

CAN A SEXUALLY DIMORPHIC LEARNED BIRDSONG BE USED FOR MALE-FEMALE RECOGNITION?

by

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(Acc. 1-VI-1998)

Summary

In many temperate zone songbirds, singing is solely a behavior of males. In these species, the singer's sex is encoded in the act of singing. However, in some species both sexes sing. For songs of these species to be used for sex recognition, the acoustic structure of songs of the two sexes needs to be dimorphic, and the dimorphism needs to be identifiable by the birds themselves. I examined the ability of acoustic sex recognition in a species in which the two sexes produce very similar songs, Northern cardinals (*Cardinalis cardinalis*). A field playback experiment was conducted to examine whether cardinals can distinguish the sex of the singer based on hearing a song. The results showed that cardinals responded differently to the songs of the two sexes, suggesting that songs of cardinals are used for acoustic sex recognition despite their remarkable similarity.

Introduction

In many species of songbirds, singing is a behavior of males. In these species, the sexual identity of the singer does not need to be encoded in the

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²⁾ I thank Peter Marler for his helpful guidance, support, advice, and comments on this research and on the manuscript. I also thank Satoshi Iseki for his excellent assistance in the field, and Doug Nelson for his advice on experimental design, and two anonymous referees for comments on the manuscript. Carol Masters Vleck generously shared her field site with me. This research was supported by the Chapman Fund of the American Museum of Natural History, Sigma Xi, and Jastro Shields Grants from UC Davis.

morphology of song, since the act of singing identifies the singer as a male. However, in some species of songbirds, both sexes sing during the breeding season. In these species, do they recognize the sex of the singer when they hear a song? In some of these species including bay wrens (*Thryothorus nigricapillus*) and red winged blackbirds (*Agelaius phoeniceus*), songs of the two sexes are very distinct and the birds have been shown to recognize the sex of the singer by hearing a song (Beletsky, 1983, 1985; Levin, 1988, 1996). However, in other species such as the cordon blue (*Uraeginthus bengalus*) and the Northern cardinal (*Cardinalis cardinalis*), songs of the two sexes sound very similar to the human ear (Laskey, 1944; Bent, 1968; Dittus & Lemon, 1969; Gahr & Güttinger, 1986), although subtle acoustic dimorphism have been identified in both cases (Gahr & Güttinger, 1986; Yamaguchi, 1996). In these latter species, whether songs are used for sex recognition is not well understood. I have explored this issue using cardinals.

Male and female cardinals sing during the breeding season (Laskey, 1944; Bent, 1968; Ritchison, 1988a). Both males and females sing throughout the breeding season (Ritchison, 1988a; Halkin, 1997). Both sexes of cardinals have eight to twelve commonly used song types within an individual repertoire. Most song types are shared among all individuals in a local population such that there is no song type unique to one sex (Halkin, 1990). Laboratory tutoring studies showed that males and females develop their songs by imitating both male and female adults during the first year of their lives (Dittus & Lemon, 1969; Yamaguchi, 1996). This shared song learning process results in male and female songs that sound almost identical. However, I have previously shown that the songs of the two sexes differ consistently and significantly in subtle acoustic features; notes and syllables repeated in female songs are less stereotyped than those of male songs, and the harmonic components of a song is louder in females than in males (Yamaguchi, 1998). These differences are likely to be the reflection of the sex differences in the circulating levels of plasma testosterone at the time of song production in the two sexes (Yamaguchi, 1996).

The sexual identity of individuals is one of the most important pieces of information exchanged between conspecifics during the breeding season. Considering that the songs of cardinals are used mainly in reproductive context (Laskey, 1944; Bent, 1968; Ritchison, 1988a, b; Halkin, 1997), it

is natural to predict that fundamental information such as sexual identity is transmitted via song. Acoustic sex recognition using songs would appear to be especially adaptive for cardinals because they inhabit dense vegetation where vision is limited. By using acoustic signals for recognition, cardinals can respond promptly and appropriately to songs without having to spend time and energy getting a conspecific singer into view. However, the task of acoustic sex recognition appears to be perceptually challenging. For cardinals to be able to recognize the sex of the singer solely based on songs, they have to be equipped with a refined perceptual capacity to distinguish the subtle acoustic differences in songs of the two sexes.

