



*Avian Behavior, Ecology, and Evolution*

## Sex-specific responses to simulated territorial intrusions provide evidence for relaxed selection pressure on female song in Orchard Orioles

### Respuestas específicas de cada sexo a intrusiones territoriales simuladas, proporcionan evidencia de una presión de selección relajada sobre el canto de hembras de *Icterus spurius*

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**ABSTRACT.** Female song has been significantly understudied compared with male song, and our understanding of how this elaborate signal trait may function has been limited as a result. Reconstruction of ancestral singing behaviors indicate that Orchard Orioles (*Icterus spurius*) have reduced female song compared with tropical ancestors. In this study, we found that male and female orioles on their breeding territories responded more strongly by all metrics to male song playback than to female song playback. Given the high degree of qualitative variation in female song and the reduced response to female playback, our results suggest that female song in Orchard Orioles may be under relaxed selection pressure. Sexual dimorphism in the complexity and frequency of songs in many lineages likely evolved as a result of a reduction or loss of female song. However, little research has investigated the functional significance of these reduced or infrequent female songs. More research is required to investigate the specific selection pressures that may be acting on female singing behavior in songbirds.

**RESUMEN.** El canto de las hembras ha sido significativamente menos estudiado en comparación con el canto de los machos, y como resultado, nuestra comprensión de cómo puede funcionar este elaborado rasgo acústico, ha sido limitado. La reconstrucción del comportamiento de cantos ancestrales indica que las hembras de *Icterus spurius* han reducido su canto en comparación con sus ancestros tropicales. En este estudio, encontramos que machos y hembras de *I. spurius*, en sus territorios de reproducción respondieron más fuertemente, de acuerdo con todas las métricas realizadas, al canto pregrabado (playback) de los machos que al canto pregrabado de las hembras. Dado el alto grado de variación cualitativa en el canto de las hembras y la respuesta reducida al canto pregrabado de las hembras, nuestros resultados sugieren que el canto de las hembras en *I. spurius* puede estar bajo una presión de selección relajada. El dimorfismo sexual en la complejidad y frecuencia de los cantos en muchos linajes probablemente evolucionó como resultado de una reducción o pérdida del canto de las hembras. Sin embargo, poco se ha investigado sobre la importancia funcional de estos cantos reducidos o infrecuentes en las hembras. Se requiere de mayor investigación para profundizar sobre las posibles presiones selectivas específicas que pueden estar actuando sobre el comportamiento del canto de las hembras en las aves canoras.

**Key Words:** *bird song; female song; Icterus spurius; Orchard Oriole; relaxed selection pressure; sexual dimorphism; song playback; songbird vocalizations*

#### INTRODUCTION

For decades, researchers in North America and Europe have studied the function and mechanisms of elaborate advertisement traits in male animals (Darwin 1871, Andersson and Iwasa 1996, Tobias et al. 2011). Researchers traditionally assumed that these traits evolved as a result of sexual selection acting on males. Thus, similar elaborate traits in female animals were often ignored or assumed to be non-functional. This male trait bias has significantly influenced the study of bird song, resulting in a long-held assumption that bird song is primarily a male trait (Podos et al. 2004, Catchpole and Slater 2008, Haines et al. 2020, Rose et al. 2022).

Sexual dimorphism in song was thought to have evolved from an ancestral state in which neither sex had elaborate song. Thus, dimorphism would have evolved when male birds gained song, and females did not. However, through literature surveys and phylogenetic analysis, researchers have determined that female bird song is widespread across the songbird phylogeny and is likely

the ancestral trait of oscine passerines (songbirds) (Odom et al. 2014, Odom and Benedict 2018). These findings challenge the traditional assumptions of the evolutionary history of bird song. Importantly, we now know that, in many cases, sexual dimorphism in song likely evolved as a result of a loss or decrease in female song (Price et al. 2009).

A growing number of studies have found support for several potential functions of female song in both oscines and suboscines. In Stripe-headed Sparrows (*Aimophila r. ruficauda*), females are more vocally aggressive than males in response to intrusions, especially to same-sex playbacks (Illes and Yunes-Jimenez 2009). Female song has also been observed to function for mate attraction: female Dusky Antbirds (*Cercomacra tyrannia*) sing different song types when they have a mate compared with when they are unmated, and it is hypothesized that the unmated “courtship” song type serves to attract a new mate (Morton et al. 2000). In duetting species, such as Rufous Horneros (*Furnarius rufus*) or Venezuelan Troupials (*Icterus icterus*), females

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participate in territory defense alongside their male partners through coordinated vocalizations and displays (Odom et al. 2016, Diniz et al. 2018). In Dusky Antbirds, both sexes overlap their mates' songs during duetting, which could warn away potential suitors (Morton 1996, Morton and Derrickson 1996). Female song may also function to maintain pair bonds in duetting species, particularly in the tropics (Langmore 1998, Hall 2004).

