. These results suggest that male–male variation in song bout length is important for mate choice among starlings. In addition, we detail the use of a novel behavioural assay for measuring female preferences that can be applied to similar behaviours in other species of songbirds.

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Female mate choice based on variation in one or more male characteristics is a fundamental component of sexual selection theory (Darwin 1871). From a proximate perspective the process of female choice is thought to act through mechanisms of female preference. A female preference can be defined as any trait that makes a female more likely to mate with one male compared to another (Kirkpatrick & Ryan 1991). Among songbirds, a large body of evidence, from both field and laboratory experiments, links variation in female behaviour and mating success to variation in male song. In particular, variation in male song bout length and repertoire size can influence female choice in a number of species (reviewed by Catchpole & Slater 1995; Searcy & Yasukawa 1996). Thus, it is reasoned that selection pressures resulting from female choice influence the evolution and maintenance of male song among these species, and that female songbirds possess preferences based on male song. We examined the proximate perceptual mechanisms of

female choice (i.e. female preferences) in a species of songbird, European starling, *Sturnus vulgaris*.

Song and Female Choice in Starlings

Variation in the total song output of male starlings corresponds to several events in their breeding cycle. Copulations between starlings are almost always preceded by bouts of male song (Eens et al. 1989; Eens & Pinxten 1995; Mountjoy & Lemon 1996). Song output in mated males peaks just prior to pairing (Hindmarsh 1984; Eens et al. 1994), with a second peak just prior to copulation (Eens & Pinxten 1990), and a third prior to egg laying (Eens et al. 1994). After laying, song ceases nearly altogether (Eens et al. 1994). Removal of the female from an active pair of starlings leads to increased male song production (Cuthill & Hindmarsh 1985; Henry et al. 1994). Additionally, the song output of unmated males is relatively constant throughout the breeding season (Kluyver 1933, cited in Eens 1997). When confronted with a conspecific female, unmated captive male starlings sing more song bouts (and sing a greater number of songs in their nestbox) than when confronted with a conspecific male (Eens et al. 1993, 1994).

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Male starlings present their songs in long and elaborate bouts (Adret-Hausberger & Jenkins 1988; Eens et al. 1989), composed of sequentially patterned song types, or motifs. Although each motif comprises several smaller notes, the pattern of notes within a given motif is fairly regular. The total number of different motifs that a male sings defines that male's repertoire size, and a single song bout rarely contains all of the motifs in a given male's repertoire. The number of different motifs contained in a bout is a function of the length of that bout, with longer bouts containing more motifs (Eens et al. 1989). Repertoire size and the mean song bout length of a male are positively correlated with age (Adret-Hausberger et al. 1990; Eens et al. 1992b; Chaiken et al. 1993; Mountjoy & Lemon 1995), and can vary considerably among individuals in the same age class (Eens et al. 1992a).

Variation among male starling songs can also be correlated with mating success. Males that spend more time singing at their nest site tend to initiate clutches sooner after pairing than males that spend less time singing (Wright & Cuthill 1992). Similarly, the first males to obtain mates tend to be those that sing longer song bouts and have larger repertoires (Eens et al. 1991). Average song bout length is also positively correlated with the number of young per male (Eens et al. 1991). In the field, there is a significant negative correlation between repertoire size and the delay between male nestbox occupation and clutch initiation, even when nest site preference is controlled (Mountjoy & Lemon 1996). Moreover, although males that mate earlier tend to be older, neither the condition of a male nor his gross morphology appears to affect the timing of mating. Male body mass and tarsus length are not significantly correlated with initial pairing dates (Eens et al. 1991). Similarly, neither male body mass, tarsus length, culmen length, wing length, nor the length of the iridescence on a male's hackle feathers are significantly correlated with the timing of clutch initiation (Mountjoy & Lemon 1996).

Measuring Female Choice

Previous studies have provided only correlational support (albeit strong) for the idea that female starlings possess preferences based on male song (reviewed by Catchpole & Slater 1995; Searcy & Yasukawa 1996), and more direct measures of female preference in starlings have been difficult to obtain. The most popular laboratory technique for directly assaying preferences in female songbirds is to count the number of copulation solicitation displays given in response to different acoustic stimuli (see King & West 1977; Searcy 1992a for review). Unfortunately, the playback of conspecific song to female starlings under experimental conditions has failed to elicit copulation solicitation displays (see Searcy 1992b; Eens 1997). Similarly, despite the fact that male song functions as an attractant in the field (Mountjoy & Lemon 1991; Smith 1995), attempts to measure female preference in the laboratory using phonotaxis have also been unsuccessful (T. Q. Gentner, unpublished data).

We present a novel behavioural assay for female preference and choice in songbirds using conspecific song

as a reinforcer. The reinforcing effects of song have been described for a number of songbirds (Stevenson 1967, 1969; Petrinovich et al. 1972; but see Dobson 1973; ten Cate 1991). Subsequent investigations of these effects have, however, been restricted to the role of song as a reinforcer in vocal acquisition (Adret 1993b), and to variation in the reinforcement strength of song as a function of photoperiod and sex (Dobson 1973; Calhoun et al. 1993). The role of song reinforcement has only recently been examined in the context of female choice (Riebel & Slater 1998).

The present study combined the reinforcing properties of conspecific song with an operant apparatus that mimicked the natural context in which female choice occurs. Approach to a nestbox serves as the operant behaviour, and the presentation of song from inside the nestbox serves as the reinforcer. The first experiment in this study examined general song-based female preferences using this technique. The second examined female preference as a function of natural variation among male conspecific songs with specific regard to the role of song bout length. The third experiment examined the sexual dimorphism of these behaviours by testing for song-based preferences in male starlings.

GENERAL METHODS

Subjects

European starlings naïve to experimental testing served as subjects. The subjects were wild caught on a farm ca. 50 km north of Baltimore, Maryland, U.S.A., in late December 1996 and January 1997. The sex of each subject was determined by the presence or absence of iris pigmentation and coloration at the base of the beak, both of which are sexually dimorphic characteristics (Feare 1984). Subjects were housed in large (90 × 48 cm and 47 cm high) flight cages that each contained two to four other starlings of the same sex. In experiments 1 and 2, the flight cages were maintained in mixed-sex aviaries containing approximately 10–40 other European starlings and zebra finches, *Taeniopygia guttata*, where the light:dark schedule was synchronized with the natural photoperiod in Baltimore. Subjects in experiment 3 were also maintained in mixed-sex aviaries, but were housed on an 8:16 h light:dark cycle prior to the experiment to maintain photosensitivity (Dawson & Goldsmith 1983). Just before the experiment, the light:dark cycle was changed to LD 14:10 h for the duration of experiment 3 (12 days). The birds had ad libitum access to food (Purina Start and Grow) and water at all times throughout the course of the study.

Apparatus

Figure 1 displays the apparatus used in this study. All experimental sessions were conducted inside a sound attenuating test chamber (IAC, New York, U.S.A.) measuring 2 × 2 m and 1.5 m high. Nestboxes (38 × 18 cm and 22 cm high) were mounted on three walls and

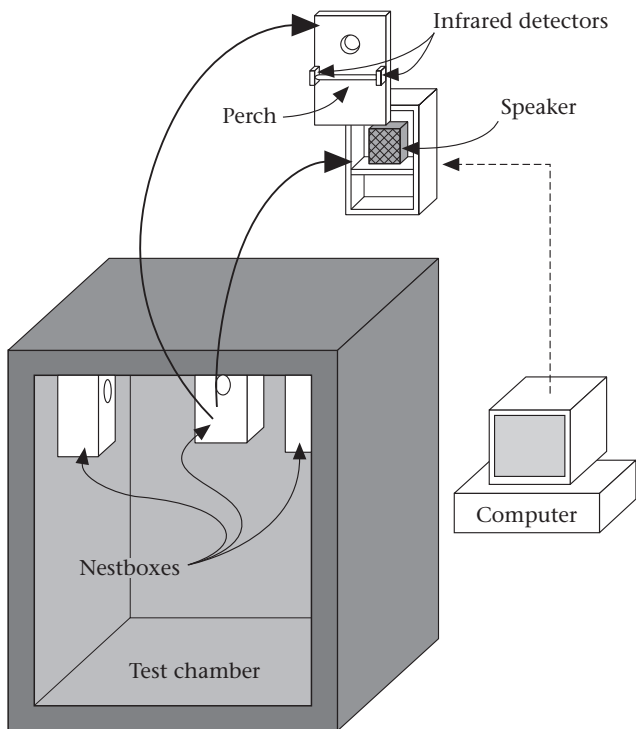


Figure 1. Schematic of the operant apparatus used to measure preferences for a variety of acoustic stimuli. A computer monitored the position of a bird inside the apparatus via infrared detectors on the perches in front of each nestbox and controlled the playback of acoustic stimuli to speakers mounted inside each nestbox. The exact layout and dimensions of the apparatus are given in the text.