In the present experiment, intrusions of male and female strangers into territories defended by breeding cardinals were simulated by broadcasting tape-recorded male and female songs via loudspeakers. I predicted that if the birds were able to recognize the sex of the singer, they would respond differently to songs of the two sexes because the members of the same sex should be perceived as competitors for limited resources, whereas the members of the opposite sex should be perceived as the resource to be pursued during the breeding season. The results show that cardinals sang more in response to hearing male songs than female songs, suggesting that they can distinguish the sex of the singer solely based on hearing a song.

Methods

Study area and subjects

The playback study was conducted in the Santa Rita Experimental Range, and Sierrita Ranch in Pima, Santa Cruz and Cochise Counties, Arizona, from May 16 to June 2, 1994. Twenty breeding pairs defending territories were used in the experiment. At the time of the experiment, 8 pairs were directly observed to be either in the nest-building or the egg-laying stage. The breeding stage of the remaining 12 pairs was unknown, although they were most likely to be in a similar stage as other pairs, because the first breeding attempts of the year, which coincided with the present experiment, tend to be synchronous.

Experimental design

I simulated territorial intrusions by broadcasting songs recorded from each sex, and compared the responses of the resident pairs. Intrusions by strange males and females are almost always met with aggressive responses by resident males and females (Laskey, 1944). Typically, a male intruder provokes an aggressive response by the resident male (Laskey, 1944; Lemon, 1967). The resident female is usually mildly aggressive to intruders of either sex, although there are a few cases of extended female-female aggression (Ritchison, 1988a;

Yamaguchi, pers. obs.). Based on published accounts of cardinal responses to intruders, I predicted that territorial males and females should respond with greater aggression towards the songs of their own sex than to those of the opposite sex, if they recognized the sex of the singer.

Playback songs

Songs used for playbacks were recorded from three male and three female cardinals breeding about 5 km distant from the study area using a Dan Gibson parabolic microphone, and a Sony TCD5M cassette tape deck. At this distance, song types sampled were shared with the subjects in the study area, and the playback songs should be perceived by the subjects as coming from non-neighborhood local birds. Non-neighborhood local birds are likely to be threatening to breeding pairs, because they are most likely to intrude and compete for limited resources such as breeding sites and mates (Lemon, 1967). Pairs of male and female songs of each of three song types were chosen as playback stimuli (Fig. 1). All three pairs of song types were recorded in a similar acoustic environment early in the breeding season, and had male- and female-typical measurements of syllable stereotypy and harmonic amplitude, the features in which male and female songs consistently differ (Table 1). All playback songs

