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## Female song is structurally different from male song in Orchard Orioles, a temperate-breeding songbird with delayed plumage maturation

### El canto de las hembras es estructuralmente diferente que el canto de los machos en *Icterus spurius*, un ave cantora que se reproduce en zonas templadas con maduración tardía del plumaje

[Michelle J. Moyer](#)<sup>1</sup>, [Evangeline M. Rose](#)<sup>2</sup>, [D'Juan A. Moreland](#)<sup>1</sup>, [Aiman Raza](#)<sup>1</sup>, [Sean M. Brown](#)<sup>1</sup>, [Alexis L. Scarselletta](#)<sup>1</sup>, [Bernard Lohr](#)<sup>1</sup>, [Karan J. Odom](#)<sup>2</sup> and [Kevin E. Omland](#)<sup>1</sup>

**ABSTRACT.** Female birds in many temperate species are thought to sing reduced or quieter songs and appear to sing less often than their male counterparts. Therefore, female song may be easily overlooked. Increasingly, researchers are recording female song in well-studied species previously assumed to have little or no female song. In this study, we document the extensive use of female song in Orchard Orioles (*Icterus spurius*), a species with delayed plumage maturation where female song had not been well-documented. Based on observations of females singing in the early breeding season, we hypothesized that female song may function for mate attraction. To formally investigate whether females sing specifically early in the season, we assessed singing rates of each sex throughout the breeding season. We also performed detailed acoustic analyses comparing male and female song structure. Females sang significantly less often than males, and female and male songs were statistically different for five of eight variables investigated, indicating that the two sexes sing acoustically distinct songs. However, females also sang more often than initially assumed, suggesting that researchers may be missing female song in other species if they are not directly searching for it, particularly in species in which yearling males and females have similar coloration. Therefore, this study highlights the need to re-explore well-studied systems. Further research is needed to determine if and how female song may function in this species.

**RESUMEN.** Se piensa que las hembras en las aves de muchas especies de zonas templadas cantan cantos reducidos o mas suaves y aparentemente cantan menos frecuentemente que los machos. Por lo tanto, el canto de las hembras puede ser fácilmente pasado por alto. Cada vez más, los investigadores graban el canto de las hembras en especies muy estudiadas en las que previamente se asumía que las hembras no cantaban o cantaban poco. En este estudio documentamos el uso extensivo del canto de las hembras en *Icterus spurius*, una especie con maduración tardía del plumaje, donde el canto de las hembras no ha sido bien documentado. Con base en observaciones de las hembras cantando temprano en la temporada de reproducción, hipotetizamos que el canto de las hembras puede utilizarse para la atracción de la pareja. Con el fin de investigar formalmente si las hembras cantan específicamente temprano en la temporada, determinamos las tasas de canto de cada sexo a lo largo de la temporada de reproducción. También realizamos análisis acústicos detallados comparando la estructura del canto de los machos y de las hembras. Las hembras cantaron significativamente menos frecuentemente que los machos y los cantos de las hembras y de los machos fueron significativamente diferentes en cinco de las ocho variables investigadas, indicando que los dos sexos cantan cantos acústicamente diferentes. Sin embargo, las hembras también cantaron más frecuentemente que lo asumido inicialmente, sugiriendo que los investigadores pueden estar pasando por alto el canto de las hembras de otras especies si no lo están buscando directamente, en particular en especies en donde los machos de primer año y las hembras tienen coloraciones similares. Por lo tanto, este estudio resaltamos la necesidad de re-explorar sistemas bien estudiados. Se requiere más investigación para determinar si el canto de las hembras tiene una función y como es esta función.

**Key Words:** *acoustic analysis; animal communication; delayed plumage maturation; female song; Icterus spurius; Orchard Oriole*

## INTRODUCTION

Historically, elaborate advertisement traits in animals have been assumed to have evolved as a result of sexual selection acting on males (Darwin 1871, Andersson and Iwasa 1996, Tobias et al. 2011). There are, however, many examples of females displaying traits that are equally or even more elaborate than those of males across a wide variety of taxa (e.g., African starling plumage, Rubenstein and Lovette 2009; butterfly coloration, Oliver and Monteiro 2011; neotropical frog calls, Serrano and Penna 2018).

The study of bird song has been dramatically impacted by this long-standing bias toward male traits. Most early prominent studies of bird song were conducted in the temperate regions of North America and Europe, and most often by male researchers, leading to geographic and sex biases in the field (Odom et al. 2014, Haines et al. 2020). As a result, singing behavior in passerines (songbirds) has been primarily attributed to males. For example, in early editions of their widely cited book *Bird Song*, Catchpole and Slater (1995:10) defined songs as “long, complex vocalizations produced by males in the breeding season” (but see

<sup>1</sup>Department of Biological Sciences, University of Maryland, Baltimore County, <sup>2</sup>Department of Psychology, University of Maryland, College Park

Catchpole and Slater 2008). However, this classic definition has been challenged by recent insights into song in both males and females.

A growing body of research shows that many female passerines also sing, and that many previous assumptions of absence are more likely a result of a failure to detect female song (Langmore 1998, Riebel 2003, Odom et al. 2014). In fact, phylogenetic reconstruction found strong evidence that the common ancestor of songbirds most likely possessed female song, indicating that song originally evolved in both sexes (Odom et al. 2014). Given the historical focus on male song, behavioral studies and analyses of female song are much less prevalent, especially in temperate species. In recent years, there have been numerous calls to investigate female song in temperate songbird species (Riebel 2003, Riebel et al. 2005, Odom and Benedict 2018). More studies quantitatively comparing male and female song are needed to truly understand the function and evolutionary history of this elaborate trait.