centred horizontally so that the base was 1.75 m off the floor of the chamber and the top of each nestbox abutted the ceiling. Except for their respective mounting positions, the three nestboxes were identical. On the front of each nestbox, centred in the upper half, was a 4.5-cm-diameter hole blocked from the inside with a piece of 5-mm wire mesh. A 12-mm-diameter dowel served as a perch, and was mounted parallel to the front of the nestbox 3 cm below the bottom of the hole and extended 5 cm from the front of the nestbox. Two infrared detectors were mounted on either end of the perch. A small speaker (Realistic, model 40-1250A) rested on a shelf inside each nestbox, 18 cm off the floor. An amplifier (Carver, TFM-6CB) powered the speakers and played analogue signals sent from the computer. Heavy black plastic sheeting covered the walls of the test chamber. Food and water were available on the floor of the apparatus at all times. A 60-W incandescent bulb suspended from the centre of the ceiling illuminated the chamber during testing. An 80-MHz/486 PC computer equipped with sound card (SoundBlaster SB16, CreativeLabs) and a digital I/O card (PIO-12, Keithley-Metrabyte) controlled the infrared detectors and the speaker inside each nestbox.

Procedure

At the start of each session, a naïve bird was placed into the testing chamber where it could fly about freely and

feed *ad libitum*. Because the only easily accessible perches were located on the front of the three nestboxes, the subjects spent their time in the apparatus either on the floor, at the feeding station, or on a perch in front of a nestbox. Alighting on one of the perches triggered the playback of a song stimulus from that nestbox. Leaving the perch after the playback of song had begun stopped that particular stimulus presentation.

Each session was divided into two blocks. During the first block, a set of songs from one male, 'male A', played from one nestbox whenever the subject landed on the perch in front of that nestbox. Another set of songs from another male, 'male B', played from one of the other nestboxes whenever the subject landed on the perch in front of that nestbox. If the subject landed on the perch in front of the third nestbox, the 'silent perch', no song played from any of the nestboxes. During the second block, we switched the perches associated with the songs of male A and those of male B so that male B's songs now played from the nestbox that had played male A's songs during the first block, and vice versa. Thus, for preferences to remain constant across both blocks of a session, subjects must alter their behaviour when the locations of the song stimuli are switched. When this happens, the subjects will appear to 'track' the preferable stimuli from nestbox to nestbox, providing a very strong demonstration that their behaviour is under direct control of the song stimuli at each location (i.e. nestbox). Because we also counterbalanced the six possible initial locations of the two stimulus classes across subjects, any position preferences due to factors other than the song stimuli cannot account for preferential behaviour in the first block of a session. Therefore, any preferences observed during the first block but not the second are also noteworthy, albeit weaker than those observed for both blocks. The location of the 'silent' perch remained fixed throughout the entire session.

After landing on a perch the subject had to remain there for a variable interval of 0.5–1.5 s before the stimulus playback actually began. This brief delay prevented transient stops on the perches from triggering stimulus presentations. Once begun, the stimulus presentation continued until either the subject left the perch, or until the song had played through to completion. If the latter, then the subject had to leave that perch and then return in order to hear another song from that same location. The particular stimulus that played from a given perch on any given trial was sampled at random without replacement from the set of all stimuli associated with that perch for that block. To complete a single block, the subject was required to produce a total of 1800 s of song and/or silence from the three perches. Sessions began in the morning, and were almost always completed in less than 8 h. Each subject was tested only once, in a single session. Two of the subjects in experiment 2 and one subject in experiment 3 needed to stay in the apparatus past sunset in order to complete the session. In these cases, the lights and apparatus were turned off and the session restarted the following day at the point where the subject had stopped.

Behavioural Measures and Analysis

For each block in a session, the computer recorded which perch was active, the playback stimulus (if any) and the time spent on that perch. We then used these data to calculate two dependent measures: (1) total time spent on each perch; and (2) the total number of visits to each perch that resulted in stimulus playback. We also calculated the total number of visits to each perch that did not result in stimulus playback, but this measure correlated closely with response measure 2 and was not used in any of the analyses. To examine responses over the course of the session, we divided the total amount of time spent on all three perches during a single block into quarters. Treating the data in this way allowed us to examine the reinforcing properties of different songs associated with each perch over the course of the session. This was intended to mimic the perceptual and cognitive constraints in more natural choice situations where females are likely to be acquiring information about songs from multiple locations (and singers) in a more parallel than serial fashion. We used repeated measures analyses of variance (ANOVA) to quantify any differences between the dependent measures for the two different classes of stimuli over the course of the session. Planned comparisons were used as necessary to examine response differences in the first and second blocks of each session. For data from individual subjects, we refer to a 'preference' as an overall level of responding at any one nestbox that is 25% above the level at any of the other nestboxes. This preference criterion is arbitrary, but none the less useful for discussing preferences among individual subjects. For data pooled across subjects, we refer to any significant differences revealed through the ANOVAs and planned comparisons as 'preferences'.

To examine the specific features of song controlling behaviour in the apparatus, we further analysed the responses to individual stimuli. We used a simple linear regression to compare the level of responding to each song bout with song bout length, the number of motifs per bout, the number of motifs in the first 10 s of each bout, and two measures of motif variability for each bout, stereotypy and the average amount of information per motif. The level of responding was normalized across subjects by dividing the amount of time that an individual spent listening to a given song by the total amount of time that that individual spent listening to all of the songs. We tested the statistical significance of each correlation coefficient using Fisher's r to Z transform. To obtain the stereotypy measure of a given bout, we first generated motif transition probability matrices for each of the two stimulus birds using a library of 21–25 song bouts from each bird. This was the same corpus of songs from which the stimuli for the subsequent experiments were drawn. These matrices contained the transition probabilities for all the ordered pairs of motifs that a given individual could sing. Thus, any song bout from a given individual could be decomposed into a sequence of ordered pairs of motifs, and a transition probability assigned to each ordered pair. We then used these

transition probabilities to calculate the total information in a given song bout, given by

$$H = - \sum_{(i=1 \text{ to } k)} P_i \log_2 P_i \quad (1)$$

where P_i is the transition probability associated with each ordered pair of motifs (1 to k) in that bout. This value, H , is the Shannon entropy (Shannon & Weaver 1949). For two song bouts with the same number of motifs, the Shannon entropy will be larger in the bout for which the transition probabilities between motifs are greater. Thus, the Shannon entropy is a measure of the transition stereotypy in a bout. However, the Shannon entropy for a given bout also increases as the number of motif transitions in a bout increases. To remove the effects of adding more motifs one can simply divide the entropy for a given bout by the number of motifs in that bout. This can be done using two different measures of the motifs in a bout. Dividing the Shannon entropy by the number of ordered pairs of motifs in a bout (k) gives a normalized measure of stereotypy per transition. Dividing by the number of unique transitions in a bout gives a similar measure of stereotypy, but one that is normalized to the number of unique transitions in a bout. We report the latter measure in this study. For the sake of comparison, we have also included a measure of the information per motif for each bout. This is obtained in a similar manner to the stereotypy index above, but instead of using the transition probabilities between motifs to calculate the entropy, one uses the probability of each motif. The two shortest song bouts (2.3 and 3.5 s) were not included in the regression analysis of the individual stimuli.

EXPERIMENT 1: SONG-BASED FEMALE PREFERENCE

Female preferences are defined as any trait that makes a female more likely to mate with a given male (Kirkpatrick & Ryan 1991). One way to demonstrate such preferences is to show that female behaviour is controlled by male-male variation in a relevant characteristic. In experiment 1 we examine very general song-based preferences in female starlings by comparing the operant responses that female starlings make to species-typical male song with their responses to the same songs played in reverse. Reversing a song transforms it in the temporal domain, while maintaining the frequency domain attributes of the signal such as bandwidth and overall spectral contour. Although it is problematic to conclude what preference for forward over reverse male starling songs (or vice versa) might mean in a functional context, most evidence suggests that starlings should be able to discriminate between these two classes of stimuli (see Chaiken et al. 1997; Gentner & Hulse 1998). Thus, the display of a behavioural preference in this experiment serves to validate the apparatus as a useful tool for investigating preferences among subtly different song stimuli, and as a first step in isolating the acoustic features of male song that might be important for more ecologically realistic female preferences.

Methods

Subjects

Eight female European starlings, naïve to all experimental procedures, served as subjects.