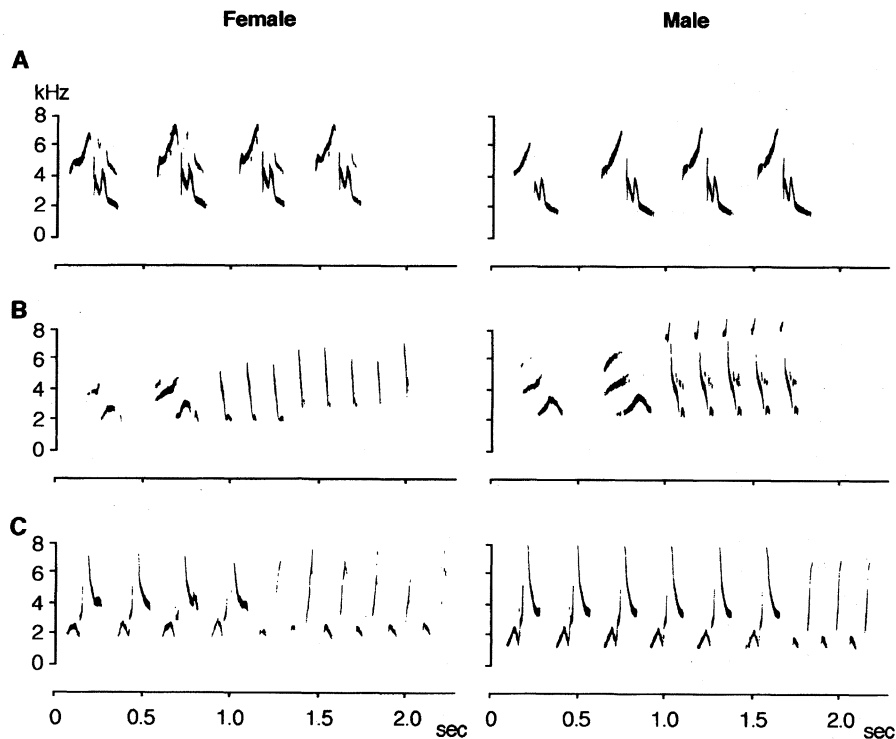


Fig. 1. Sound spectrograms of three pairs of playback songs. The songs in the left column are songs of females, those in the right column are songs of males.

TABLE 1. Sex differences in syllable repetition accuracy and harmonic amplitude difference for the three pairs of playback songs used in the playback experiment

Parameters	Playback stimuli					
	A		B		C	
	female	male	female	male	female	male
Syllable repetition accuracy	0.768	0.90	0.654	0.963	0.792	0.929
Harmonic amplitude difference (dB)	30.0	41.3	29.4	31.3	8.2	19.3

Spectrograms of the songs are shown in Fig. 1. Syllable repetition accuracy is an average cross correlation coefficient of three pairs of consecutive syllables sampled from a song. The parameter ranges from zero to one, and indicates how accurately syllables are reproduced within a song. Harmonic amplitude difference is a difference between the sound amplitude at fundamental frequency and at the second harmonic overtone. A larger parameter indicates greater pure tonality of a sound.

recorded were sung by birds in solo, not as a part of a duet as mated cardinal pairs sometimes do. The repeated use of a relatively small number of exemplars in this experiment may be viewed as pseudoreplication (Kroodsma, 1989). However, when cardinals sing, they tend to sing all song types equally frequently, and the song types used are context independent (Lemon & Chatfield, 1971; Ritchison, 1988b). Thus, I assumed that the three song types used were representative of all the song types used by birds in the study site. In support of this assumption, post hoc tests showed that there was no difference in responses to the three different song types used (see below). Playback tapes were synthesized using SIGNAL sound analysis software (Engineering Design, 1987) such that all 6 songs had an equal average amplitude.

Playback procedure

Each pair of subjects was presented with both male and female songs on the same day separated by an interval of two hours. The order of song presentation was balanced so that half of the subjects heard the female song first, and half heard the male song first. Each subject pair heard only one of the three pairs of stimuli.

The stimulus tapes were played on a loudspeaker (Acoustic Research Powered Partner 570) which was placed about 15 m inside the subjects' territorial boundary and 1.5 m above the ground in a tree. The sound level was adjusted to 75 dB at 1 m in front of the speaker to approximate the natural amplitude of a singing bird (Realistic Sound Level Meter model 33-2050). Playback experiments began 1-2 hours after sunrise, when I found birds to be most active in defending their territories. I initiated each experiment only after subjects had been silent for more than two minutes.

An experimental trial consisted of a 5 min playback period followed by a 5 min silent period. During playback a male or female song was repeated 30 times, once every 10 s. The behavior of the subjects was monitored throughout the trial period by one or two observers sitting motionless behind vegetation about 25 m away from the speaker. Songs emitted by

male and female subjects were recorded using a Dan Gibson parabolic microphone with a Sony TCD5M cassette deck by the observers.