Previous studies across a variety of species have found that female song may be less elaborate in some acoustic characteristics compared to male song. For example, female Least Flycatchers (*Empidonax minimus*) sing songs with lower frequency ranges and shorter inter-note intervals than male songs (Kasumovic et al. 2003). Montane White-crowned Sparrow (*Zonotrichia leucophrys*) female song reportedly lacks terminal elements, and sounds “softer and quavering” compared to male song (Baptista et al. 1993:522). Because of such findings, female songs in temperate migratory species appears to be thought of either as rare (Morton 1996, Langmore 1998, Riebel 2003, Slater and Mann 2004) or, when present, less frequent and complex than male song (Hoelzel 1986, Baptista et al. 1993, Brunton and Li 2006, Price et al. 2009). Additionally, in temperate regions, females can be relatively quiet and visually cryptic (Price 2019), likely contributing to increased detection of and researcher bias toward male songbirds (Bennett et al. 2019).

However, a comparable number of studies have found that female song is similar to or more elaborate than male song, in both tropical and temperate regions (e.g., Arcese et al. 1988, Johnson and Kermott 1990, Pavlova et al. 2005, Price et al. 2008, Illes and Yunes-Jimenez 2009, Campbell et al. 2016, Odom et al. 2016, Reichard et al. 2018, Rose et al. 2018). In Venezuelan Troupials (*Icterus icterus*), females sing more often during the day than males, and the number and syntax of syllables is similar in female and male songs, indicating similar overall song structure (Odom et al. 2016). Eastern Bluebird (*Sialia sialis*) females sing acoustically equivalent songs to males according to five common measures of song variation (Rose et al. 2018). Recent studies have increased our knowledge of the presence of female song in temperate regions (e.g., Halkin 1997, Krieg and Getty 2016, Hathcock and Benedict 2018, Rose et al. 2018, Heaphy and Cain 2021).

In both temperate and tropical species, pre-existing scientific biases and plumage patterns may lead to misidentifying singing females as males. Female song in many tropical species can be missed as a result of males and females displaying sexually monomorphic plumage (Webb et al. 2016, Odom and Benedict 2018). In temperate species, female song may be missed because of delayed plumage maturation, when one or both sexes does not acquire their adult plumage patterns or coloration until after their

first breeding period (Hawkins et al. 2012). In many species, yearling males retain immature plumage which is, in varying degrees, similar to female plumage (Lyon and Montgomerie 1986, also see Patchett et al. 2021).

Orchard Orioles (*Icterus spurius*) are a migratory, temperate breeding icterid (a member of the New World blackbird family, Icteridae) found across the eastern United States and into parts of Canada and Mexico during their breeding season (May–July). The species is sexually dichromatic, and females and older males can be easily distinguished in the field. However, males have delayed plumage maturation: yearling males have similar olive-green plumage to females, only differing from females in the black coloration on their face and throat (Scharf and Kren 2020). Most tropical icterids have female song, and ancestral state reconstruction indicates that this family historically had this behavior (Price et al. 2009, Odom et al. 2015). However, female song in temperate orioles has been significantly understudied. Male Orchard Orioles sing extensively, and their vocalizations have been described in detail (e.g., Sturge et al. 2016). There are several mentions of female song in this species (Enstrom 1992, Scharf and Kren 2020) but these accounts are brief or anecdotal. Our lab had been conducting field work on Orchard Oriole breeding behavior for over five years, but we did not notice female song in this species until our work on tropical species primed us to look for female song. These initial observations of females singing occurred primarily in the early weeks of the breeding season, suggesting that female song in this species may primarily function in mate attraction (Gahr and Güttingery 1986, Eens and Pinxten 1998, Langmore 2000, Austin et al. 2021). If this is the case, we predict that female song rates will be highest at the beginning of the breeding season, before they have found a mate, and decline after incubation begins.

In this study, we collected high-quality recordings of male and female song in Orchard Orioles, and analyzed the song structure of both sexes using eight measures of acoustic variability. Additionally, we compared song production rates from both sexes across the breeding season. To date, there have been no published detailed analyses of female Orchard Oriole vocalizations.

## METHODS

We conducted fieldwork at 24 field sites across central Maryland (Howard, Prince George’s, Carroll, and Baltimore Counties; Appendix 1). We obtained recordings for 48 males and 32 females. All recordings were made with a Marantz PMD 661 recorder and a Sennheiser ME67 or ME66 shotgun microphone with K6 powering module. We recorded orioles between 6:00 am and 12:00 pm from 28 April to 30 June 2020, and 29 April to 30 June 2021. Females were distinguished from adult males by lack of any chestnut coloring, and from yearling males by the absence of prominent black coloration on their face and throat. Sex identification was confirmed by observation of females performing sex-specific breeding behaviors, such as nest-building and incubation (Ligi and Omland 2007, Scharf and Kren 2020). We excluded songs recorded within 500 m of each other to avoid re-recording the same individual, unless multiple individuals were observed simultaneously. Given what we know about typical territory size in Orchard Orioles and our knowledge of movements of marked birds, 500 m was well beyond the distance of any likely movement (Ligi and Omland 2007, Dowling and Omland 2009).

### Song rates

Once an individual was clearly visible, regardless of whether it was singing or not, we began recording for up to 10 minutes or until the location of the bird was no longer known, with an average recording length of 9 min 4 sec. All songs produced by the focal individual during the recording period (that we directly observed) were used to calculate song rates (songs/min). Multiple recordings (comprising a total time of 10 minutes or less) were combined as a single observation if taken within the same 30-minute period, beginning with the longest segment of continuous recording. Rate observations from a given location or individual were only included once within a seven-day period. One rate observation of at least five minutes from each individual was selected at random to calculate average singing rate for each sex (male rate observations = 48, female rate observations = 27). We then performed an independent samples t-test to compare average male and average female rates (Appendix 2).

Finally, we investigated if there was any effect of time of year on the song rate of either sex. We fit linear and quadratic models to the individual-level rate data and calculated their  $R^2$  values to determine how much variation in rate could be explained by time of year.