Stimuli

Eight complete song bouts from a single male European starling were used to create the two stimulus sets used in experiment 1. The recording procedure used to acquire the original songs from the male starlings is detailed elsewhere (Gentner & Hulse 1998). The eight songs were transferred from digital audio tape to a computer for manipulation with SoundDesignerII software (Digidesign-Avid, Menlo Park, California, U.S.A.). We down-sampled each song from 48 to 44.1 kHz at 16-bit resolution, and fed the result through a high-pass filter with a 200-Hz cutoff frequency to remove low-frequency noise. Then we completely reversed the order of the samples in each song and saved the results as a PCM sound file. To control for any processing artefacts, we repeated the reversal process to regenerate the forward versions of the songs. We again saved the results as separate PCM sound files. The reversal process was such that for a sound file containing samples taken at times t_1 to t_k , any sample in the original sound file, t_n , goes to position $t_{(k-n+1)}$ in the reversed sound file. This procedure yielded two stimulus sets derived from the same eight songs: a 'forward' set, in which each song played in its originally recorded order, and a 'reverse' set, in which each song played 'backwards'. The recording procedure used to acquire the original songs from the male starlings has been detailed elsewhere (Gentner & Hulse 1998).

Results

The subjects displayed a preference for the forward songs. As shown in Fig. 2, the subjects spent significantly more time on the perch associated with the forward songs than on the perch associated with the reverse songs during the first block of each session (ANOVA: $F_{1,7}=9.112$, $P<0.05$). However, during the second block the subjects spent roughly the same amount of time on the perches associated with the forward and the reverse songs (ANOVA: $F_{1,7}=0.353$, NS). When the data for both blocks were combined the difference between the amount of time spent on each perch was not significant (ANOVA: $F_{1,7}=4.865$, NS). Of eight subjects, seven showed a significant preference for either the forward or reverse songs during the first block of the session. Of those seven, six preferred the forward songs over the reverse songs, spending an average of 34.5% more time on the perch associated with the forward songs. Although the subjects spent more time listening to the forward songs than the reverse songs during the first half of the session, they visited each nestbox roughly the same number of times. That is, the number of responses to forward and reverse songs did not differ significantly during either block one (ANOVA: $F_{1,7}=1.397$, NS), block two (ANOVA: $F_{1,7}=1.234$, NS), or over the entire session (ANOVA: $F_{1,7}=3.258$, NS).

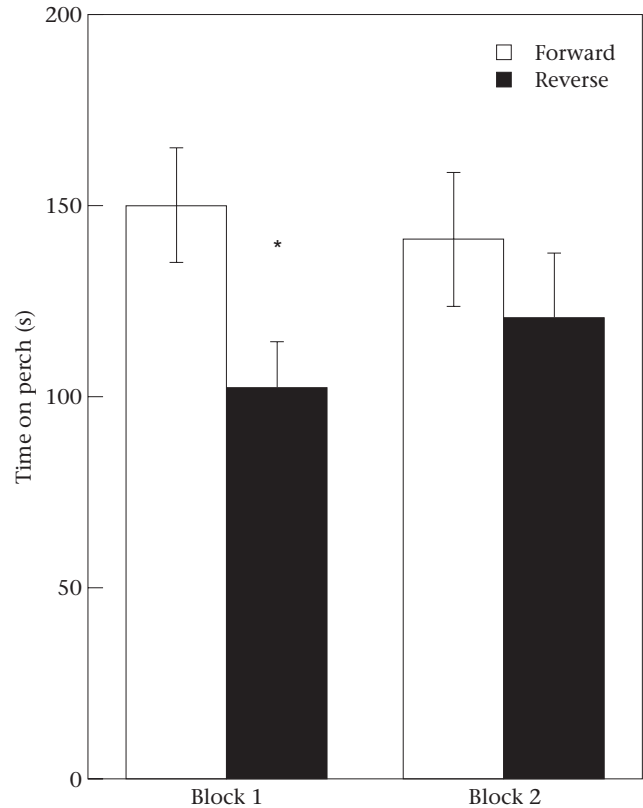


Figure 2. Mean \pm SE time spent on each of the perches associated with the forward and reverse songs during the first and second block of the session in experiment 1 (ANOVA: * $P<0.05$).

Neither measure of responding showed reliably consistent changes over the course of the session (Fig. 3). The total amount of time spent on the perches playing both the forward and reversed songs did not change significantly over the course of the session (repeated measures ANOVA for main effects: block 1: $F_{3,21}=0.855$, NS; block 2: $F_{3,21}=1.81$, NS). Similarly, the total number of responses made to both perches did not change significantly over the course of the session (ANOVA: $F_{3,21}=0.169$, NS; $F_{3,21}=2.63$, NS). In addition, we failed to observe any differential changes in responding over the course of the session that were specific to either the forward or reverse song perches. The subjects spent roughly the same amount of time on the forward and reverse song perches over each of the four quarters in both blocks (ANOVA: interaction with quarter for block 1: $F_{3,21}=1.106$, NS; interaction with quarter for block 2: $F_{3,21}=0.308$, NS). Similarly, subjects made roughly the same number of responses to the forward and reverse song perches over each of the four quarters in both blocks (ANOVA: interaction with quarter for block 1: $F_{3,21}=0.841$, NS; interaction with quarter for block 2: $F_{3,21}=2.211$, NS).

Discussion

The results of experiment 1 suggest that female European starlings possess preferences for naturally

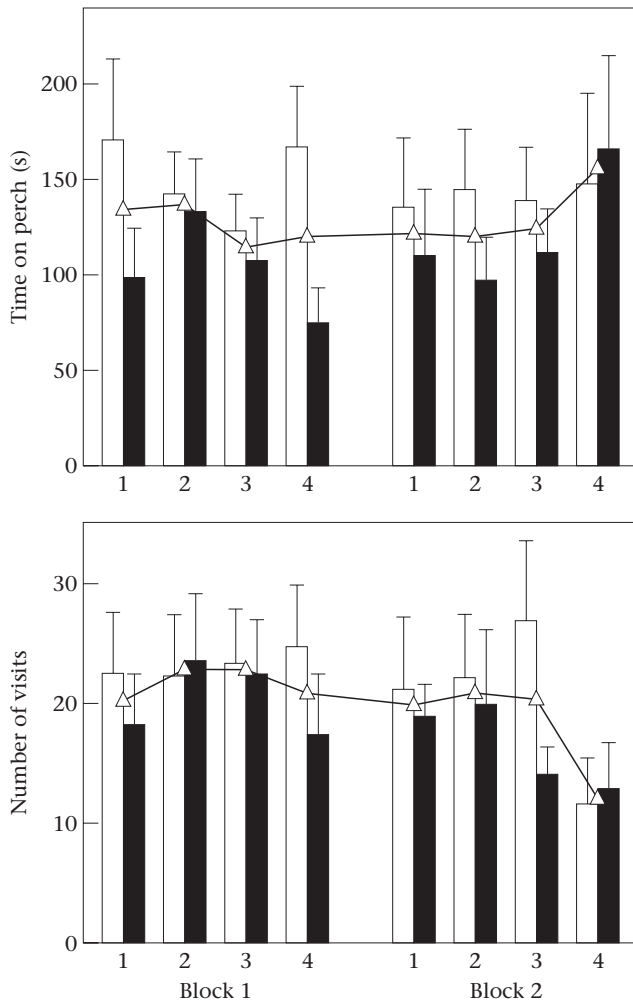


Figure 3. Mean+SE time spent on the perch in front of the nestbox that played either forward (□) or reverse (■) songs during the first and second block of the session in experiment 1. Data from each block was divided into quarters to examine variation in responding over the course of each block. The line connects means (△) for each quarter.

ordered conspecific male song. During the first block of each session the subjects spent more time at the nestbox associated with forward songs than at the nestbox associated with those same songs played in reverse. Because the experimental design controlled for initial position preferences by varying the location of the forward and reverse songs between sessions, we conclude that subjects allocated their time (at least during the first block) on the basis of the acoustic stimuli associated with each particular nestbox. The degree of stimulus control was not very robust, however, as this preference did not withstand switching the locations of the forward and reverse songs halfway through the session. Although the tendency in the data from the second block was the same as that in the first, there was no statistically significant difference between the times spent on the forward and reverse song perches during the second block. It is unlikely that the weakness of this preference for forward over reverse

song is the result of a failure to discriminate between the two classes of songs, as discrimination was surely present during the first block of the session when the females did show preferences. Earlier studies have also demonstrated that starlings are capable of discriminating very subtle differences between songs (Chaiken et al. 1997; Gentner & Hulse 1998). Therefore, although reversing the natural order of male starling song is sufficient for females to discriminate between different songs and display a preference, it does not appear to be a drastic enough change to preclude responses to those songs altogether. Based on these results it would be unwise to rule out the hypothesis that reversed song contains at least some of the acoustic features that females use to establish song-based preferences.

