PATTERNS OF PAIR BOLDNESS AND MATE RECOGNITION IN SONG SPARROWS (*MELOSPIZA MELODIA*)

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Western Carolina University in partial fulfillment of the
requirements for the degree of Master of Science in Biology.

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ABSTRACT

PATTERNS OF PAIR BOLDNESS AND MATE RECOGNITION IN SONG SPARROWS (*MELOSPIZA MELODIA*)

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Individual variation in behaviors such as aggression or boldness has the potential to influence the survival and reproductive success of individual animals. For example, individual variation in boldness means that some individuals will be more explorative and risk-tolerant than others, which could allow them to be more active in the face of danger or disturbance. Individual variation in boldness has been studied in many species, but fewer studies have examined patterns of boldness in mated pairs of animals. For many seabird species and some songbirds, mates tend to have similar levels of boldness and other personality characteristics. This suggests that coordination and decreased conflict between mated parents may allow for more effective care of young, leading to increased reproductive success. Boldness in male song sparrows (*Melospiza melodia*) has been shown to be both variable among individuals and repeatable for individuals, but similar work has not been conducted on females of the species. To determine if song sparrows within mated pairs have similar, different, or unrelated levels of boldness, we conducted Carolina wren (*Thryothorus ludovicianus*) alarm call playback experiments, simulating the presence of a predator, on the territories of 30 song sparrow pairs. At 24 of these territories, we conducted at least two playback trials to determine if boldness is repeatable for males and females. In a second study of the ability of song sparrow pairs to coordinate their boldness responses, we used playback experiments of song sparrow alarm calls to investigate whether males can distinguish between the calls of a stranger and their mate. Boldness and male response to a mate versus stranger were measured as the average distance from the playback speaker and the number of calls produced by each subject bird. Boldness, as measured by both distance from the speaker and number of calls, was repeatable for individuals and variable among individuals for males and females. Males and females produced a similar number of calls on average, although there was not a significant correlation between the number of calls produced by males and females within pairs. This suggests that the number of calls produced by one mate is unrelated to the number of calls produced by the other. Within a mated pair, males and females had similar levels of boldness as measured by average distance from the speaker. Song sparrows may mate assortatively for boldness or modify their behavior to match their mate's. In response to alarm calls of a stranger versus a mate, male song sparrows got closer to the speaker in response to calls from a stranger, suggesting that males can recognize their mate's alarm calls, which could allow for greater coordination when responding to threats on their territory.

INTRODUCTION

Personality in the context of animal behavior is described as consistent individual differences in behaviors such as aggression and boldness (Carere & Eens, 2005; Groothuis & Carere, 2005). Aggression, behavior intended to harm or intimidate an individual ((Harris & Siefferman, 2014), often relates to the defense of territories, nests, or young (Burtka & Grindstaff, 2015). Boldness refers to the likelihood of an individual to engage in risk-taking behavior (White et al., 2013) and may present itself as willingness to explore novel objects or spaces (Hall, 1934; Carere et al., 2005), activity in the presence of predators (Sih et al., 2003; Bell, 2005), or willingness to approach and inspect predators (Magurran et al., 1985; Pitcher et al., 1986). Individual variation in boldness can have a significant impact on the stress levels and reproductive success of individual birds (Atwell et al., 2012; Patrick & Weimerskirch, 2014).

A study on dark-eyed juncos (*Junco hyemalis*) found that individuals that displayed more exploratory behavior, a component of boldness, had lower stress hormone (corticosterone) levels (Atwell et al., 2012). Prolonged high stress hormone levels have been correlated with lower fitness, particularly survival (Breuner et al., 2008). In black-browed albatrosses (*Thalassarche melanophris*), shyer males and bolder females had higher reproductive success (Patrick & Weimerskirch, 2014). The personalities of birds within a mated pair may also impact reproductive success. For example, within most black-legged kittiwake (*Rissa tridactyla)* pairs, mates had similar boldness, which suggests assortative mating, with individuals choosing mates similar to themselves (Collins et al., 2019). However, kittiwake reproductive success was more strongly influenced by individual boldness than pair similarity. Pairs with two bold mates had the highest reproductive success, followed by bold-shy and then shy-shy pairs. Contrastingly, in eastern bluebirds (*Sialia sialis*), pair personality may have a stronger impact on reproductive success. Several studies on eastern bluebird aggression, measured as nest defense behavior or territorial aggression, found evidence for assortative mating based on aggression and higher reproductive success for pairs that mated assortatively (Harris & Siefferman, 2014; Burtka & Grindstaff, 2015). These studies suggest that increased coordination and decreased conflict between assortatively mated parents allows for more effective care of young, leading to increased reproductive success.