In many species, however, female song is reduced compared with male song or absent entirely (Austin et al. 2021, Moyer et al. 2022, Price et al. 2023). Little research has been conducted on the consistent selective pressures that may lead to a reduction or loss of female song under particular ecological conditions. Female song may be costly in certain contexts, such as increasing predation risk for females singing from their nests (Kleindorfer et al. 2016, Stracey et al. 2023). Additionally, new evidence suggests that complex female song may be lost even if duetting behavior is maintained (Price et al. 2023). Price et al. (2009) found that loss or reduction of female song in the New World blackbird family Icteridae is associated with a change from tropical to temperate breeding. This loss of female elaboration in association with migratory behavior can also be seen with elaborate plumage patterns: in the oriole genus *Icterus*, phylogenetic reconstructions indicate that the loss of elaborate female plumage was associated with the evolution of migration (Friedman et al. 2009).

One member of the genus *Icterus* is the Orchard Oriole (*Icterus spurius*), a migratory songbird species that breeds across much of central and eastern North America (May–July). Male orioles arrive on the breeding grounds in late April–early May and establish territories, with females arriving later (Scharf and Kren 2022). A proportion of males, particularly yearling males, remain unmated for the duration of the season (Enstrom 1993, Scharf and Kren 2022). In addition to songs, Orchard Orioles produce several types of calls, including “chatter” calls and alarm “jeets.” Alarm jeet calls are typically produced in aggressive contexts and most often by males (Sturge et al. 2016, Scharf and Kren 2022). Orchard Oriole females have completely lost elaborate plumage (Hofmann et al. 2008) and can be easily visually distinguished from mature males in the field by plumage coloration. Yearling male plumage closely resembles that of females, with the exception of black feathers around their bill and throat (Scharf and Kren 2022).

Previously, our team analyzed the song rate and structure of both male and female Orchard Orioles during the breeding season, and we found that females sang significantly less frequently than males (Moyer et al. 2022). Furthermore, we found that female song was acoustically different from male song for five of the eight variables that we measured and showed significantly more variation than male song for all eight variables (Moyer et al. 2022). However, we noted that further studies were required to determine if there are functional differences in male and female oriole song, and if the birds can distinguish between male and female vocalizations.

Playback experiments are a common method for observing animal reactions to stimuli in their natural environment, particularly to simulated territorial intrusions (Kroodsma 1989, Illes and Yunes-Jimenez 2009, Cain et al. 2015). To determine if Orchard Orioles can distinguish between male and female songs, we conducted playbacks with male and female oriole song in the

field and observed the orioles' responses. The results of these experiments may also serve to help us assess potential functional hypotheses for female song in Orchard Orioles. For example, if male and female songs both elicit strong responses under similar conditions, male and female Orchard Oriole song may serve similar functions. However, if female song is nonfunctional in the traditional male contexts of territory defense or mate attraction/guarding, we might not expect either sex to respond strongly to female song playback.

Below we provide a summary of potential playback responses that would support specific functions of female song.

1. Female song functions in territory defense: if females primarily defend against rival females, females should approach the speaker more closely and signal more aggressively to female song playback than to male song playback. If females defend against any intruders, they should respond to the playback of both sexes. It is possible that males will also respond aggressively to female song if they consider other females a territorial threat, in which case males should also approach and produce aggressive vocal signals in response to female song.
2. Female song functions for mate attraction: males, particularly yearlings who are unmated, should be attracted to female song and respond positively by approaching the speaker or singing more frequently.
3. Female song functions for mate guarding: females, but not males, should respond more aggressively to female song than to male song if they are attempting to ward off a rival female or prevent their mate from interacting with other females. Females should also sing at higher rates in response to female song.
4. Female song is not functional or has reduced function in traditional male song contexts: neither sex should respond strongly to female song.

Distinct functions for female song do not necessarily have exclusive predictions. However, interpreting our results in light of the above predictions is a good first step toward determining potential functions for female song in Orchard Orioles.

## METHODS

### Simulated territorial intrusions

We conducted playback studies between 0600–1130 EDT at field sites across central Maryland (Howard, Prince George's, Carroll, and Baltimore counties; Append. 1) in May and June of 2021 and 2022. We recorded observations during each playback using a Marantz PMD 661 recorder and a Sennheiser ME67 or ME66 shotgun microphone with K6 powering module. We played all song playback stimuli using an iPod Classic and an Anchor Audio AN-MINI speaker covered by a thin brown speaker cloth for camouflage. We set the speaker volume at a constant level to produce each signal at an amplitude of approximately 85 dB at one m, measured using a Radio Shack 33-2050 sound level meter with a C-weighting curve and a fast time response. Before each playback trial, the ambient noise level, temperature, wind speed, and all other metadata were recorded by both observers to ensure that playback volume would not be obstructed.

We placed a speaker on the ground below a tree  $\geq 5$  m in height near the center of an Orchard Oriole territory, and stationed two observers 20 m away. Observers were stationed roughly at 90 degrees from each other in areas that provided the clearest line of sight to the playback area. We placed flags 5, 10, 20, and 50 m away from the speaker along the N, S, E, and W axes to improve the accuracy of distance estimates. We determined that 50 m was the radius in which vocal behaviors could be reliably monitored and attributed to the playback stimuli. If the bird left the 50 m observation radius during the trial, we did not include any of their behaviors produced outside of that radius in the analysis, as the behaviors could not be reliably attributed to specific individuals.