In addition to providing a first step in isolating the acoustic features that are important for song preferences among female starlings, the results of experiment 1 also provide independent support for our dependent measures of preference. The lengths of the song bouts associated with each nestbox in experiment 1 were identical, yet the amount of time that the subjects spent at each location showed significant variation. This should be kept in mind during the subsequent experiments in which the lengths of song bouts at each nestbox varied. In addition, the 'listening time' measure used here is qualitatively similar to the phonotactic responses used to assess female preference in other species of songbirds (see Searcy & Yasukawa 1996 for review), in that both measures rely on a temporal integration of the female's position with reference to a song source. The importance of providing spatially distinct song sources when measuring female choice is addressed in the General Discussion.

EXPERIMENT 2: FEMALE PREFERENCES FOR VARIATION IN SONG BOUT LENGTH

Given that female starlings responded to variation between the songs associated with different nestboxes in experiment 1, it is reasonable to consider how these preferences may be based on more realistic variation between the songs of conspecific males. Variation among male starling songs is extensive and has been correlated in several studies with mating success (see Introduction; Eens 1997 for review). In particular, variation in male repertoire size and song bout length appears to be important for female preference and choice. In experiment 2 we use the operant preference apparatus to test the hypothesis that female starlings possess preferences based on variation between the songs of conspecific males. Specifically, we examine the relative importance of variation in song bout length.

Methods

Subjects

Thirteen female European starlings, naïve to all experimental procedures, were used as subjects.

Table 1. Stimuli for experiment 2

Stimulus	Bout length (s)	Motifs per bout		Mean motifs heard	
		Unique	Repeated	Unique	Repeated
A-1	54.4	43	28	10	3
A-2	53.3	40	32	9	4
A-3	57.8	45	30	10	4
A-4	60.5	46	40	7	6
A-5	68.4	37	59	8	5
A-6	60.5	49	43	14	2
B-1	44.3	35	18	3	0
B-2	12.1	3	3	3	0
B-3	28.9	20	15	3	0
B-4	26.2	21	13	5	2
B-5	42.3	34	17	5	1
B-6	2.3	1	0	1	0
A-7	27.0	27	17	8	3
A-8	29.9	24	18	6	4
A-9	3.5	3	0	3	0
A-10	40.3	36	17	5	2
A-11	14.3	14	8	4	6
A-12	37.1	34	21	6	5
B-7	52.4	44	17	6	0
B-8	56.7	42	20	3	0
B-9	53.1	38	19	3	0
B-10	59.1	33	14	1	0
B-11	58.2	40	20	2	0
B-12	51.4	41	21	7	1
Long A (1–6)	55.6	105	39	38	4
Short A (7–12)	25.4	84	23	20	3
Long B (1–6)	55.2	76	19	10	0
Short B (7–12)	26.0	74	11	15	1

The first letter of each song bout refers to the bird (male A, male B) that sang that specific bout. Shown are the numbers of unique and repeated motifs contained in each song bout and the mean number of unique and repeated motifs contained in the portion of each song bout that the subjects heard during the mean playback time for a given stimulus set (see results for experiment 2; Fig. 7). The last four rows show data for each stimulus set, in which case 'bout length' is a mean.

Stimuli

We used 24 complete song bouts, 12 from each of two male European starlings (male A and male B), to create four stimulus sets. Each song was recorded and manipulated as in experiment 1.

We selected the song bouts used in experiment 2 from larger samples on the basis of their length and the repertoire size of the putative set. We then divided the 24 song bouts into four sets of stimuli. The six shortest song bouts from each of the two males composed two different stimulus sets, referred to as Short A and Short B, and the six longest song bouts from each of the two males composed two other stimulus sets, referred to as Long A and Long B. Table 1 shows the stimuli used in this experiment along with several measures for each bout and for each of the four stimulus sets. Note that the Long A and Short B sets vary in song bout length (55.6 and 26.0 s, respectively) and repertoire size (105 and 74, respectively). The Long B and Short A sets show a similar variation in song bout length (55.2 and 25.4 s, respectively), but the variation in repertoire size is much smaller and in the opposite direction (76 and 84, respectively).

Procedure

Using the same general procedure as outlined above, we exposed the first six subjects to the Long A and Short B stimulus sets, and the remaining seven subjects to the Short A and Long B stimulus sets. This design allowed us to dissociate preferences based on male song bout length from those based on male repertoire size or other individually specific song features. Given a clear preference among both groups of subjects, there are four possible conclusions. (1) Females in the first group prefer the Long A songs, and those in the second group prefer the Long B songs, suggesting that female preference varies directly with male song bout length, and that repertoire size is less important. (2) The first group prefers the Short B songs and the second group prefers the Short A songs, suggesting that male song bout length is not important and that repertoire size has a small effect on female preferences. (3) Both groups of subjects prefer the songs of male B, suggesting that neither song bout length nor repertoire size affect female preference, but that a feature specific to the songs of male B is important. (4) Females in both groups of subjects prefer the songs of male A, suggesting that female preference varies directly with male repertoire

size, and/or that some other feature specific to the songs of male A was preferred. Note that if repertoire size alone controls preference behaviour, then initial preferences should be established for the Long A songs over the Short B songs in the first group, and then abolished altogether in the second group when repertoire size is removed as a differential cue.

Results

Females tested with the Long A and Short B songs showed a preference for the Long A songs, and those tested with the Long B and Short A songs showed a preference for the Long B songs.

Time on perch

The subjects showed a strong preference for the long songs (Fig. 4). Of the 13 subjects in this experiment, 11 (84.6%) showed preferences at or above the level of our criterion. All of these 11, preferred the long songs over the short songs, spending a mean \pm SE of $65.4 \pm 10.8\%$ more time on the perch that produced the longer songs. Subjects in both groups spent significantly more time 'producing' (i.e. playing back) the longer song bouts than the shorter song bouts (ANOVA: $F_{1,11}=23.114$, $P<0.0005$). Importantly, this preference was evident throughout the session even though the actual location of the long and the short song bouts switched between block 1 and block 2 (ANOVA: block 1: $F_{1,11}=5.656$, $P<0.05$; block 2: $F_{1,11}=5.112$, $P<0.05$). The effect of song bout length was also consistent between the two groups (ANOVA: $F_{1,11}=1.85$, NS) despite the fact that each group was tested with different stimuli in which the opposite individual sang the longer songs.

The subjects spent roughly the same total amount of time on the perches throughout the session (see Fig. 5), but as each block progressed they spent significantly less time on the perches that produced the shorter songs, while the time they spent on the perches that produced the longer songs remained unchanged (ANOVA: $F_{3,33}=4.455$, $P<0.01$). This pattern of responding provides evidence of learning over the course of the session, and suggests that the subjects were under strong stimulus control throughout the entire session. That is, when the locations of the long and the short songs were switched halfway through the session, the response pattern of the subjects changed accordingly. Subjects began responding more to the perch that had previously been associated with the shorter songs (but was now associated with the longer songs), while responding less to the perch that had previously been associated with the longer songs (but was now associated with the shorter songs). The lack of significant differences between the patterns of responding to the long and short perches between the two blocks of the session (ANOVA: $F_{3,33}=0.069$, NS) provides evidence for the maintenance of this preference, despite the switch in the location of the long and short songs.