Pair personality and its implications for reproductive success have not been well studied in songbirds. While the small number of studies suggest that songbirds tend to mate assortatively for personality and that assortative mating is associated with higher reproductive success, the majority of studies on pair personality in birds have focused on long-lived, monogamous, seabird species. There is not yet enough data to determine if such patterns exist in songbird pairs more generally.

Song sparrows (*Melospiza melodia*) are socially monogamous songbirds found in a variety of habitats across North America. Males of this species hold and defend territories through the breeding season and pairs share young-rearing responsibilities on the male's territory (Hughes & Hyman, 2011). Many studies have confirmed that male song sparrow aggression and boldness are variable among individuals and repeatable for individuals. There are also interesting trends in boldness and aggression across urban to rural gradients, with urban males tending to be bolder and more aggressive than their rural counterparts (Evans et al., 2010; Scales et al., 2011; Hyman et al., 2013; Myers & Hyman, 2016; Fossett & Hyman, 2021). However, the behavior of female songbirds is often understudied compared to male songbirds, and the only study to date to measure boldness in female song sparrows was not able to determine individual repeatability due to small sample size (Higgins et al., 2022). As a result, patterns of boldness within pairs and how song sparrow boldness may influence their reproductive success is not known. Song sparrows may mate assortatively for boldness, as coordination and cooperation within pairs can have reproductive benefits as seen in other songbird species (Harris & Siefferman, 2014; Burtka & Grindstaff, 2015).

In this study, patterns of boldness among song sparrow pairs were determined using Carolina wren (*Thryothorus ludovicianus*) alarm call playback. Heterospecific alarm call playback simulates the presence of a predator in or near a bird's territory but avoids simulating the presence of a conspecific territorial intruder. Many species, including song sparrows, will respond to the alarm calls of Carolina wrens, and wren alarm calls have been used in previous studies to measure song sparrow boldness (Hyman et al., 2013; Myers & Hyman, 2016). Song sparrows that produce more alarm calls and get closer to the playback speaker will be considered bolder than those that produce fewer calls and stay further from the speaker.

Similarity in mate boldness may be the result of assortative mating. Alternatively, pair similarity may occur when mates coordinate their behavior. In order to find a mate with similar boldness, or to match a mate's behavior, an individual must be able to discriminate between individuals and recognize their mate, especially their calls. Many studies have shown that captive songbirds can recognize the vocalizations of their mate. Captive male and female zebra finches (*Taeniopygia guttata*) produced more calls in response to their mate than to a familiar finch that was not their mate, suggesting that they can recognize their mate's calls (Vignal et al., 2004; Vignal et al., 2008). Perez et al. (2015) found that in a captive population, female zebra finches have increased corticosterone levels and behavioral changes in response to calls from their mate who had received corticosterone treatment to simulate elevated stress levels. No changes were observed in females in response to calls from an unfamiliar male who had received the same treatment. This provides evidence that zebra finch mates can recognize their mate's calls which allows them to coordinate their behavior. Captive female zebra finches have also been shown to prefer their mate's song compared to a familiar male's song based on approach duration (Miller, 1979). In captive populations, male and female eastern silvereyes (*Zosterops lateralis*) also recognize their mate's calls and can distinguish between calls from their mate, neighbors, and strangers, producing the most calls in response to their mate (Robertson, 1996).

While there are studies on wild birds that demonstrate their ability to recognize their mate's calls, these studies primarily focus on non-songbird species (Buhrman-Deever et al., 2008; Berg et al., 2011; Dentressangle et al., 2012; Curé et al., 2016). Berg et al. (2011) found that female green-rumped parrotlets (*Forpus passerinus*) approached their nest cavity entrance more often in response to their mate's calls than to a non-mate's; they responded preferentially towards their mate. In a different study, male and female blue-footed boobies, (*Sula nebouxii*) were shown to have individually distinct calls and to be able to recognize their mate's calls (Dentressangle et al., 2012). For brown-throated conures (*Aratinga pertinax*), individuals were more likely to respond, responded more quickly, and produced more calls in response to their mate than to other roost members (Buhrman-Deever et al., 2008).