We created playback treatments for three stimulus categories from previously collected high-quality recordings: male Orchard Oriole songs, female Orchard Oriole songs, and male Eastern Bluebird (*Sialia sialis*) songs as a heterospecific control. The geographic range of Eastern Bluebirds broadly overlaps that of the Orchard Oriole during the breeding season (Gowaty and Plissner 2020), but this species is not closely related enough to be considered a potential sexual competitor, so we did not expect any response to this treatment. For each of the three playback stimulus categories, we chose four individual stimuli, each one containing a single song from a unique individual so that we would have multiple exemplars for each stimulus class as a means to test for the effects of pseudoreplication in playback treatments (Kroodsma 1989). All songs were recorded during the breeding season (late April–June), digitized at 44.1 kHz, and high-pass filtered to remove background noise (Moyer et al. 2022).

Each Orchard Oriole tested received one playback treatment of each of the three types (male song, female song, bluebird song) in a given trial. Within a trial, each individual playback treatment lasted 2.5 min, with one recorded song repeated every 10 sec, corresponding to the singing rate in natural (i.e., in the absence of any stimuli) male Orchard Oriole song bouts. Between each of the playback treatments, we enforced a 10-min interstimulus period to reduce carryover effects from one treatment type to the next. Thus, a playback session with a given individual or pair resulted in approximately 47.5 min total trial time, including a 10-min prestimulus observation period. The playback treatment used for each trial was randomly selected without replacement from our recordings of four males, four females, and four bluebirds, with the stipulation that no song recorded from a given site was used in playbacks at that location. The bluebird treatment was always played first as a control, as we expected no response to this treatment, and the male and female oriole playbacks were alternated in order. Song playback stimulus treatments were created in Audacity® audio editing and recording software (V.3.0.2, Audacity Team 2021). All playback experiments were performed at least 1 km away from one another, or  $\leq 1$  km if the focal males were of different age classes and therefore visually distinguishable.

As noted above, a proportion of males remain unpaired throughout the breeding season, but no unpaired females were ever observed, so trials were performed on pairs and solo males. Before beginning a trial, we ensured that there was only one pair or individual male visible and that there were no neighbors that were likely to enter the observation area. If there was a known nest, the speaker was placed at least 20 m from the nest tree. On one occasion where the nest was located after the experiment had

already commenced, and the speaker was less than 20 m from the nest, the bird's distance from the speaker was only recorded when the bird was not on the nest.

During the course of our playback experiments, we modified two aspects of our original experimental design to streamline our data collection and increase our overall sample size. For the first ten playback trials, we began with 10 min of prestimulus observation, where any target behaviors were noted by both observers. After none of the responses that were typically given during playback experiments (speaker approach, alarm jeets, etc.) were observed, we ceased including this prestimulus assessment period for all subsequent trials. Similarly, after no such responses were observed during the bluebird control treatment in the first 38 playback trials, we conducted all subsequent trials with only male and female Orchard Oriole playbacks (Appendix 2, 3). In total, we conducted 59 playback trials (34 to both males and females, 25 to solo males).

### Playback analysis

During each playback stimulus, we recorded the number of jeets and the number of songs produced by each responding oriole, as well as the closest approach to the speaker. Only one female produced an alarm jeet during any of the playback trials, so this response variable was scored only in males. One traditional approach for interpreting count data, such as the number of jeets, would be to model the data using a Poisson regression and Generalized Linear Mixed Models (GLMMs). However, there was a high proportion of zeros in the data. Appendix 4 shows the proportion of zeros expected for a typical Poisson distribution compared with the distribution observed in this data set.

Given the high proportion of zeros we observed compared with what would be expected from typical count data, we chose to analyze playback responses using zero-inflated “glmmTMB” package in R, v.4.2.1 (Brooks et al. 2017). We built five models to compare responses to each playback type: (1) Male Jeets ~ Playback Sex, (2) Male Songs ~ Playback Sex, (3) Female Songs ~ Playback Sex, (4) Male Closest Approach ~ Playback Sex, and (5) Female Closest Approach ~ Playback Sex. For all five models, we included the order of playback type provided as a fixed effect, and the date, the identity of the responding bird, and the pair status (of males, as all females observed were paired) as random effects. To obtain *F* statistics and *p* values for both random and fixed effects, we ran a one-way ANOVA on the model output. The Male Songs model failed to converge with all effects, so we determined the combination of effects that resulted in the lowest AICc and ran a simplified model, which did not include date or order.

Zero-inflation can obscure patterns in the data and make it difficult to detect underlying trends. Instead of reporting average values for each response, which would be lower than expected as a result of the high proportion of zeros, we converted the three behaviors of the focal male and the focal female (when present) to binary variables. For a given individual, each response behavior was scored as “yes” if they performed that behavior (sang at least one song, produced at least one alarm jeet, or came within 20 m of the speaker, respectively) during a playback trial. Individuals that did not perform that behavior were scored as a “no” response for that variable. Converting these responses to binary variables allowed us to more accurately assess overall response to playbacks, despite the large proportion of zeros in the data.

As an additional analysis to confirm our model results, we compared each of these binary response variables using Fisher's Exact Tests. We also compared male and female responses to oriole playbacks vs. the bluebird control playback for the 38 trials that received the control stimulus. Finally, we quantified the song rates of responding birds during playbacks to compare with the natural rates reported in Moyer et al. (2022). All statistics were computed using R, v4.3.2 (R Core Team 2023).