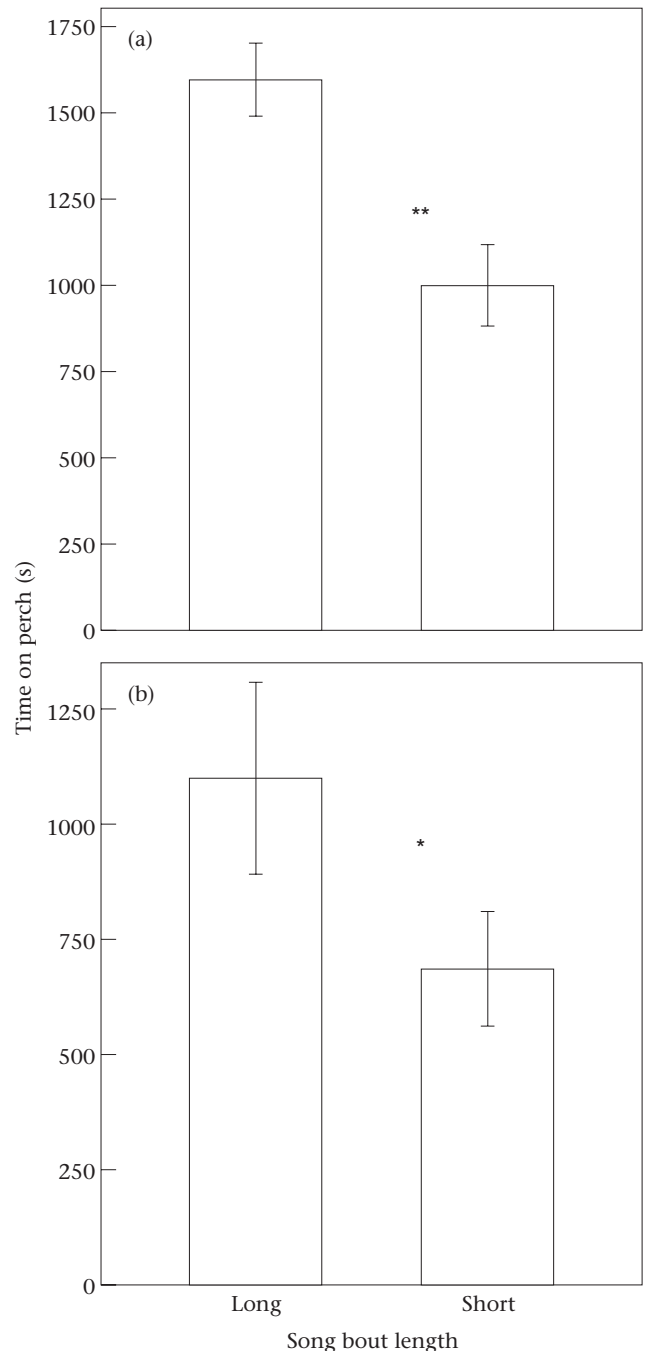


Figure 4. Mean \pm SE time spent on the perch in front of the nestbox that played either long or short song bouts in experiment 2. (a) Subjects exposed to the Long A and Short B songs. (b) Subjects exposed to the Short A and Long B songs. (ANOVA: * $P<0.05$, ** $P<0.005$).

Number of responses

There was a general tendency for the number of responses to decrease, or habituate, as the block progressed (ANOVA: $F_{3,33}=5.376$, $P<0.005$; Fig. 6), and this habituation was mainly restricted to the shorter songs. As each block progressed, subjects made significantly fewer responses to the perch that played the shorter songs (ANOVA: $F_{3,33}=4.389$, $P<0.05$), whereas the number of

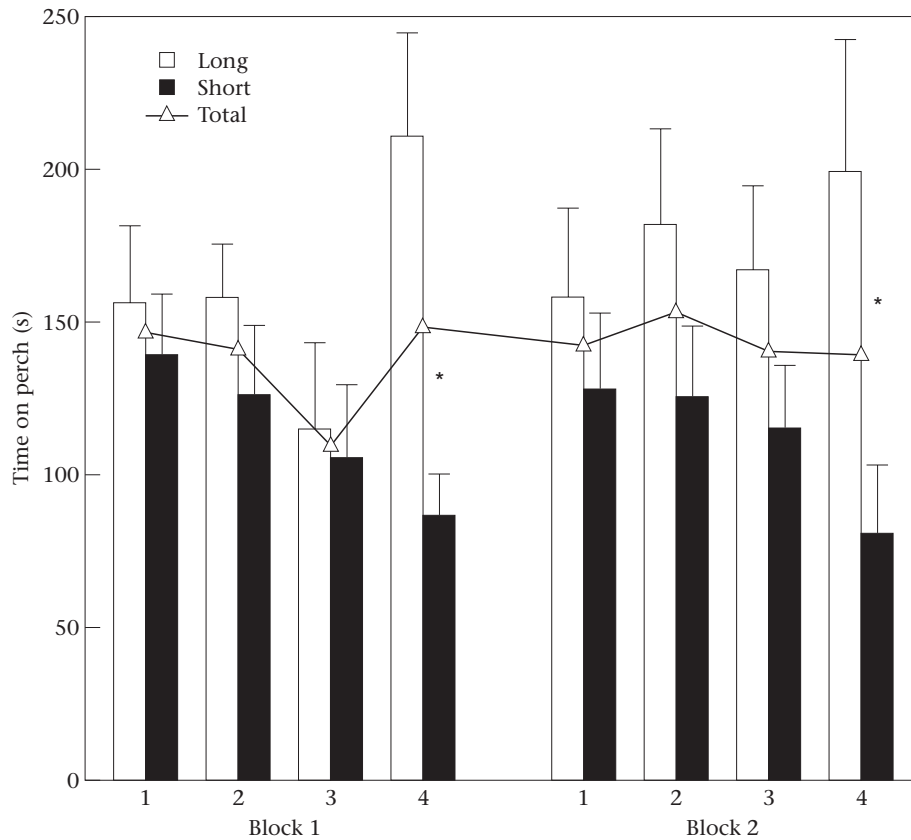


Figure 5. Mean \pm SE time subjects spent on the perch in front of the nestbox that played either long or short song bouts over the course of the session in experiment 2. The session was divided into quarters, and the data from both the first and second blocks are shown separately. The line connects the combined means for each quarter and shows that the overall level of responding was consistent across the session. ANOVA: quarter*song bout length: * $P < 0.01$.

responses to the perch that played the longer songs did not change significantly over the course of the session (ANOVA: $F_{3,33} = 1.315$, NS). However, overall, the number of responses made to the perches associated with the long and the short songs did not differ significantly (ANOVA: $F_{1,11} = 0.028$, NS).

Playback length

The playback time for each stimulus was controlled by the length of time that the bird remained on a given perch for that trial, and most subjects rarely, if ever, generated the playback of an entire song (see Fig. 7). In addition, the average length of the stimulus presentation on any single trial was significantly higher for the long songs than for the short songs (ANOVA: $F_{1,11} = 6.737$, $P < 0.05$), and the mean \pm SE playback lengths for both the long (9.6 ± 0.77 s) and the short song bouts (6.9 ± 0.63 s) were well below the mean song bout lengths for their respective stimulus sets (see inset Fig. 7, Table 1). Furthermore, the frequency histograms for the playback lengths (Fig. 7) indicate that the distribution of response lengths was unimodal, and that only a few stimulus presentations lasted for the entire duration of either type of song bout.

Correlates of female preference

Several measures of each song were correlated with the amount of time that the subjects spent listening to each

of those songs (see Table 2). The amount of time subjects spent listening to each song was positively correlated with song bout length (Pearson product-moment: $r = 0.730$, $N = 21$, $P < 0.0001$), the number of motifs in a bout (Pearson product-moment: $r = 0.706$, $N = 21$, $P < 0.0005$), and the stereotypy of that song bout (Pearson product-moment: $r = 0.539$, $N = 21$, $P < 0.05$). There was also a high level of significant covariance among many of the song bout measures (Table 2). For measures such as the total number of motifs in a bout, the number of unique motifs in a bout and song bout length, the correlation was not unexpected. However, the significant correlation between song bout stereotypy and bout length (Pearson product-moment: $r = 0.564$, $N = 21$, $P < 0.01$), the number of motifs per bout (Pearson product-moment: $r = 0.710$, $N = 21$, $P < 0.005$) and the number of unique motifs per bout (Pearson product-moment: $r = 0.483$, $N = 21$, $P < 0.05$) was unexpected, because our method for calculating the stereotypy of a bout was independent of the number of motifs (see Methods). The amount of time spent listening to a given song bout was not correlated with the total number of motifs in the first 10 s of a bout (Pearson product-moment: $r = 0.012$, $N = 21$, NS), the number of unique motifs in the first 10 s (Pearson product-moment: $r = 0.083$, $N = 21$, NS), or the average amount of information per motif (Pearson product-moment: $r = 0.432$, $N = 21$, NS).

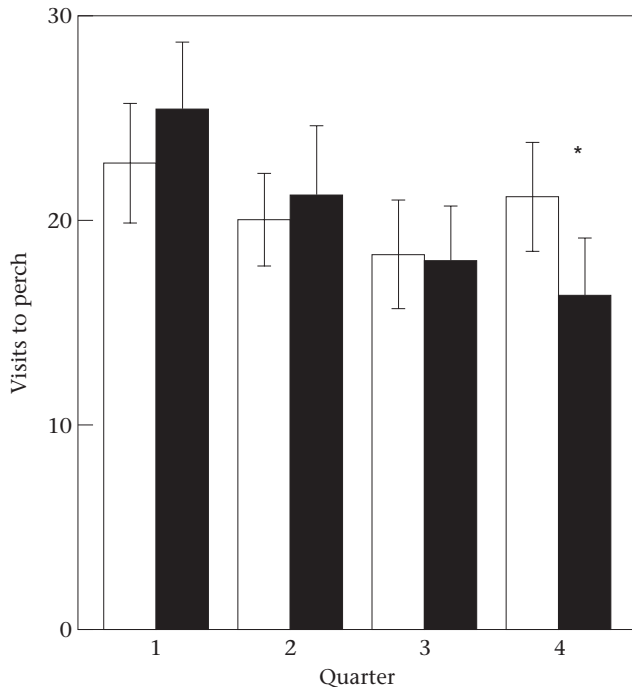


Figure 6. Mean \pm SE number of flights made to the perch associated with either long (\square) or short (\blacksquare) song bouts over the course of the session in experiment 2. The session was divided into quarters and the data from the first and second block are combined. There was a general tendency for the number of responses to decrease as the session progressed (ANOVA: $*P < 0.005$), mainly due to the significant drop in responding to the perch associated with the short songs (see text).