There is less evidence of mate recognition based on vocalizations in wild songbird populations. In song sparrows specifically, females have been shown to respond most strongly to their mate's song compared to song from neighboring males and strangers (O'Loghlen & Beecher, 1999). Additionally, male song sparrows can distinguish between songs from neighboring males and strangers, although this is somewhat dependent on the location of the song's source and stability of neighboring territory boundaries (Stoddard et al., 1990; Stoddard et al., 1991). While there is evidence that female song sparrows can recognize their mate's song and that males can distinguish between other male individuals' songs, we do not yet know if male song sparrows can recognize their female mate's alarm calls.

The health and reproductive success of songbirds may be influenced by components of their personality including boldness. Boldness has been shown to be variable and individually repeatable for male song sparrows. However, female boldness and the patterns of boldness within song sparrow pairs are not well understood. Therefore, in this study, we examined patterns of boldness within song sparrow pairs by measuring the response of both the male and female within each pair to heterospecific alarm call playback. Additionally, we investigated the ability of male song sparrows to discriminate between alarm calls from their mate and calls from a female stranger. We hypothesized that within song sparrow pairs, the male and female would have similar levels of boldness as measured by number of calls and average distance from the speaker in response to heterospecific alarm call playback. This similarity would indicate that there is a reproductive benefit to pair cooperation and coordination. Additionally, we predicted that the number of calls and average distance from the speaker in response to heterospecific alarm call playback would be individually repeatable for both males and females. Finally, we predicted that males would have different responses to calls from a female stranger versus their mate. If males have a different response to calls from their mate versus a stranger in one or both response measures (number of calls and average distance from the speaker), this would indicate that they are able to recognize their mate from their alarm calls, which could allow for greater coordination of responses to danger.

METHODS

From February through June of 2022, we used baited live traps and mist nets to capture 71 song sparrows on the Cullowhee campus of Western Carolina University. We banded each bird with unique number and color band combinations. During this time frame, we mapped territories using observations of territorial behavior, primarily song, by males. We determined whether birds from each territory were male or female based on behavior (primarily male song), and if banded during the breeding season, the presence of a brood patch on females or cloacal protuberance on males. We monitored territories throughout the breeding season, April through August 2022, for the presence of a mated pair and the status of any young. We used the begging calls of young, and adult alarm calls to locate song sparrow nests with hatched young. We conducted boldness assays using heterospecific alarm call playback after a pair's young had fledged, but before juveniles were independent.

Boldness can be assessed by the number of alarm calls produced by adults in response to a heterospecific alarm call or the approach distance to the location of the heterospecific alarm call. Alarm calling is typically associated with the approach and inspection of predators as birds investigate potential danger and initiate mobbing behavior (Hurd, 1996; Templeton & Green, 2007). The closer to the source of an alarm call that a bird gets and the more alarm calls it produces in response, the bolder that individual is (Hyman et al., 2013; Myers & Hyman, 2016). Heterospecific alarm call playback was the preferred method for testing boldness in this study since flight initiation distance, a commonly used measure of boldness, may primarily measure habituation to humans and conspecific alarm calls may be interpreted as territorial intrusions (Hyman et al., 2013; Myers & Hyman, 2016; Fossett & Hyman, 2021).

Boldness Trials

From May through August 2022, we used heterospecific alarm call playback experiments to determine the boldness of the male and female for 30 song sparrow pairs. We placed a small speaker in the approximate center of the territory, ensuring 8-10 meters of vegetation were present on either side. We placed flags marking 2-meter intervals up to 10 meters away on opposite sides from the speaker. We then moved 20-25 meters away from the speaker to observe. If the song sparrow pair was not present at a territory upon arrival, we waited 30 minutes for the birds to arrive. If birds did not arrive after 30 minutes, that territory was not tested on that date. Once both the male and female were present on the territory, we waited 5 minutes before starting the alarm call playback to ensure that any response was due to the heterospecific alarm call playback rather than the presence of the human observer.

We played six minutes of Carolina wren alarm calls from the speaker. A single observer recorded the alarm calls made by both members of the song sparrow pair for 9 minutes, 6 minutes during playback and 3 minutes afterwards using a MARANTZ PMD661 MK II solid state recorder and Sennheiser shotgun microphone. While recording, we stated whether the call came from the male, female, or neighbor when possible. We also stated the distance from the speaker for each bird every time they moved locations. If a bird was between two flags, we recorded their distance from the speaker as the integer between the flags. For example, if a bird was between the 2- and 4-meter flags, we recorded their distance from the speaker as 3m. If a bird was in line with a flag, we recorded their distance as that integer (in line with 4m flag, distance = 4m). If a bird was beyond the 10-meter flag, we recorded their distance as 11m. There was at least one day between boldness trials for neighboring territories. For each recording, we counted the number of calls made by the male and the female. Additionally, we calculated the average distance from the speaker for the 9-minute period for each bird based on the distance from the speaker for each bird at 30 second intervals.