### Opportunistic observations of natural female song in context

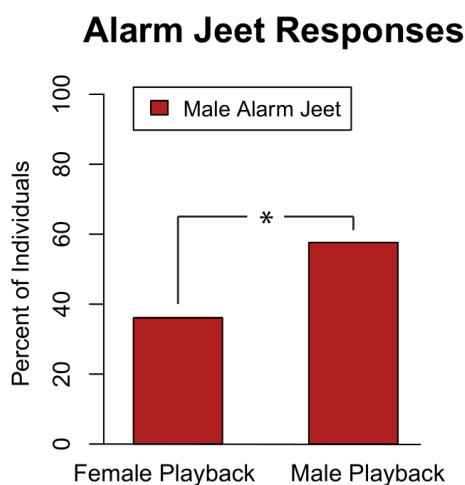
We recorded opportunistic observations of females singing throughout the breeding season and noted the contexts in which they sang. Orchard Oriole pairs ( $n = 28$ ) were recorded for 20-min observation sessions between 6:00 am and 12:00 pm at least once per week for 4 wks in May and June 2022. The location and behaviors of the female, her mate, and any additional orioles nearby were noted "before" (the 10 sec preceding a female song) and "during/after" (the duration of song the song and the period immediately after, totaling 10 sec) each female song. These behavioral observations were compared across all females to determine if there were any consistent contexts in which female song was most often produced and if there were any consistent responses observed from conspecifics in the "during/after" period. Natural female song rates (calculated as songs/min during the 20-min observation) during each stage of the breeding season (pre-building, building, incubation, and provisioning) were also quantified and compared to determine if song rate was associated with breeding stage.

## RESULTS

### Simulated territorial intrusions

Males produced significantly more alarm jeets in response to male playback than to female playback ( $F_{1,57} = 5.10, p = 0.028$ ; Fig. 1). There was significant individual variation in male jeets ( $F_{56,57} = 1.65, p = 0.032$ ). Both paired ( $n = 24$ ) and unpaired ( $n = 23$ ) males produced alarm jeets more often in response to male playback, with solo males producing significantly more alarm jeets overall ( $F_{1,57} = 6.84, p = 0.011$ ; Table 1).

**Fig. 1.** Percentage of males who produced an alarm jeet during male playback ("Yes"  $n = 34$ ) and female playback ("Yes"  $n = 21$ ). \* indicates significant difference according to the zero-inflated GLMM.



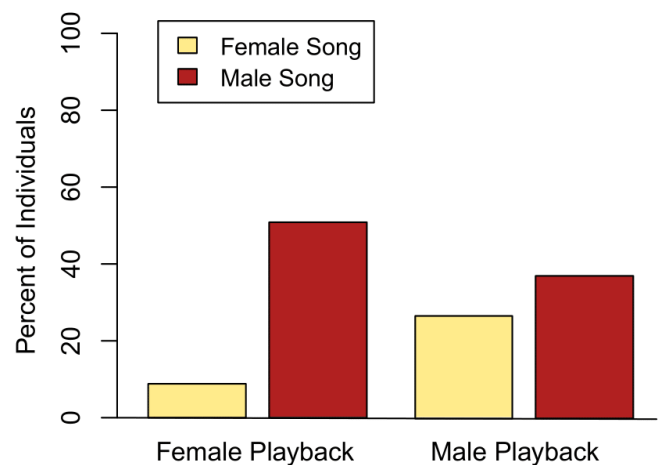
**Table 1.** Mean number ( $\pm 1$  standard deviation) of male alarm jeets in response to male and female playback.

Responding male condition	Pair status	
	Paired	Solo
Male playback	7.38 $\pm$ 13.32	12.92 $\pm$ 14.72
Female playback	3.29 $\pm$ 6.53	8.04 $\pm$ 13.69

There was no significant difference in male song responses to male vs. female playback ( $F_{1,58} = 1.26, p = 0.267$ ; Fig. 2), although there was significant individual variation ( $F_{57,58} = 1.66, p = 0.028$ ). Male song rates were low overall—only 50.8% of males sang during female playback, and only 37.3% of males sang during male playback (Fig. 2). Male song rates were lower than the natural rates observed in Moyer et al. (2022) (Table 2).

**Fig. 2.** Percentage of males and females who sang at least once during male playback (Male "Yes"  $n = 22$ , Female "Yes"  $n = 9$ ) and female playback (Male "Yes"  $n = 30$ , Female "Yes"  $n = 3$ ).

## Song Responses



**Table 2.** Male song rates during male and female playbacks compared with the natural song rates reported in Moyer et al. (2022). Rates shown are songs/min  $\pm 1$  standard deviation.

	Observed male rate during playback (songs/min)	Natural male song rate (songs/min) (Moyer et al. 2022)
Female playback	0.97 $\pm$ 1.63	2.35 $\pm$ 2.43
Male playback	0.71 $\pm$ 1.38	

Females did not sing significantly more often to either type of playback ( $F_{1,33} = 1.51, p = 0.228$ ), but date had a significant effect, with females singing more earlier in the breeding season ( $F_{1,58} = 13.43, p = 0.001$ ; Fig. 2). In response to female playback, 9.1% of females sang, and 27.3% of females sang in response to male playback (Fig. 2). However, female song rates during both playbacks were similar to the natural rates observed in Moyer et al. (2022) (Table 3).