### Acoustic measurements

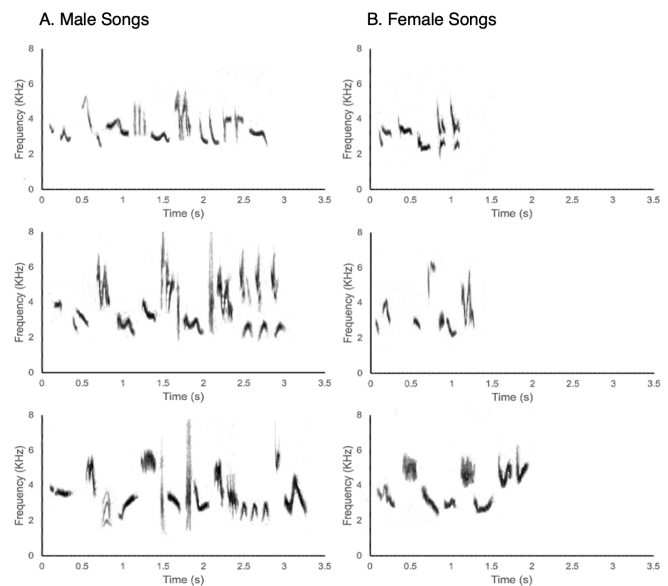
We analyzed all songs in RAVEN Pro v1.6.1 (Center for Conservation Bioacoustics 2019) using a 512 pt DFT (Discrete Fourier Transform) for a frequency resolution of 124 Hz. Song elements that were at least 0.01 s apart were defined as distinct syllables (Hagemeyer et al. 2012). We classified vocalizations as songs if they comprised at least three syllables and were at least one second apart from other vocalizations (Fig. 1). We measured nine acoustic characteristics from three songs from each individual bird (male recordings  $n = 30$ , female recordings  $n = 15$ ). The three-song limit was chosen to achieve the maximum sample size of high quality female songs. Three songs was the minimum number of high quality songs that were recorded from most females. Importantly, we used the same sampling scheme for males and females, which prevents biases toward either sex.

Syllable-level measurements were averaged for each song to create a song-level dataset, and grand-means of the measurements from all three songs from each individual bird were calculated to create an individual-level dataset. The first five measurements were taken at a syllable level: syllable duration (s), minimum frequency (Hz), maximum frequency (Hz), bandwidth, and number of inflection points. The remaining four measurements were taken from the full song: song duration (s), syllable rate (syllables/s), number of syllables, and percent pause (the percentage of the song comprising silence). Minimum and maximum frequency were defined as the frequencies delineating 5% and 95% (low to high) of the spectral energy in the selected syllable, respectively, with bandwidth as the difference between these two frequencies). The number of inflection points were derived from peak frequency contours in Raven, which tracks modulations in frequency of a signal over time.

Preliminary analyses indicated that adult and yearling male songs did not differ significantly (Appendices 3, 4, 5), so we grouped these for the remainder of the tests. We assessed correlations among acoustic variables and removed one within each pair of highly correlated variables with a Pearson correlation value of  $R$

$= 0.8$  or greater (Appendix 6; Asuero et al. 2006). There was one pair of highly correlated variables: full song duration and number of syllables ( $R = 0.95$ ). Therefore, we excluded number of syllables from the final analyses (Andersen and Bro 2010). Full song duration and syllable rate were log-transformed, and a squared transformation was applied to minimum frequency and percent pause to meet the assumptions of homoscedasticity (Jan et al. 2014). One outlier at the individual level and two at the song level were removed from minimum frequency to ensure a normal distribution. To ensure that the transformed data reflected accurate patterns, each of these four variables were also tested using the non-parametric *nparLD* package in R (v.2.1), which approximates an ANOVA-type statistic using the  $F$ -distribution with adjusted degrees of freedom (see Noguchi et al. 2012 for more details; Appendix 7). In order to confirm relationships between our variables, we also performed a principal components analysis (PCA) using the individual-level dataset; this confirmed the correlations between pairs of variables and further supported our removal of the number of syllables variable (Appendix 8).

**Fig. 1.** Song spectrograms for three male (A) and female (B) Orchard Orioles (*Icterus spurius*). Males sang significantly longer songs with higher maximum frequencies, greater percent pause, and greater bandwidth than females. Female songs had longer syllables than male songs. Examples of short, medium, and long songs are shown for each sex.



To test for individual and sex differences in acoustic characteristics, we performed a repeated measures ANOVA using the song-level dataset, with individual nested within sex (to account for within-sex variation) for the three songs from each individual. Post-hoc Bonferroni corrections, traditional and sequential (Holm 1979) were calculated to account for multiple comparisons.

We also quantified sex-specific variance for each variable of the individual-level dataset using coefficients of variation because acoustic traits under strong selection are more likely to show less variance (Barton 1990, Nowicki et al. 2001, Reinhold 2011, but

**Table 1.** Untransformed means for each acoustic characteristic used for analysis, calculated from the individual-level dataset. Repeated measures ANOVA revealed Orchard Orioles (*Icterus spurius*) males (n = 45) and females (n = 15) differed significantly for five out of the eight variables after Bonferroni correction (bolded p-values for  $p < 0.05$ ; Table 2).

Song Characteristics	Female mean	± SE	Male mean	± SE	Bonferroni corrected p-value
Full song duration (s)	1.26	± 0.07	2.84	± 0.13	<b>&lt; 0.0001</b>
Syllable duration (ms)	130	± 10.00	120	± 0.00	<b>&lt; 0.0001</b>
Syllable rate (syl/sec)	5.96	± 0.24	5.40	± 0.10	1.0000
Minimum frequency (Hz)	3072	± 75	2985	± 30	1.0000
Maximum frequency (Hz)	4120	± 79	4251	± 54	<b>0.0035</b>
Bandwidth (Hz)	1048	± 67	1265	± 43	<b>&lt; 0.0001</b>
Peak freq. contour - number of inflection points	3.64	± 0.30	3.41	± 0.21	1.0000
% Pause	0.34	± 0.02	0.36	± 0.01	<b>0.0007</b>

see Houle 1992). All statistics were computed using R, Version 3.6.3 (R Core Team 2021) or IBM SPSS Statistics for Windows, Version 26.0.

## RESULTS

### Song rates

Male Orchard Orioles sang significantly more often than females; on average, males sang  $2.35 \pm 2.43$  songs/min, while females sang  $0.14 \pm 0.35$  songs/min (Fig. 2;  $p < 0.0001$ ,  $T = -4.68$ ). Of the 27 females for which we have rate observations, we observed 16 of them singing (59%). Of the 48 males for which we have rate observations, we observed 43 of them singing (90%). There was no significant effect of time of year on song rate for either sex: linear regression models to investigate the relationship between song rate and time of year resulted in  $R^2$  values of 0.035 and 0.0009 for females and males, respectively. Quadratic models resulted in  $R^2$  values of 0.0418 and 0.0014 for females and males (Appendix 4).