Discussion

The results of experiment 2 demonstrate that female behaviour is controlled by song bout length. The subjects spent significantly more time at the perches associated with the longer songs, regardless of the location of those perches, and the subjects' rates of responding habituated more quickly to the perch associated with the shorter songs than to the perch associated with the longer songs. The differential control exerted by the long and short songs, in turn, provides direct evidence that song preferences in female starlings are based on intraspecific variation in male song bout length. Moreover, changes in the pattern of responding over the course of the session suggest that by the end of each block females had learned to associate both long and short song bouts with specific nestboxes, and that they actively withheld responding to the perch that played the shorter songs. The associative nature of the behaviour observed in the apparatus demonstrates a strength of this procedure in its ability to measure changes in female preference over time, and is consistent with the interpretation that female starlings make an active choice between conspecific males.

At first, one might interpret the results of experiment 2 to suggest that female starlings attend to male–male variation in song bout length when allocating their time among spatially distinct song sources. On this basis, one might then argue that song bout length is the relevant

dimension controlling the rate of habituation at each location. However, closer inspection of the data suggests that such an interpretation is too simplistic. Although one can control preference by associating song bouts of varying lengths with each nestbox, the absolute length of a song bout, per se, does not appear to be the relevant feature controlling reinforcement at each perch. Females rarely, if ever, stayed on a perch long enough to hear an entire song bout, and the mean length of a playback at either location (long or short) was well below the mean length of the song bouts associated with that perch (see Fig. 7). Thus, subjects did not appear to use estimates of the absolute duration of the song bouts (or for that matter estimates of the total repertoire size) to control the distribution of their behaviour. Nevertheless, as each block in the session progressed, a clear preference for the longer song bouts emerged (see Fig. 5). Moreover, the amount of time subjects spent listening to each of the individual song bouts was positively correlated with both the length of that bout and the number of motifs in that bout (see Table 2). Because of the relatively short duration of the average playback, the actual feature, or features, controlling habituation at each nestbox appears to be available long before the length of a song bout or the total number of motifs in a bout can be obtained. One potential feature that females may have access to directly without having to hear an entire song bout is the transition stereotypy between motifs, which may account for the pattern of correlation coefficients among response time, repertoire size and song bout length.

Both the stereotypy and the amount of information per motif are functions of the frequency of occurrence of motifs in a bout. They differ in that the stereotypy is sensitive to transitions between pairs of motifs, whereas the information/motif is sensitive to the frequency of single motifs. Motif transitions are more predictable in high-stereotypy song bouts than in lower-stereotypy song bouts, and song bout length and repertoire size are both directly correlated with stereotypy. Therefore, although the acoustic variability of the signal increases with longer repertoires, it is constrained by the organization of sequential motifs within a bout. The high correlation between female preference and stereotypy may reflect a perceptual sensitivity to large-scale (i.e. multiple motif) temporal properties of a song bout. Female choice could increase both repertoire size and song bout length, but in a manner that is constrained by the preference for 'structured variation' between motifs. Sensitivities to motif transition probabilities have been observed for starlings engaged in vocal recognition tasks (Gentner & Hulse 1998). In addition, the volume of the song control nucleus HVC is correlated with song bout length (but not repertoire size) in male starlings (Bernard et al. 1996). This suggests that singing longer song bouts carries with it a morphological constraint that is not simply related to having a larger repertoire. However, in the present study, stereotypy was not manipulated as an independent variable, and thus the importance of stereotypy as a feature in female starling preference and choice remains unknown. Further work determining the number of trials required for an accurate estimate of stereotypy, and how this

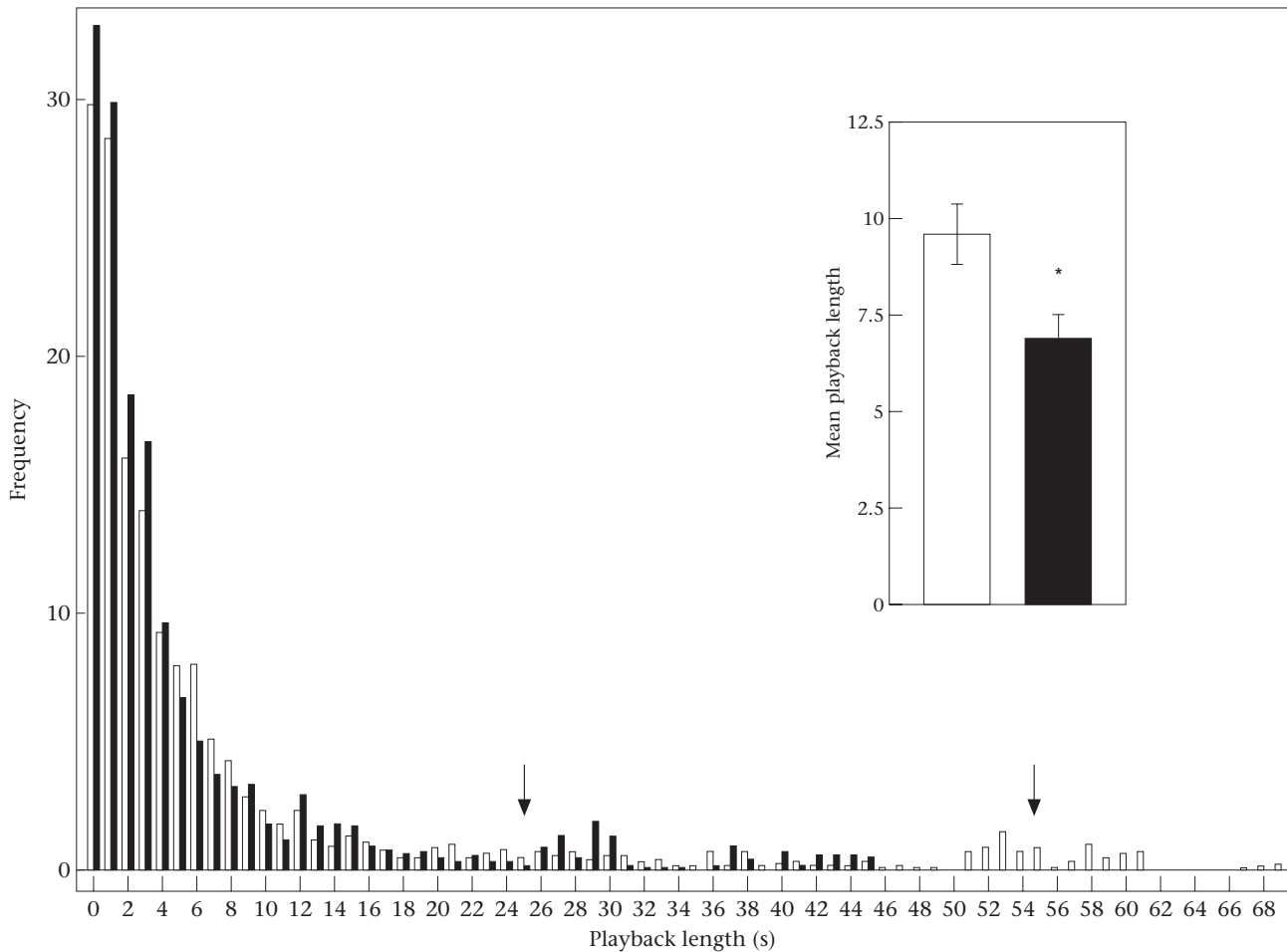


Figure 7. Frequency histogram of playback lengths for the trials that played either a long (□) or short (■) song bout in experiment 2. Note that the distribution is unimodal and that relatively few stimulus presentations lasted for the entire duration of either type of song bout. The arrowheads mark the mean lengths for the short (25.8 s) and long song bouts (55.4 s). The inset shows the mean length of a playback for both the long and short song bouts (ANOVA: $*P < 0.05$).

compares with the time course of the location-specific habituation observed in experiment 2 will be important.