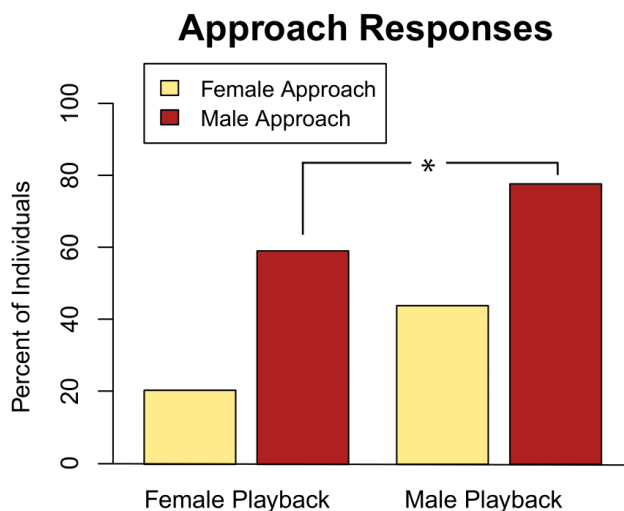


**Table 3.** Female song rates during male and female playbacks compared with the natural song rates recorded in Moyer et al. (2022). Rates shown are songs/min  $\pm$  1 standard deviation.

	Observed female rate during playback (songs/min)	Natural female song rate (songs/min) (Moyer et al. 2022)
Female playback	0.09 $\pm$ 0.34	0.14 $\pm$ 0.35
Male playback	0.18 $\pm$ 0.31	

Males were significantly more likely to approach within 20 m to male playback ( $F_{1,57} = 4.82, p = 0.0322$ ; Fig. 3). Females did not approach differentially to male playback vs. female playback ( $F_{1,33} = 1.69, p = 0.203$ ; Fig. 3). All model results were confirmed by the Fisher's Exact Tests (Append. 5).

**Fig. 3.** Percentage of males and females who came within 20 m of the speaker during male playback (Male "Yes"  $n = 46$ , Female "Yes"  $n = 15$ ) and female playback (Male "Yes"  $n = 35$ , Female "Yes"  $n = 7$ ). \* indicates significant difference according to the zero-inflated GLMM.



For the 38 trials that included bluebird control playback, males and females were significantly more likely to approach and males were significantly more likely to jeet in response to male playback than to the control playback (Append. 2, 3). Males were significantly more likely to approach to female playback than to the control playback, but for all other response variables, the response to female playback was similar to the response to control playback (Append. 2, 3)

**Opportunistic observations of natural female song in context**

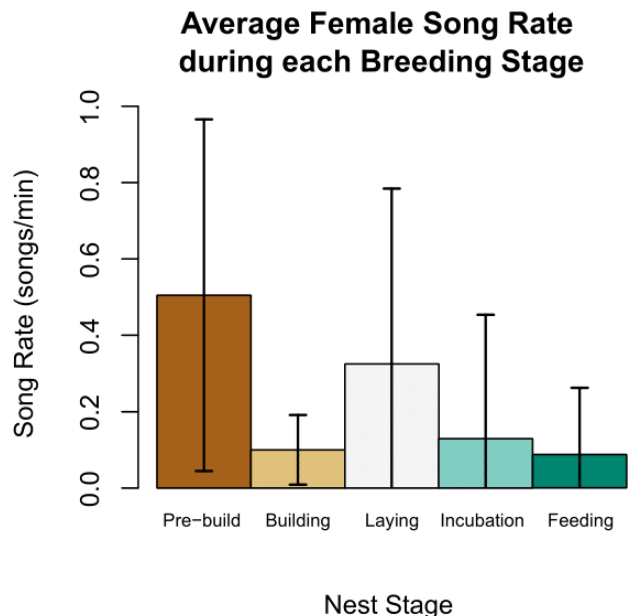
Opportunistic observations of natural singing behavior did not indicate any specific contexts in which female song was consistently produced (Table 4). Females sang in a variety of contexts, including during aggressive interactions with other individuals or following songs from a mate, but there were no strong associations with specific behavioral interactions, conditions, or responses. Song rate comparisons across the

breeding stage indicated that female song rates were highest during the pre-building stage of the nest cycle, although there was a great deal of individual variation (Fig. 4). Female song rates are naturally significantly lower than those of males, with females singing roughly 95% less frequently than males (Moyer et al. 2022).

**Table 4.** Results of naturalistic female song observations. The number of times that specific condition occurred, such as the female exhibiting aggressive behavior, was counted during the 10 sec before a female song and the 10 sec including and following a female song.

Condition	Number of instances	
	Before female song	During/after female song
Male present	7	12
Additional oriole present	7	10
Female aggressive behavior	4	5
Female chasing/following mate	2	5
Copulation behaviors	0	1
Another oriole singing	3	3

**Fig. 4.** Average female song rate from a given 20-min observation period, with one rate chosen from each female at random. Bars on each graph represent one standard deviation.