### Acoustic measurements

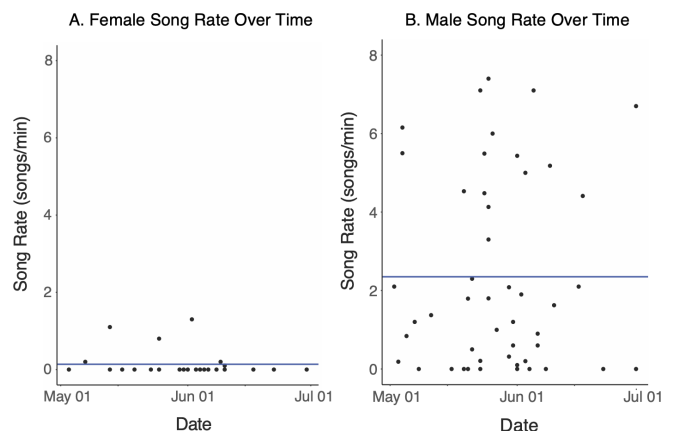
After Bonferroni correction, the nested ANOVA determined that female and male Orchard Oriole songs differed significantly for five of the eight variables measured (Tables 1, 2; Appendix 9; Figs. 1, 3). Female songs were shorter ( $F_{1,88} = 744.00$ ,  $p < 0.0001$ ), had lower maximum frequency ( $F_{1,88} = 13.49$ ,  $p = 0.0035$ ), and smaller bandwidth ( $F_{1,88} = 38.88$ ,  $p < 0.0001$ ). Male songs had shorter syllables ( $F_{1,88} = 8.21$ ,  $p = 0.0435$ ) and a greater proportion of pause ( $F_{1,88} = 16.98$ ,  $p = 0.0007$ ). For the remaining three variables, females and males did not differ significantly.

Females showed greater variation for all eight variables, as calculated by an average of each individual's coefficient of variation (Table 3). The coefficient of variation is calculated using the mean, which removes any effect of scaling differences between the songs of each sex.

## DISCUSSION

Female Orchard Orioles sang often throughout the breeding season, and their songs were structurally distinct from those of males for five of the eight variables we investigated. Female songs were easily distinguishable by ear in the field, reinforcing these data.

**Fig. 2.** (A) Female (n = 27) and (B) male (n = 48) Orchard Orioles (*Icterus spurius*) song rates during the course of the breeding season are shown above, beginning in early May, and ending at the end of June. Averages are indicated by the straight blue line on each plot. Females sang an average of  $0.14 \pm 0.35$  songs per minute. Males sang an average of  $2.35 \pm 2.43$  songs/min, which was significantly more than female orioles ( $p < 0.001$ ). For some individuals, we were able to record them for more than one 10-minute window. For this figure, one rate observation was chosen from each individual at random. Thus, across all observations, more individuals sang than are shown in the figure.



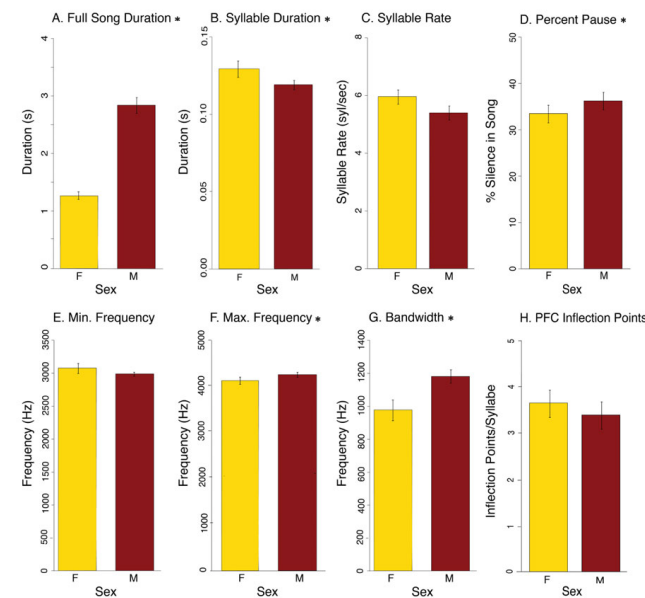
Our study presents the first formal documentation of female song in Orchard Orioles. Female song in this species had rarely been recorded or described previously. We did not notice females singing in our first five years of fieldwork on this species, and there are only brief mentions of potential female song in the literature. For example, Enstrom (1992) stated that females sang a single short song phrase that was recognizably distinct from male song and was produced much less frequently. In the Birds of the World species account there is only one short sentence about this possibility: “Females may sing occasionally” (Bent 1958, as cited in Scharf and Kren 2020). Even people who have studied the species extensively have expressed skepticism at the suggestion that females sing regularly (W. C. Scharf, *personal communication*).



**Table 2.** Repeated measures ANOVA results for the song-level dataset (with individual nested within sex). Orchard Orioles (*Icterus spurius*) male and female songs were significantly different for five of the eight variables after Bonferroni correction. Full song duration and syllable rate were log-transformed, and a squared transformation was applied to percent pause and minimum frequency. Bonferroni correction was performed, correcting the P-value output. Sequential Holm-Bonferroni was also performed (see Table S2). Within-sex variation was significant for all eight variables, which was expected given the amount of individual variation observed in the field. Positive effect direction indicates that males have a greater value for that variable. (Values significant at  $p < 0.05$  shown in bold.)