Although the design of experiment 2 did not test explicitly whether male repertoire size controls female preferences, the results are not consistent with the idea that repertoire size influences female choice in this species. If female starlings do attend to male–male

variation in repertoire size as a primary cue, then the strong preference observed for the Long A over Short B songs should have been abolished when subjects were exposed to the Long B and Short A songs. The repertoire size for the Long A songs was much larger than that for the Short B songs, whereas the repertoire sizes for the Long B and Short A songs were approximately equal (Table 1).

Table 2. Correlation matrix for experiment 2

	Response time	Song bout length	Motifs/bout	Unique motifs/bout	Motifs in 1st 10 s	Unique motifs in 1st 10 s	Stereotypy	Info./motif
Response time	1.0	0.730*	0.706*	0.745*	0.012	0.083	0.539*	0.432
Song bout length		1.0	0.856*	0.874*	-0.187	-0.190	0.564*	0.387
Motifs/bout			1.0	0.880*	0.232	0.235	0.710*	0.250
Unique motifs/bout				1.0	-0.018	0.059	0.483*	0.481*
Motifs in 1st 10 s					1.0	0.847*	0.240	-0.276
Unique motifs in 1st 10 s						1.0	0.361	0.009
Stereotypy							1.0	0.519*

Matrix showing pairwise Pearson product-moment correlation coefficients between several measures of each song bout including the amount of time that the subjects spent listening to that song (normalized for each subject, see Methods). *Significant correlation, $P < 0.05$.

However, despite the fact that the repertoire sizes of these sets of song bouts did not differ, females showed a strong preference for the Long B over the Short A songs. In fact, the preference shown by the subjects tested with the Long B and Short A songs was in the opposite direction from that expected if male–male variation in repertoire size controls how females allocate their time among the different nest-boxes. Similarly, the data do not support the idea that females use an estimate of repertoire size drawn from the beginning of the song bout (i.e. ‘subrepertoires’; see Table 1). Any reliance on the subrepertoire measures leads to the same contradiction of the present results as the putative use of the full repertoire sizes. Thus, subrepertoire sizes are unlikely to have had an important effect on behaviour in the operant apparatus. None the less, it is important to reiterate that the influence of repertoire size was not examined explicitly in the present study, as none of the conditions tested preference when song bout length was held constant while repertoire sizes associated with different perches were varied. In addition, it is also unlikely that vocal characteristics specific to either singer affected behaviour in the apparatus. If female behaviour in the apparatus was dependent upon some individually specific characteristic in a given male’s songs then females in both groups of subjects should have been attracted to the songs of the same male. This was not the case, as the females in the two groups showed preferences for the songs of the opposite male.

Another interpretation of the data from experiment 2 is that subjects spent more time listening to the longer songs, simply because those songs could play for longer periods of time on any given trial; that is, male song may function as a passive attractant (sensu Parker 1983). The absence of any statistically significant difference between the overall number of responses to each perch is consistent with this idea, and suggests that subjects responded randomly, simply stopping to listen to whatever song happened to play, for as long as it happened to play. However, two facts argue against this simple interpretation. First, if the subjects responded randomly throughout the session, then one would expect consistent and stable differences in the response measures for both perches over the course of the session. This was not the case. Instead, the difference in mean listening times between the perches associated with the long and short songs broadened over the course of each block (see Fig. 5). Second, the rate of responding habituated more quickly at the perch associated with the shorter songs than at the perch associated with the longer songs. Again, if song functioned as a passive attractant one would expect stable differences throughout the session. Instead, the fact that multiple measures of responding showed changes over the course of the session that were unique to specific nestboxes (habituation occurred more rapidly to the perch playing the shorter song bouts) suggests that the behavioural processes underlying female choice require a period of associative learning. That is, females must learn to associate certain songs with certain locations. In the context of this associative learning, the reinforcing properties of the songs at each location then give rise to differential behaviour observed (i.e. preference). Our data

support the conclusion that longer song bouts possess greater value as reinforcers than do shorter song bouts.

EXPERIMENT 3: MALE PREFERENCES FOR VARIATION IN SONG BOUT LENGTH

To broaden the external validity of the results from the first two experiments and to argue that behaviour in the apparatus generalizes to the environmentally rich contexts in which female choice actually occurs, it is important to examine the sexual dimorphism in these behaviours. In experiment 3 we tested the hypothesis that male starlings show behavioural preferences based on male conspecific songs by exposing male starlings to the same stimulus sets used with the first group of subjects in experiment 2.

Methods

Subjects

Seven male European starlings, naïve to all experimental procedures were used. Capture and animal care was identical to that in experiments 1 and 2. Prior to use in this experiment the subjects were housed in a mixed-sex aviary for 4 months on an LD 8:16 h cycle to maintain photosensitivity (Dawson & Goldsmith 1983). One week prior to the start of the experiment, we moved the subjects to a same-sex aviary and changed the photoperiod to an LD 14:10 h cycle. The maximum exposure that any subject had to this long-day photoperiod, prior to running in the experiment, was 2 weeks.

Stimuli

The stimuli used in this experiment were the same as those used for the first set of subjects in experiment 2: long song bouts from male A, and short song bouts from male B.

Results

Of the seven subjects in this experiment, four (57.1%) showed preferences at or above the level of our criterion. Of those four subjects, two spent more time listening to the longer song bouts, and two spent more time listening to the shorter song bouts, spending a mean \pm SE of $37.1 \pm 2.2\%$ more time at one or the other nestboxes. When the data from all of the subjects in experiment 3 were combined, there was no indication of a preference for either the long or the short song bouts. There were no significant differences in either the mean amount of time spent on the two perches that produced the longer and shorter song bouts or in the number of responses made to each of those two perches (ANOVA: $F_{1,6}=2.361$, NS). Furthermore, this lack of a preference was consistent across both blocks of the session. The mean amount of time spent at the perches associated with long and short song bouts was roughly the same during both the first (ANOVA: $F_{1,6}=0.884$, NS) and the second (ANOVA: $F_{1,6}=0.012$, NS) block of the session. Likewise, there were

no significant differences in the number of flights made to each of two perches during either the first (ANOVA: $F_{1,6}=3.91$, NS) or second ($F_{1,6}=0.361$, NS) block of each session.

Also consistent with the fact that the males showed no preferences for the song bouts associated with each location was the fact that both measures of responding stayed relatively stable over the course of the session. There was no significant variation associated with the amount of time that the subjects spent at both perches over the course of the session (ANOVA: $F_{1,6}=0.004$, NS), or with the number of responses that the subjects made to both perches over the course of the session (ANOVA: $F_{1,6}=3.044$, NS). We also failed to see any differential habituation of responding associated with either the long and short song bout perches over the course of the session, as was observed in experiment 2. As the session progressed, the subjects spent roughly the same amount of time on each perch (ANOVA: interaction between time on each perch*quarter of session: $F_{3,18}=0.580$, NS) and made roughly the same number of responses to each perch (ANOVA: response*quarter of session: $F_{3,18}=0.936$, NS).

Discussion

The results of experiment 3 provide no evidence that male European starlings possess reliably predictable behavioural preferences for male starling song bouts that vary in length. The lack of a significant preference for either the longer or the shorter song bouts also implies that male starlings do not display song preferences for conspecific male repertoire size or for individual singers. Although we observed no statistically significant differences in either measure of male responding, there was a tendency, particularly during the first block of the session, for subjects to respond at higher levels to the perch that played the shorter songs. Whether this general tendency is reliable given a large enough sample, and thus whether male starlings possess song-based preferences, remains a topic for future investigation. It is possible that male starling preferences for conspecific song may be observable under different experimental conditions where additional behaviours are measured. In any case, we have demonstrated that the robust preference for long song bouts displayed by female starlings is a sexually dimorphic behaviour.

GENERAL DISCUSSION

Our results suggest several important conclusions regarding the proximate perceptual mechanisms that underlie female preference and choice in European starlings. First, we have provided direct evidence that female starlings display preferences based on male–male variation in conspecific song. Second, we have demonstrated that preference behaviour among females can be brought under direct control by varying the length of song bouts presented at different locations. Third, we have demonstrated that this preference for longer song bouts is

restricted to females. This study also provides a general demonstration of how the reinforcing properties of conspecific song can be used to assay female song perception via the natural behaviours associated with mate choice. This, in turn, highlights the role of associative learning processes in the proximate mechanisms of female mate choice among songbirds.