**DISCUSSION**

By all three metrics of response, male and female Orchard Orioles responded to song playback from both sexes. Although the responses to male and female playback only differed significantly for two of the five variables, all five variables showed stronger responses to male playback than to female playback. Males produced significantly more alarm jeets and approached within 20 m of the speaker significantly more often when exposed to male song than to female song. "Male songs" was the only male

variable that was not significantly different between male and female playback. The production of alarm jeets likely represents a more reliable male response to a territorial intrusion than songs, as male Orchard Oriole song rates often decreased in response to territorial intrusions and were lower than the natural rates previously observed (Moyer et al. 2022). Females did not sing or approach significantly differently to male vs. female playback. Responses from both sexes to female playback was low overall, and did not differ significantly from responses to the control playback for all variables except male approach.

Below we summarize the proposed hypotheses for female song and how well they are supported by the observed responses to playbacks:

1. Female song functions in territory defense: We found low support for this hypothesis. Although males did produce alarm jeets, which suggests that female song could be a threatening signal to territory owners, they did so significantly less often in response female playback than to male playback. In addition, females did not respond more strongly to female song than to male song, nor did they sing at higher rates than natural levels in response to territorial intrusion from either sex.
2. Female song functions for mate attraction: We found low support for this hypothesis. Males did approach to playbacks of female song. However, they also produced alarm jeets, which are generally an aggressive signal. Furthermore, unpaired males also produced more alarm jeets than paired males in response to female song, which contradicts the hypothesis that female song might be an attraction signal to birds seeking a mate. Females did sing more in response to male song, but this difference was not significant. However, females did sing more earlier in the breeding season according to the Female Song model and female rate observations, which could suggest that female song may be important for coordination of early breeding activities.
3. Female song functions for mate guarding: We found low support for this hypothesis. Females did not respond more aggressively to female song than to male song. Females also did not typically sing in response to female song, as would be predicted if females were guarding their mates.
4. Female song is not functional or has reduced function in traditional male song contexts: We found moderate support for this hypothesis. If female song were non-functional as a territorial or mate attraction signal, we would not expect either sex to respond to a simulated territorial intrusion playback experiment using female song. However, female song may be similar enough to male song in acoustic characteristics to elicit some response in that context, regardless of sex.

Naturalistic observations of female song did not provide evidence for any consistent function of female song, which suggests that this behavior may be under relaxed selection pressure. However, we have observed individual females using song in a number of clearly identifiable specific contexts. For example, two females sang continuously for over 2 min while engaged in a physically aggressive altercation (*personal observation*, MJM). On several

occasions, we observed females singing at a rapid rate while two nearby males attacked one another (*personal observation*, KEO). Furthermore, several females have been observed singing on the nest when their mates were nearby (*personal observation*, MJM), a behavior that may or may not be adaptive (Leonard 2008, Kleindorfer et al. 2016, Stracey et al. 2023). Finally, we noticed that males usually sang in long bouts, often from exposed areas such as treetops or the edge of bushes, whereas females did not typically sing from any particular prominent locations (*personal observations*, MJM, KEO). It is possible, therefore, that female song may be functional for some individuals in specific contexts but is not under strong selection for a consistent function across all females. Relaxed selection pressure on female song is also supported by the increased variation that we documented previously in the acoustic structure and syllable usage of female song (Moyer et al. 2022; Moreland et al., *unpublished manuscript*).

Few studies have examined direct selective pressures against female song or reduced selection pressure in favor of retaining female song. Orchard Orioles are New World blackbirds (Icteridae), and Price et al. (2009) demonstrated that across the Icterid phylogeny, loss of female song was associated with temperate breeding and the gain of migratory behavior. If the environmental conditions shift so that that the benefits of a given trait no longer to outweigh the costs, then evolutionary theory predicts that the trait will be selected against and will be lost or reduced (Lahti et al. 2009). Conversely, the trait could simply no longer be selected for, which is predicted to lead to increased variation in trait expression (Lahti et al. 2009, Reinhold 2011).

In migratory species, pair bonds and territories are more ephemeral, and female song may not be maintained in favor of other energetic requirements (Price 2009, Logue and Hall 2014). For Orchard Orioles, the natural history characteristics associated with temperate breeding, such as a shorter breeding season, lack of year-round territory defense, or the energetic constraints of migration could have led to a reduction in female song structure and functionality.

Importantly, elaborate female coloration, which may have been an important signal for territory, mate, or resource competition in an ancestral oriole, has been completely lost in this species (Hofmann et al. 2008). This is the only species of oriole that has no solid black and no pure patches of color in adult females. Similarly, Orchard Oriole female song is acoustically reduced (shorter, smaller bandwidth, less frequent) compared with male song, which may indicate an analogous reduction in selective pressure for competitive vocal signaling. In Carolina Wrens (*Thryothorus ludovicianus*), a species with long-term pair bonds and sedentary behavior, complex female song was still lost, which suggests that there may yet be additional unknown factors impacting the maintenance of female song (Price et al. 2023).

## CONCLUSION

Further research in both tropical and temperate species is needed on the specific selection pressures that may be shaping female song in comparison with male song, particularly given the documented relationships between female song and tropical natural history characteristics (Price 2009). This study demonstrated that female song in Orchard Orioles may be functional in specific contexts, but playback experiments and

naturalistic observations suggest relaxed selection on female song for functions traditionally associated with male song, such as territory defense and mate attraction. Whereas both males and females approached and sang in response to both playback stimuli, the overall responses to male song were stronger than to female song. Compared with male song, female song is reduced both in its production (Moyer et al. 2022) and in the degree of response it elicits (present study). Therefore, the retention of female song in specific contexts likely is not under strong positive selection in this species.