Because song is used in male-male (Laskey, 1944; Lemon, 1967), as well as in female-female agonistic encounter (Ritchison, 1988a; Yamaguchi, pers. obs.), the number and type of songs emitted by the subjects within a trial were used to estimate the aggressiveness of the subjects in response to playback songs. However, the sexual identification of the responding singer was often not possible due to the density of the vegetation. Although it is possible to distinguish the songs of males and females acoustically (Yamaguchi, 1998), the quality of sound recordings made during playback trials was inadequate for the kind of sound analyses used to distinguish the songs of the two sexes due to the low signal-to-noise ratio. Thus, to evaluate overall response of the pair, the combined number and type of songs sung by male and female subjects were used for further analysis. Because females sing in response to playback much less frequently than males (Ritchison, 1988a), I predicted that the combined singing response of a subject pair would still be greater to a male song than to a female song, if the sex of the playback song is recognized, at least by males. Furthermore, as reported below, a subset of the data with singers visually identified was used to examine directly which member of the pair played an active role in singing back to the playback stimuli.

Data analysis

Numbers of songs emitted by each pair in response to male and female songs were counted and compared using a 2-tailed Wilcoxon signed rank test. The Kruskal-Wallis test was used to determine whether there was any significant difference in responsiveness to the three song types used as stimuli.

To test for possible differences in song type matching in response to male and female song playback, I compared the proportion of response songs that matched male and female playback songs using the Mann-Whitney U test. In addition, I counted the number of trials during which at least one song that matched the stimulus song type was sung, and compared the response to the male and female songs using Fisher's exact test.

Singing subjects were visible for 39.8 percent of all songs recorded. When one member of the pair was singing in view, the singing activity of his or her mate could also be determined because pairs almost always stay in close proximity at the beginning of the breeding season when the playback experiment was carried out (Ritchison *et al.*, 1994). The number of songs sung by each member of the pair while they spent equivalent amounts of time in view were also compared using the Wilcoxon signed rank test.

Results

Three out of 20 breeding pairs did not respond at all to the playback trials. Eleven pairs responded to female playback songs, and 15 pairs responded to male playback songs, and 9 pairs responded to both.

Combining male and female responses, subjects sang significantly more songs in response to male songs than in response to female songs (Fig. 2).

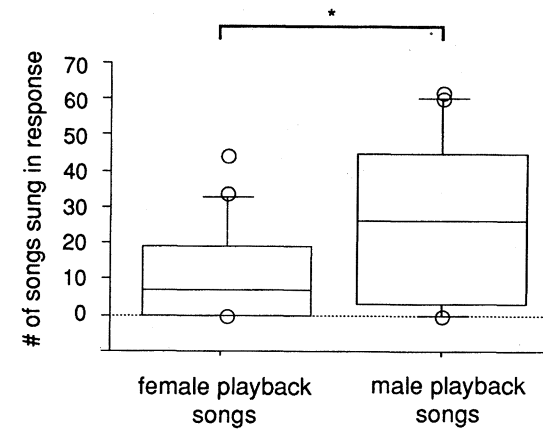


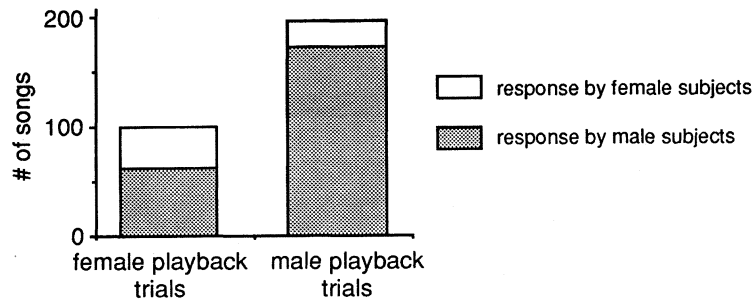
Fig. 2. Box plots showing the number of songs sung by subjects in response to hearing male and female playback songs. Each horizontal bar indicates 10, 25, 50, 75, and 90 percentile of the data set, and empty circles indicate outliers. The singing response difference is statistically significant (Wilcoxon signed rank test, $z = -2.154$, $p = 0.031$).