Song Characteristics	Between Sexes (Uncorrected, $p = 0.05$ )	Between Sexes (Bonferroni Corrected, $p = 0.05$ )	Within Sex Variation	Power	Effect Size (Cohen's $D$ )	Effect Direction
Full song duration (s)	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	1.000	2.877	+
Syllable rate (syl/s)	0.5220	1.0000	<b>&lt; 0.0001</b>	0.068	-0.088	-
Syllable duration (ms)	<b>0.0054</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	0.348	-0.351	-
Minimum frequency (Hz)	0.9100	1.0000	<b>&lt; 0.0001</b>	0.051	-0.014	-
Maximum frequency (Hz)	<b>0.0004</b>	<b>0.0035</b>	<b>&lt; 0.0001</b>	0.054	0.420	+
Bandwidth (Hz)	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	0.909	0.739	+
Peak freq. contour - number of inflection points	0.2450	1.0000	<b>&lt; 0.0001</b>	0.107	-0.156	-
% Pause	<b>0.0001</b>	<b>0.0007</b>	<b>&lt; 0.0001</b>	0.522	0.452	+

**Fig. 3.** Average Orchard Orioles (*Icterus spurius*) female and male values (+ SE) for each acoustic characteristic measured. Female and male songs were significantly different for five of the eight variables after Bonferroni correction (indicated by \*).



In this species, delayed plumage maturation likely contributed to this lack of reporting on female song. Female Orchard Orioles have uniform olive-green plumage; yearling males look similar but have black feathers on their face and throat. In the past, researchers may have attributed female song to yearling males that they did not get a good view of, or that had little black coloration. In our case, we had excellent views of each of the females we recorded and observed many of the females that sang performing female-specific behaviors, including nest-building and incubation.

Typically, definitions of bird song distinguish long and complex songs from shorter and simpler calls (Catchpole and Slater 2008). We defined songs based on acoustic structure and context. Operationally, we defined song as vocalizations containing more than three syllables; some birds were observed producing song fragments with fewer syllables, but these were usually produced by males immediately before singing a full song, or in agonistic contexts (Moyer, *personal communication*). Songs were only produced by adult birds, not by fledglings, which are only reported to make contact calls or begging calls (Jaramillo and Burke 1999). Additionally, the songs performed by females clearly differ from other types of simple vocalizations used by Orchard Orioles including chucks, chips, chatters, and one note whistles (Appendix 10; Jaramillo and Burke 1999, Sturge et al. 2016).

#### Song rates

Females sang at significantly lower rates than males throughout the breeding season. Like males, females produced song well into June, and neither linear nor quadratic models testing the effect of time of year on song rate explained the variation in song rate found in the data. Our speculation that females might mostly sing upon returning to the breeding grounds proved unfounded, as statistically there was no evidence of a decline in song production as the breeding season progressed (similar to Cain and Langmore 2015). Thus, our hypothesis that female song in Orchard Orioles mainly functions in mate attraction was not supported. Alternative hypotheses, such as territory defense or pair-bond maintenance, will be directly tested in the future using playback experiments.

#### Acoustic measurements

We found that female and male Orchard Oriole songs were statistically different for five of the eight acoustic variables measured: full song duration, syllable duration, maximum frequency, bandwidth, and percent pause (Tables 1, 2). These results provide strong evidence that female song elements in Orchard Orioles are structurally distinct from male song elements. Many previous studies in temperate species have concluded that female song is structurally simpler than male song (Arcese et al.

**Table 3.** Coefficients of variation of each sex for each variable, calculated from the song-level dataset. Female Orchard Orioles (*Icterus spurius*) showed more variation than males for all eight acoustic traits measured.

	Full song duration (s)	Syllable rate (syl/sec)	Syllable duration (ms)	Minimum frequency (Hz)	Maximum frequency (Hz)	Bandwidth (Hz)	Peak freq. contour - number of inflection points	% Pause
Female	13.99	12.15	12.10	6.11	5.85	18.36	37.50	14.27
Male	13.28	7.45	8.10	3.78	3.33	11.19	17.64	8.37

1988, Baptista et al. 1993, Price et al. 2009, but see Rose et al. 2018, Kornreich et al. 2021). Before we can begin to determine how or if these sex-specific differences are relevant to Orchard Orioles, it will be important to investigate if males and females can even perceive sex-specific differences in vocalizations.

### Song function

Perhaps as a result of the historical bias toward studying males in a sexual selection context, rarely observed elaborate traits have sometimes been assumed to be nonfunctional, a vestigial side effect of selection acting on males. Darwin wrote that elaborate female traits were maintained through inheritance of male ornamentations, which he proposed evolved primarily for mate attraction and male-male status signaling (Darwin 1871, Andersson and Iwasa 1996, Tobias et al. 2011). Other early studies proposed that female song is a nonfunctional, short-term behavior resulting from hormonal shifts such as increased androgen (Kern and King 1972, also see Langmore 1998). In Chestnut-sided Warblers (*Setophaga pensylvanica*), one study concluded that female song was likely a non-functional trait because it was produced so rarely (Byers and King 2000). However, only 5% of the Chestnut-sided Warbler females sang in that study, compared with 50% of the Orchard Oriole females we observed. This is a conservative estimate given our strict conditions required to include songs in the acoustic analysis data, because only individuals directly observed singing were included. In other words, if a bird was singing slightly obscured in a tree, even if a female was known to be there, we did not include these songs in the acoustic analysis. Furthermore, to maximize sample size, rate observations were stopped after 10 minutes in order to record new individuals. Thus, it is likely that a much larger proportion of females sang than reported.

In some cases, however, infrequent communication signals can also carry complex information (Hauser and Nelson 1991, Wilkins et al. 2020). For example, female Canyon Wrens (*Catherpes mexicanus*) sing significantly less often than males, but female song in this species has been clearly documented to function for resource and territory defense (Hathcock and Benedict 2018). Female song in Song Sparrows (*Melospiza melodia*) has been referred to as a “rare, but normal aspect of female behavior” and is thought to function primarily in female-female conflict (Arcese et al. 1988:49). Male Eastern Bluebirds sing 20 times more frequently than females (Rose et al. 2018), but female song has been shown to function for pair-bond maintenance (Rose et al. 2019). Many important behaviors, such as egg laying or forming long-term pair bonds, can occur quite rarely in the life of an organism but are clearly functional.