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#### Author Contributions:

MJM, BL, and KEO conceived the idea and designed the experiments. KEO supervised the research. MJM, EO, EKB, AR, BAM, and KEO collected the data, and MJM, NKN, JL, BAM, and KEO analyzed the data. MJM and KEO wrote and edited the paper.

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#### Data Availability:

Raw song and rate data have been deposited online at FigShare (<https://figshare.com/>), under data identifier <https://doi.org/10.6084/m9.figshare.26496574.v1>.

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#### LITERATURE CITED

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## Appendix 1.

List of field sites in central Maryland. All observations were performed between 6:00 am and 12:00 pm from April 29th – June 30th, 2021, and May 2<sup>nd</sup> – June 24<sup>th</sup>, 2022.

### **Howard County**

Alpha Ridge Park, Marriottsville, Maryland

Blandair Regional Park, Columbia, Maryland

Bon Secours, Marriottsville, Maryland

Castlebridge Road, West Friendship, Maryland

Centennial Park – Ellicott City, Maryland

Crest Lawn Memorial Gardens, Marriottsville, Maryland

Font Hill Wetlands Park, Ellicott City, Maryland

Howard County Conservancy, Woodstock, Maryland

Howard County Living Farm Heritage Museum, West Friendship, Maryland

Guilford Park, Jessup, Maryland

Lime Kiln Middle School, Fulton, Maryland

Maple Lawn Business District, Fulton, Maryland

Meadowbrook Park, Ellicott City, Maryland

Rockburn Branch Park, Elkridge, Maryland

Schooley Mill Park, Highland, Maryland

Sewells Orchard Ponds, Gaithersburg, Maryland

Sharp's at Waterford Farm, Brookeville, Maryland

Warfield Pond Park, Glenwood, Maryland

Woodmark Lake, West Friendship, Maryland

**Baltimore City County**

Druid Hill Park, Baltimore, Maryland

Masonville Cove Environmental Education Center, Baltimore, Maryland

**Baltimore County**

Arbutus Library, Arbutus, Maryland

BWI Nursery, Linthicum Heights, Maryland

Gwynnbrook Wildlife Management Area, Owings Mills, Maryland

Meadowood Regional Park, Timonium, Maryland

Oregon Ridge Park, Cockeysville, Maryland

Patapsco Valley State Park – Avalon, Halethorpe, Maryland

Southwest Area Park, Baltimore, Maryland

St Louis Church, Clarksville, Maryland

Quarry Lake, Pikesville, Maryland

University of Maryland, Baltimore County Campus, Catonsville, Maryland

**Carroll County**

Gaither Road, Eldersburg, Maryland

**Montgomery County**

Burtonsville Elementary, Burtonsville, Maryland

Lake Hallowell, Olney, Maryland

Olney Manor Park, Olney, Maryland

Wheaton Park, Silver Spring, Maryland

**Prince George's County**

Bentley Park, Laurel, Maryland

Fairland Regional Park, Laurel, Maryland

Grace Baptist Church, Laurel, Maryland

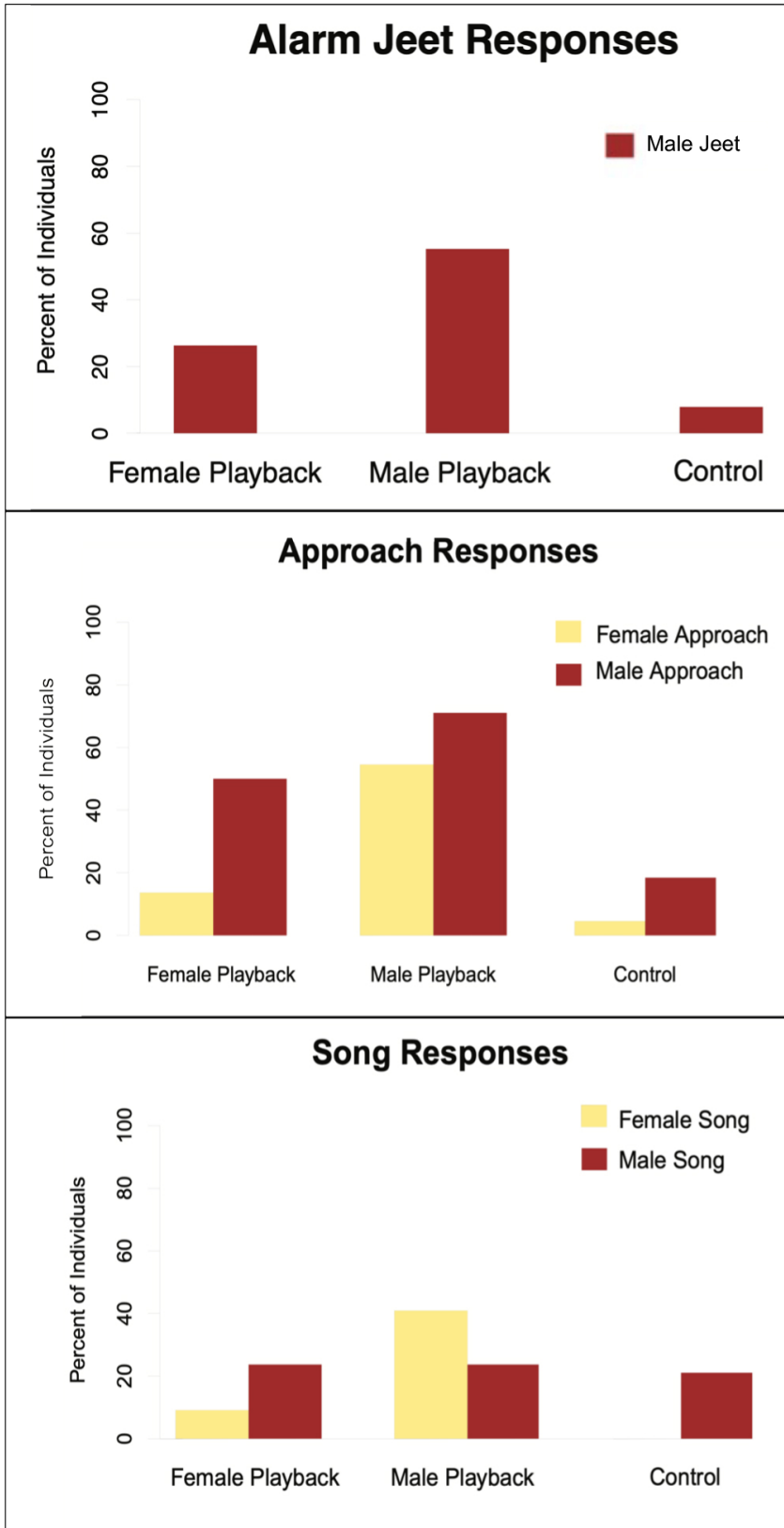
Lake Artemesia Park, College Park, Maryland