There was no difference in the responsiveness to the three pairs of playback song types that were used for the experiment ($H = 1.428$, $p = 0.490$).

On average, 27.5 ± 14.0 (mean \pm standard error)% of songs sung by the subjects matched the female playback songs, and $36.9 \pm 10.4\%$ matched the male playback songs. There was no significant difference in the proportion of songs that matched the playback song types of males and females ($U = 67.5$, $p = 0.436$). Moreover, when the number of trials in which at least one song matched the playback song types were compared, there was no significant difference (60% match to male songs, 27% match to female songs, $p = 0.132$).

In 19 trials, the observers were able to visually identify the sex of the singers for at least some of the songs sung during the trial (8 female playback trials, 11 male playback trials) as summarized in Fig. 3A. The number of songs with a visually identified singer accounted for 39.8 percent of all songs recorded in the 26 playback trials that elicited any singing response. Subject females sang 21.3%, and males sang 78.7% of the songs with visually confirmed singers. Four females were observed to sing in response to female playback songs and three females were observed to sing in response to male playback songs. In contrast, six males were observed to sing in response to female songs and nine males were observed to sing

(A)



(B)

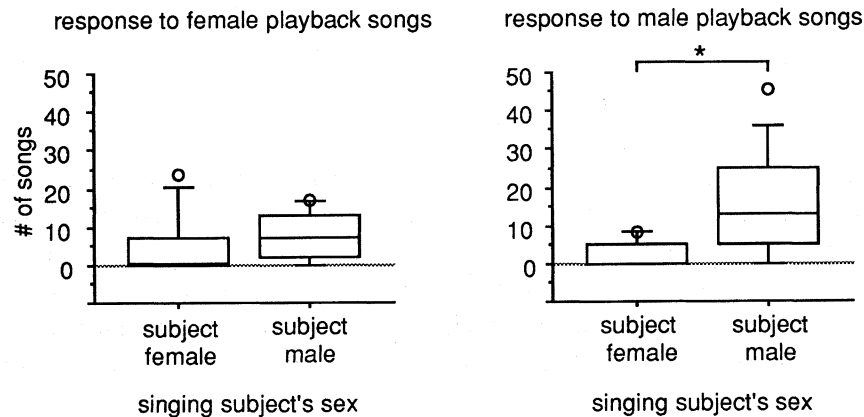


Fig. 3. (A) The total number of songs sung by visually identified singers during the playback experiment. These observations accounted for 39.8% of the total number of songs recorded during all the playback trials. (B) Box plots showing the number of songs sung by visually identified males and females during male and female playback sessions. Males sang significantly more than females did in response to male playback songs (Mann-Whitney U test, $U = 17.5$, $p = 0.005$), but the two sexes sang similar numbers of songs in response to female playback songs (Mann-Whitney U test, $U = 22.0$, $p = 0.29$).

in response to male songs. Thus, both sexes sang in response to songs of both sexes. During the visual observation, females sang 4.8 ± 3.1 (mean \pm standard error) songs in response to female songs and 2.2 ± 1.1 songs in response to male songs, whereas males sang 7.6 ± 2.3 songs in response to female songs and 15.6 ± 4.2 songs in response to male songs (Fig. 3B). Within this set of observations, males and females sang similar numbers of songs in response to female songs, but males sang significantly more than females did in response to male songs (Fig. 3B).

I conclude that cardinal pairs, taken as a unit, are able to distinguish the songs of the two sexes, responding more to male songs than to female songs by singing, but not by song matching. In addition, the results suggest that males are most responsible for the differences in singing responses observed.

Discussion

Sex recognition or mature male recognition?