Thus, our result documenting that females sing less often than males in Orchard Orioles does not necessarily indicate a lack of adaptive utility for female song. Given that female songs have different acoustic structure from male songs, vocalizations in this species could potentially serve as a sexual identity signal or have distinct functions for each sex (Riebel et al. 2019).

### Evolutionary implications

Differing selection pressures on each sex may lead to evolutionary changes in acoustic production. For instance, there are many examples of passerine males with higher frequency songs obtaining greater mating success, as measured by overall male quality, increased female response, and/or higher rates of paternity (Ratcliffe and Otter 1996, Christie et al. 2004, Byers 2007, Cardoso et al. 2007, Ripmeester et al. 2007). In contrast, frequent female song may actually carry fitness costs: in Superb Fairywrens (*Malurus cyaneus*), females who sang more often were more likely to lose eggs to nest predators, likely by alerting predators to their location (Kleindorfer et al. 2016, but see Odom et al. 2021).

Sex-specific vocalizations may also assist in sex identification during territory defense. For example, Baptista et al. (1993) suggested that male White-crowned Sparrows were able to distinguish between the songs of females and territorial males by recognizing the shorter, simpler female songs as non-threatening. Likewise, male Red-winged Blackbirds (*Agelaius phoeniceus*) recognize the vocalizations of their mates, whereas females are apparently unable to tell other females apart (Beletsky 1983a, 1983b).

As mentioned, many scientists have historically proposed that elaborate female traits were only maintained via genetic correlation with males, resulting in females maintaining male-like traits without any fitness benefits (Darwin 1871, Muma and Weatherhead 1989, Amundsen 1999). In order to investigate sex-specific selection pressures on acoustic traits, we compared variances in male and female songs to determine if females had significantly greater variance, which would be expected if female song was subject to relaxed selection pressure (Barton 1990, Reinhold 2011). Females displayed greater variation for all eight variables (Table 3).

However, recent studies examining selection pressures in songbirds have suggested that greater variability in female songs may be selected for if female vocal signals are more limited in diversity and duration (Collins et al. 2009, Wilkins et al. 2020). Variation in vocal behavior may also reflect variation in competitive interactions (Cain and Ketterson 2012, Cain et al.

2015, Austin et al. 2021, also see Tobin et al. 2019). Additionally, if female songs are important for sexual or individual identity, increased variation might be expected (Sandoval and Escalante 2011, Hahn et al. 2013). Social selection has also been shown to play an important role in the evolution of elaborate advertisement traits in both males and females, as many behaviors function in social contexts outside of mating and the breeding season (Tobias et al. 2011, Lyon and Montgomerie 2012). More research into the sex-specific selection pressures that Orchard Orioles face is needed to determine how or if the variability of female songs affects female fitness.

#### Future directions

Since the publication of work demonstrating ancestral female song (Odom et al. 2014), we have evaluated the presence or absence of female song in two oriole species: the Puerto Rican Oriole (*Icterus portoricensis*, Campbell et al. 2016), a tropical species for which female song was expected, and the Orchard Oriole, which breeds in temperate regions where female song has been thought to be rare or absent (Stutchbury and Morton 2001, Price et al. 2009). Both studies clearly demonstrate the presence of female song. Two other temperate long-distance migratory oriole species, the Bullock's Oriole (*I. bullockii*) and the Baltimore Oriole (*I. galbula*), have already been documented to have female song (Miller 1931, Beletsky 1982, Jaramillo and Burke 1999). Examining female song in the Orchard Oriole provides further evidence of the ubiquity of female song in *Icterus* and provides more evidence that female song is ancestral in the genus (Price et al. 2009, Campbell et al. 2016).

As mentioned, our results do not support the hypothesis that female song in this species mainly functions to attract mates. In the future, we plan to investigate the behavioral contexts in which male and female song is produced in more detail. We will use playback experiments to observe and quantify the response of Orchard Orioles to conspecific song, in order to better understand the singing behavior of both sexes. The results of this work will allow us to test alternative hypotheses about how song may or may not function in this species.

Documentation and analysis of song in female and male songbirds for a wide range of species is needed to accurately understand the global distribution and evolutionary history of this complex trait (Odom et al. 2014, Odom and Benedict 2018, Roper 2018). Female song has been especially understudied in temperate species, so studies like ours can help bridge this gap of knowledge and develop a more complete picture of the distribution of female song geographically and phylogenetically.

Prior to this work, there was no reliable documentation of female song in Orchard Orioles, which highlights one of the significant downfalls of the interface between scientific bias and field work as a tool for documenting behavior. In species such as Orchard Orioles, the female-like yearling male plumage led some researchers to assume that female song was produced by yearling males. It is critical to our understanding of female bird song and the evolution of complex acoustic signaling that field biologists are aware of possible biases toward assuming male-only song (Haines et al. 2020).

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#### Author Contributions:

*M.J.M., E.M.R., A.L.S., and K.E.O. conceived the idea and designed the experiments. K.E.O. supervised the research. M.J.M. and K.E.O. collected the data, M.J.M., D.A.M., A.R., and S.M.B. analyzed the data. M.J.M., E.M.R., B.L., K.J.O., and K.E.O. 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.19214334.v1>.*

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## **Appendix 1**

List of recording sites in central Maryland. All recordings were obtained between 6:00 am and 12:00 pm from April 28<sup>th</sup> - June 30<sup>th</sup>, 2020, and April 29<sup>th</sup> – June 30<sup>th</sup>, 2021.