The Proximate Basis of Mate Selection

One proposed mechanism for female choice among songbirds is the selective habituation to different male songs (Searcy 1992a). According to this theory, male repertoires function to increase song variety, which in turn, prevents an otherwise more rapid habituation of female copulation solicitation behaviour. Males with larger repertoires (i.e. more variable song) are able to solicit copulations more successfully and thus gain a selective advantage over males with smaller repertoires. Female preference is exercised in this context via the selective habituation to particular repertoires. Conversely, increasing male repertoire size can be understood as a method for avoiding the general property of sensory systems to habituate to a given stimulus (Searcy 1992a). This theory is consistent with the large body of work demonstrating that female songbirds are likely to give more copulation solicitations to song composed of larger than smaller repertoires (Searcy & Yasukawa 1996).

In the present study, variation in repertoire size was correlated with the amount of time that subjects spent listening to each song bout (see Table 2). However, variation in the repertoire sizes associated with each perch cannot explain the distribution of responding between the two perches. Similarly, although variation in male song bout length could be used to control the behaviour of females in the apparatus, it appears that song bout length was not the feature of song to which females actually attended, because the mean playback length was significantly less than the average length of the bouts at each location. To explain the pattern of results observed in experiment 2, then, we propose that female starlings may attend to the statistical probabilities associated with transitions between pairs of motifs. Although this idea requires further testing, it does fit with the available data from starlings showing that both males and females attend to the transitions between pairs of motifs when recognizing the songs of conspecific singers (Gentner & Hulse 1998). Moreover, this idea is consistent with the data from copulation solicitation assays as outlined above, because both theories posit a sensitivity to the variability in male song. We suggest that this sensitivity may have an upper boundary in that songs cannot exceed a certain degree of randomness without losing their effectiveness as sexual attractants. Thus male starlings may sing longer songs in order to attract females to specific locations more efficiently, while tailoring the variability of their motif transitions so as not to exceed the upper limit on attractiveness once females are nearby. Alternatively, the statistical regularity of motif transitions may be an emergent property of longer songs (and larger repertoires) that females use to assess a singer's age

quickly. Among starlings, age is positively correlated with both song bout length and repertoire size (Adret-Hausberger et al. 1990; Eens et al. 1992b; Chaiken et al. 1993; Mountjoy & Lemon 1995). Because male starlings can have very large repertoires, variation in song bout length may be a more efficient way of capturing the relevant variation in motif variability than sorting song on the basis of repertoire size.

Assaying Female Preference and Choice

The claim that females choose among a range of potential mates on the basis of phenotypic variation in song implies a set of minimum constraints for the behavioural and neural mechanisms that underlie such a 'choice'. First, females must be able to detect male song from the variety of other sounds with which it is likely to occur, such as ambient noise and heterospecific vocalizations. Second, females must discriminate among multiple conspecific male songs, and associate particular songs either with particular males or particular locations in space. Third, these song-singer (or song-location) associations must be retained in memory long enough to allow for their use in some later decision-making process. From a proximate standpoint, then, female choice can be viewed as a composite of several different cognitive processes, and it stands to reason that female preference (sensu Ryan & Rand 1993) acting at one level of processing may not extend to other levels. The operant reinforcement technique presented in this study provides a new tool for assaying female preference and choice in songbirds, and is amenable to the investigation of preferences at various cognitive levels.

Although the application of operant techniques to questions of female choice has not been exploited, the general reinforcing properties of conspecific song have been well known for some time (Stevenson 1967, 1969; Petrinovich et al. 1972; but see Dobson 1973; ten Cate 1991). Most research on the reinforcing properties of song has been directed at questions of song acquisition, and these studies strongly suggest that, at least in zebra finches, reinforcement is an important aspect of song acquisition (Adret 1993a). The value of song as a reinforcer also appears to play an important role in the context of intersexual communication. Among canaries, *Serinus canaria*, the potency of song as a reinforcer differs between sexes (Calhoun et al. 1993). Additionally, among both white-crowned sparrows, *Zonotrichia leucophrys*, and canaries, the reinforcing properties of song also vary as a function of photoperiod (Dobson 1973; Calhoun et al. 1993). It is often difficult to understand how general process theories of learning and reinforcement affect the behavioural ecology of a particular species. However, in the present study both the reinforcer (song) and the operant behaviour (approach to a nestbox) translate easily to natural contexts where song often functions among starlings as an attractant (Mountjoy & Lemon 1991; Smith 1995).

The operant method presented in this paper requires the experimenter to make very few assumptions about the behaviour under examination, and to that extent

differs significantly from more 'traditional' measures of female preference, such as the copulation solicitation display and phonotaxis (reviewed by Searcy 1992a). The task in the operant preference apparatus presents the female with an active, zero-sum choice between male songs that are spatially and temporally distinct. The choice to spend time at any one location has an associated cost in that it is also a choice not to spend time at other locations. In addition, the songs are actively sampled at a frequency and duration that is completely controlled by the subject. In passive playback designs, such as the copulation solicitation assay, the only 'choice' typically presented to the subject is whether to respond in a particular manner to a given stimulus. Responding to any one stimulus in a particular manner does not necessarily preclude responses to other stimuli. The passive playback design also forces the experimenter to make a priori assumptions about the appropriate frequency and duration of stimulus exposure, and these assumptions need not follow a pattern consistent with the sampling strategies employed by prospecting females in the field. Therefore, although the results of passive playback designs are clearly informative with regard to questions of general female preferences, their extension to questions of female choice is inferential. There are, however, advantages to measuring clearly sexual behaviours, such as copulation solicitations (see Searcy 1992a), and it is not our intention to imply that these advantages should be overlooked. Rather, we want only to point out that the procedures used for most copulation solicitation studies make it difficult to determine the level of cognition (i.e. discrimination, categorization, recognition or choice) at which preference mechanisms (such as the habituation outlined above) might be acting to elicit differential female behaviour. It is likely that our understanding of female choice will be dramatically improved by examining females in experimental contexts that force them to use their preferences to make realistic choices. In this regard, it would be very informative to measure copulation solicitation displays using the type of operantly controlled stimulus presentation we have demonstrated here. This would allow one to make a clear dissociation between the potency of certain songs in attracting females to specific locations, and their potency in eliciting more straightforward sexual responses.

Another advantage of our operant apparatus is that the subjects did not require an exogenous dose of oestradiol, as is the case for many species with the copulation solicitation display (but see King & West 1977; Searcy & Marler 1981; Searcy 1992b). Instead, we relied on the general preference among songbirds to spend time on perches rather than on the ground, and attempted to make the perches attractive to both sexes by placing them in front of nestboxes. The success of this apparatus with starlings is likely due, in part, to the particular mating strategies employed by both sexes in this species. Similar nestboxes (without the electronics) have successfully attracted breeding pairs of European starlings at nearby field sites (G. F. Ball, personal communication), and the nestbox (or site) plays an important function in mate attraction (Mountjoy & Lemon 1996). Male starlings will

often sing in their nestboxes as prospecting females approach, and increase the amount of time spent at the nestbox (as well as the amount of time spent singing) when introduced to a female (Eens & Pinxten 1990). Our results suggest that any position preferences among females are secondary to the attractive properties of song at a given location, as the patterns of their responding easily transferred to different perches when the location of the stimuli was changed. This level of stimulus control is consistent with data from the field, where although some nest sites appear to be more preferable than others, males with more sexually attractive songs do not necessarily occupy those more preferable sites (Mountjoy & Lemon 1996). This suggests that the benefits of female choice in starlings are related to issues of male quality rather than resource-holding potential.

Additionally, the operant apparatus presented in this study enables the researcher to measure a large amount of sensitive behaviour over an extended period of time. Because of this, the data obtained are capable of revealing very subtle behavioural differences, particularly in the strategies that female songbirds employ when prospecting for potential mates (e.g. best-of-*n*, Janetos 1980; sequential search, Real 1990). It is not known what decision-making strategy female starlings use when searching for mates in the wild. The fact that females in the present study continued to sample from different locations throughout the session suggests a best-of-*n* approach, but the apparatus needs to be expanded beyond two playback locations in order to test this hypothesis adequately. In the event of complete habituation (i.e. a loss of responding) to one or more playback locations, a sequential search or threshold model might be surmised. Alternatively, with a suitably large array of playback locations one might be able to track the employment of hybrid search strategies in which certain locations are no longer visited, but sampling continues at some subset of sites.

Finally, by varying the rate of reinforcement through more extensive manipulations of the songs associated with different nestboxes, one can study the distribution of preference behaviour in even more precise ways than we did here. In particular, the use of operant techniques to investigate the reinforcing properties of male song would allow female-choice literature to be integrated with existing psychological models of choice (Herrnstein 1961; Real 1991). One such model, known as the 'matching law' (Herrnstein 1961), provides a theoretical relationship between the value of reinforcement at different locations and the distribution of behaviour. Thus, once the relevant stimulus dimensions for reinforcement are known, one could use the matching law to formulate testable predictions about the underlying neurobiological mechanisms for female choice.

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