Does the differential singing response given by subject pairs signify that they recognized the sex of the singer? Active singing responses given mostly by males in response to male playback songs are interpreted as a strong territorial defense behavior. Thus, it is most likely that the subject cardinals recognized male playback songs as a male intruder and sang back at the speaker to defend their territories. However, it is not clear whether the moderate amount of singing given by males and females in response to female playback songs indicate that they were recognized as intruder females.

I have argued elsewhere that female cardinal songs are somewhat neotenic and similar to songs of immature males (Yamaguchi, 1998). The greater amplitude of upper harmonics and lower syllable stereotypy of female songs are characteristics of the songs of immature males in other species (Podos *et al.*, 1995). This raises the possibility that songs of females and immature males are not distinguishable by cardinals. Instead of sex recognition, the differential response observed in this study may demonstrate cardinals' ability to distinguish songs of reproductively mature males from those of all other individuals.

While further study is necessary to clarify how female playback songs were perceived by male and female cardinals, the subject females' response offers an indication that female playback songs may have been recognized at least by females as an intruding female. As described above, visual identification of the singing subjects were available at part of the time during both male and female trials. Based on this set of data, females, on average, sang more songs during female playback trials than in male playback trials. This may suggest that females recognized female playback songs as a female intruder and took an active role in defending territory by singing back to the speaker more frequently than they did during male playback trials. Thus, the present data suggest the ability of acoustic sex recognition by cardinals, although other interpretations mentioned above could not be ruled out.

Which member of the pair recognized the difference?

The contribution of the two members of the pair to the differential responses observed are difficult to disentangle. The discrimination ability of one member of the pair could be better than that of the other. Based on the subset of the data with visually identified singers, the differential response observed appeared to result largely from the differential responses of males to the songs of the two sexes. Visual observation during playbacks revealed that almost 80% of all the songs produced by identified singers were sung by males. Among the songs of male subjects, 74% were given in response to male playback songs, apparently reflecting the males' ability to discriminate between songs of the two sexes and to sing differently in response. But it is possible that the observed response difference by males is in fact a reaction to females that discriminate between songs of the two sexes, and sang differently in response, or behaved differently in some other way not measured in my study. The results do not permit me to make a separate judgment about the discrimination abilities of males and females, which may be the same or may differ, as shown in other species (*e.g.* Vos, 1995).

What acoustic cues were used for discrimination?

Although cardinals' ability to recognize the sex of the singing conspecifics was predicted on a functional bases, the finding was still surprising because the songs of males and females sound so similar to the human ear. The

two acoustic parameters that are shown to distinguish the songs of males and females, syllable stereotypy and harmonic amplitude difference, could be the cues used by birds for sex recognition shown in this study. I do not yet know whether these features are actually used in discrimination or whether other, as yet unidentified, features are also required. Which cues are used, whether a single cue is sufficient or whether a combination of all the cues is necessary for birds to recognize the sex of the singer needs to be determined. In addition, it would be of interest to examine whether the cardinals are born with this recognition ability, or they need to acquire the skill during development.

Acoustic communication of songbirds species in which both sexes sing has received little attention to date. These communication systems appear to be more complicated than those of species in which songs are mostly sung by males, simply because there are as twice as many birds involved in singing activity during the breeding season. One of the fundamental questions that arises when examining these communication systems is whether sexual identity of individuals is revealed by singing. While acoustic sex recognition using songs would appear to be highly adaptive, the recognition task seems perceptually challenging in species such as cardinal in which both sexes develop very similar learned songs (Yamaguchi, 1996). The result of the present study showed that cardinals are in fact capable of discriminating songs of the two sexes based on hearing a song. While it is still not clear whether the discrimination ability shown in this study truly represents their ability to recognize the sex of the singer, or their ability to distinguish songs of adult males from those of all others, the present study shows that cardinals are equipped with perceptual capacity to discriminate songs of the two sexes despite their similarity. Other species in which both sexes learn to sing very similar songs may have evolved similar abilities, and may use them to recognize the sex of the singer.

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