### **Howard County**

Blandair Regional Park, Columbia, Maryland

Castlebridge Road, West Friendship, Maryland

Centennial Park – Ellicott City, Maryland

Font Hill Wetlands Park, Ellicott City, Maryland

Howard County Conservancy, Woodstock, Maryland

Howard County Living Farm Heritage Museum, West Friendship, Maryland

Laurel Park, Laurel, Maryland

Layhill Park, Aspen Hill, 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

Sharp's at Waterford Farm, Brookeville, Maryland

Wilde Lake, Columbia, Maryland

Woodmark Lake, West Friendship, Maryland

### **Baltimore County**

Cromwell Valley Park, Parkville, Maryland



Masonville Cove Environmental Education Center, Baltimore, Maryland

Patapsco Valley State Park – Avalon, Halethorpe, Maryland

Southwest Area Park, Baltimore, Maryland

University of Maryland, Baltimore County Campus, Catonsville, Maryland

### **Carroll County**

Gaither Road, Eldersburg, Maryland

### **Montgomery County**

Wheaton Park, Silver Spring, Maryland

### **Prince George's County**

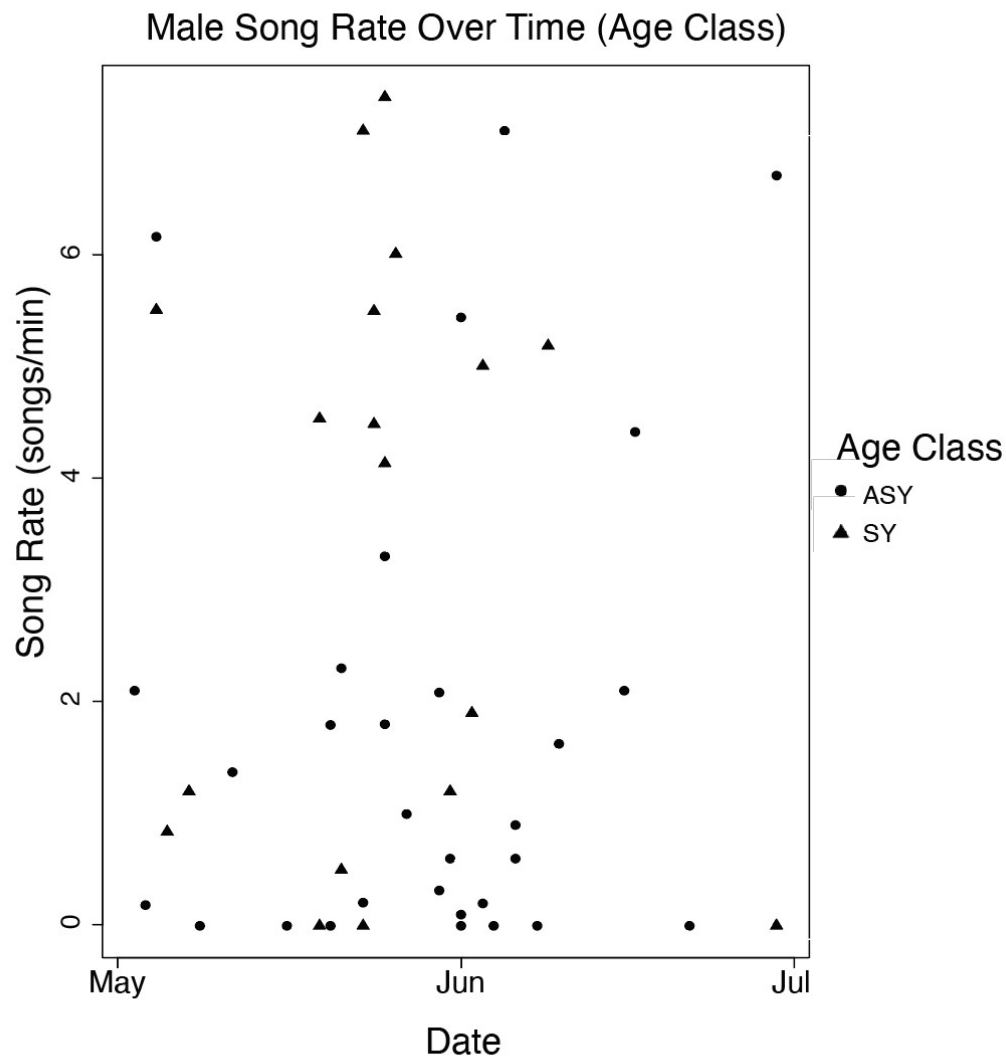
Patuxent Research Refuge – South Tract, Laurel, Maryland

## Appendix 2

P-values from an ANOVA comparing the male and female song rates with sex, season and interaction included. These results supported the findings that time of year did not affect the song rate of either sex (Appendix 4).

Variable	ANOVA (p = 0.05)
Sex	<0.0001
Date	0.772
Sex:Date	0.974

### Appendix 3



## Appendix 4

$R^2$  values and p-values for linear and quadratic models fit to rate data. One observation was taken at random from each individual. ASY and SY males were also modeled separately to determine if age class affected rate patterns (Appendix 3). Neither model type explained significant variation in song rate for either sex, so rate does not seem to be affected by the period of the breeding season in which the recording was collected.

Model Type	Linear $R^2$	Quadratic $R^2$	Linear Significance of Fit ( $P = 0.05$ )	Quadratic Significance of Fit ( $P = 0.05$ )
Female	0.035	0.001	0.348	0.691
Male (combined)	0.042	0.001	0.840	0.438
ASY Male	-0.004	0.084	0.355	0.117
SY Male	-0.059	0.010	0.808	0.363



## Appendix 5

P-values from T-tests between ASY and SY male Orchard Orioles. The males did not differ significantly by age class for any of the variables studied, so the two age classes were grouped for all further analyses.

Variable	T-test p-value	Bonferroni corrected p-value
Full song duration (s)	0.339	1.000
Syllable duration (ms)	0.923	1.000
Syllable rate (syl/sec)	0.159	1.000
Number of syllables	0.957	1.000
Minimum frequency (Hz)	0.488	1.000
Maximum frequency (Hz)	0.087	0.783
Bandwidth (Hz)	0.283	1.000
Peak freq. contour – number of inflection points	0.332	1.000
% Pause	0.954	1.000

## Appendix 6

Univariate correlations ( $R$ ) for the nine original acoustic variables, calculated from the song-level dataset. Correlation values were considered strong above 0.80, resulting in one pair of strongly correlated variables (bolded): Full Song Duration/Number of Syllables. In order to avoid measuring redundant elements, the number of syllables variable was excluded from final analyses.

[illegible]

## Appendix 7

Several variables did not meet the ANOVA assumption of homoscedasticity and were transformed. The below table demonstrates that the raw, transformed, and non-parametric (*npardL*D, Noguchi et al. 2012) *P*-values for these variables reflected the same statistical patterns, even when correcting for multiple comparisons.