Laurel Park, Laurel, Maryland

North Laurel Park, Laurel, Maryland

Patuxent Research Refuge – South Tract, Laurel, Maryland

Appendix 2.



Responses to male and female playback compared to the control playback, Eastern Bluebird song. As predicted, the control playback did not elicit a response from either sex, and was thus not included after the first 38 trials.

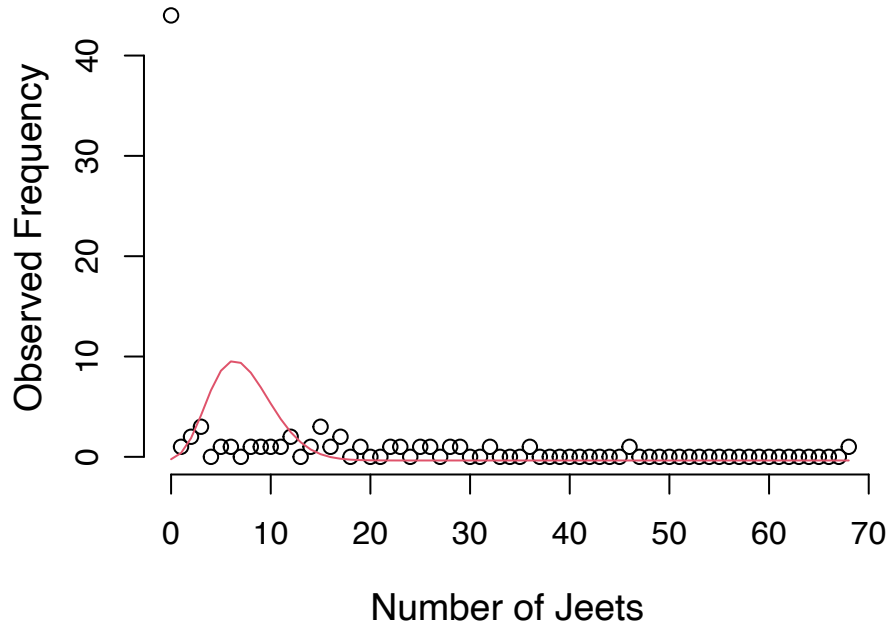


Appendix 3. Fishers Exact Test results comparing responses to each sex of oriole playback with the Eastern Bluebird control (n=38 trials). Bold values indicate significance at  $p \leq 0.05$ .

Response Variable	Male Playback vs. Control	Female Playback vs. Control
Male Approach	<b>&lt;0.0001</b>	<b>0.0072</b>
Female Approach	<b>0.0008</b>	0.6069
Male Song	1.0000	1.0000
Female Song	<b>0.0014</b>	0.4884
Male Jeets	<b>0.0042</b>	0.7879

Appendix 4.

### Observed vs. Expected Count Frequencies (Male Jeets)



Observed and expected frequencies of male jeets under a traditional Poisson count distribution. The red line demonstrates the Poisson model, with the circles representing the observed counts of male jeets. This data showed a large proportion of zeros (more than 40) that a traditional Poisson model would not accurately capture.

Appendix 5. Results of Fisher's Exact Tests comparing binary responses to male and female playback. All Fisher's Exact results agreed with the results of our zero-inflated GLMMs. Bold values indicate significance at  $p \leq 0.05$ .

Response Variable	Fisher's Exact p-value
Male Approach	<b>0.046</b>
Female Approach	0.068
Male Song	0.194
Female Song	0.108
Male Jeets	<b>0.026</b>