Song Characteristic (ANOVA)	Raw <i>P</i> value	Transformed <i>P</i> -value	Raw Bonferroni <i>P</i> -value	Transformed Bonferroni <i>P</i> value	Untransformed, non-parametric <i>P</i> -value
Full Song Duration	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Syllable Rate	0.3730	0.5220	1.0000	1.0000	0.6204
Minimum Frequency (Hz)	0.7730	0.9100	1.0000	1.0000	0.5070
% Pause	0.0001	<0.0001	<0.0001	0.0007	0.0576

## Appendix 8

Principal Component Analysis factor loadings for each of the four components with eigenvalues >1. Loadings greater than 0.60 were considered salient to a factor, resulting in four variables with high factor loadings in PC1 (bolded): full song duration, number of syllables, and bandwidth. PC1 was the only component that was significantly different between males and females (t-test p-value < 0.001). Duration and number of syllables are clearly correlated, and longer songs with greater numbers of syllables are also stochastically more likely to span a broader bandwidth. The results of this PCA provide further support for the removal of number of syllables from the final analysis in order to avoid redundancy. Furthermore, given that PC1 only explained 34.8% of the between-sex variation, it may be that some acoustic differences between sexes may be subtle and distributed across numerous variables. The remaining PCs are not easily interpretable based on the factor loadings for each variable. It is possible that other dimensions of song analysis would reveal other differences.

Song Characteristics	PC1	PC2	PC3	PC4
Full song duration (s)	<b>0.919</b>	<0.100	0.185	<0.100
Syllable duration (ms)	-0.249	0.743	0.420	<0.100
Syllable rate (syl/sec)	-0.265	<0.100	-0.905	<0.100
Number of syllables	<b>0.934</b>	-0.140	<0.100	<0.100
Minimum frequency (Hz)	<0.100	-0.107	<0.100	0.954
Maximum frequency (Hz)	0.526	0.278	0.274	0.677
Bandwidth (Hz)	<b>0.648</b>	0.386	0.331	<0.100
Peak freq. contour – number of inflection points	0.139	0.865	0.190	<0.100



% Pause	<0.100	-0.829	0.422	<0.100
Percent variance explained	34.82	24.45	14.27	11.35

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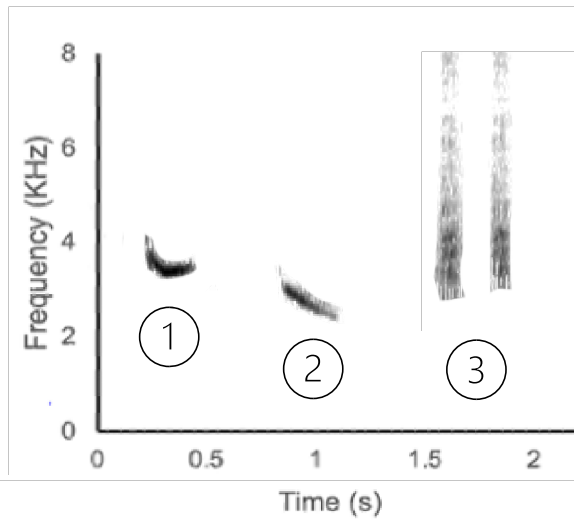
## Appendix 9

ANOVA results for all eight variables used in final analyses: raw, and Bonferroni corrected by traditional Bonferroni with different  $p$  cutoff or adjusted  $p$  values, and sequential Bonferroni (i.e. Holm-Bonferroni). All three methods of correcting for multiple comparisons produced statistically similar results.

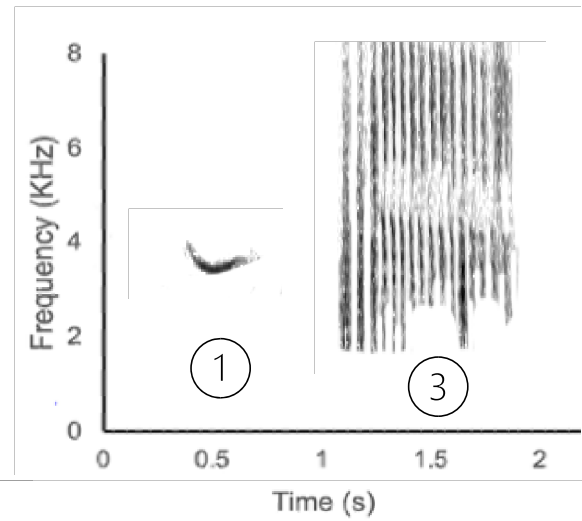
Song Characteristics	Raw ( $p = 0.05$ )	Bonferroni ( $p = 0.0072$ )	Bonferroni ( $p = 0.05$ )	Holm-Bonferroni ( $p = 0.05$ )
Full song duration (s)	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
Syllable duration (ms)	<b>0.0054</b>	<b>0.0054</b>	<b>0.0435</b>	<b>0.0218</b>
Syllable rate (syl/sec)	0.5220	0.5220	1.0000	1.0000
Minimum frequency (Hz)	0.9100	0.9100	1.0000	1.0000
Maximum frequency (Hz)	<b>0.0004</b>	<b>0.0004</b>	<b>0.0035</b>	<b>0.0022</b>
Bandwidth (Hz)	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
Peak freq. contour – number of inflection points	0.2450	0.2450	1.0000	1.0000
% Pause	<b>0.0001</b>	<b>0.0001</b>	<b>0.0007</b>	<b>0.0005</b>

## Appendix 10

### A. Male Vocalizations



### B. Female Vocalizations



Spectrographic examples of calls produced by males (A) and females (B). These syllables were produced in aggressive contexts by both sexes and are clearly qualitatively different from the songs we analyzed. Note that these figures are composites of calls given by different individuals at different times and are just shown to represent the range of vocalizations produced.

1: “Jeet” (Jaramillo and Burke 1999, Sturge et al. 2016)

2: “Whistle note” (Note: we have only observed males producing this call type, all other call types were produced by both sexes.)

3: Chattering