A song sparrow that produced more calls and got closer to the speaker was considered bolder than one who made fewer calls and stayed further from the speaker. We conducted at least 2 boldness trials at 24 territories in order to analyze individual repeatability for number of calls and distance from the speaker. These repeatability trials were between 1 to 3 days apart depending upon weather conditions and the presence or absence of birds on the territory within the 30-minute window for that day.

For recordings with calls that could not be determined as having come from the male, female, or neighbor during the trial, we analyzed calls using the Batch Correlator tool in Raven Pro 1.6 (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology (KLYCCBCLO), 2023). We recorded alarm calls from individual birds at close proximity to use as known calls for this analysis. The spectrograms of unknown calls from each trial were compared to spectrograms of 6-12 known calls each from the male and female and a correlation value between 0 and 1 was produced for each comparison. A correlation value of 1 would mean the two spectrograms are identical, while a spectrogram of 0 would mean the two spectrograms have no similarities. We determined whether unknown calls came from the male or female based on which known call they shared the highest correlation value with. Unknown calls that did not have higher correlation values for one bird or the other from the territory were not included in final counts. If more than 5% of calls for a recording were unknown, that recording was not used for number of call comparisons.

Mate Recognition Trials

From June through July 2022, we conducted conspecific alarm call playback experiments for 22 male song sparrows to determine if males can distinguish between calls from a stranger versus their mate. For each territory, we produced an audio file of alarm calls from the female. Files were edited in Raven Pro 1.6 (KLYCCBCLO, 2023) to include 3 minutes of calls with a call every 1.5 seconds. Files were also edited to be as similar in decibels to one another as possible. Recognition trials were conducted in the same way as the boldness trials except for the following differences. Two trials were conducted per territory, one using alarm calls from the male's mate, and one using calls from a stranger (a female from a territory in a non-adjacent section of campus). For each territory, we randomly selected which trial type (mate or stranger) would be conducted first. These recognition trials were between 2 to 3 days apart. We recorded the number of calls and distance from the speaker for each male over 6 minutes: 3 minutes of playback and 3 minutes afterward.

Ethical Note

All protocols were approved by the Institutional Animal Care and Use Committee of Western Carolina University (IACUC Protocol # 2022-03-30-01). All bird banding and trapping was conducted under a US Department of the Interior Federal Bird Banding Permit (Permit # 23626).

STATISTICAL ANALYSES

We conducted all statistical analyses in R and RStudio (R Core Team, 2021; RStudio Team, 2022).

Individual Boldness - Repeatability

We determined repeatability for male and female number of calls and average distance from the speaker using Pearson correlations, analysis of variance (ANOVA), and intraclass correlation coefficients (ICC). We produced Pearson correlations to compare Trial 1 versus Trial 2 number of calls and average distance from the speaker for both males and females. We used intraclass correlation coefficients to determine the proportion of total variance attributed to among individual variance for the number of calls and average distance from the speaker for both males and females using the ICC package in R (Wolak et al., 2012; Wolak, 2022). We conducted an ANOVA to determine if the within trial variance was greater than the between trial variance for male and female number of calls and average distance from the speaker. For individuals for which there were more than two usable trials, we randomly selected two trials to compare for repeatability.

Boldness within Pairs

We produced Pearson correlations and conducted paired Student's t-tests to compare male versus female number of calls and average distance from the speaker. The order of trials had a significant impact on female song sparrow response to playback for both number of calls and average distance from the speaker. Females produced fewer calls and stayed further from the speaker in the second boldness trial compared to the first boldness trial (Number of Calls: Paired Student's t-test: $p= 0.002$, $t= 3.530$, $df= 22$; Avg. Distance from the Speaker: Paired Student's t-test: p= 0.017, t=-2.564, df= 23; Table A1). For male song sparrows, the order of trials had a significant impact on their average distance from the speaker in response to playback, but not for number of calls. Males stayed further from the speaker in Trial 2 compared to Trial 1 (Paired Student's t-test: p= 0.036, t=-2.222, df= 23; Table A1). Additionally, for 6 territories, we only conducted one trial. Therefore, for comparisons between males and females, statistical analyses were based on the first trial for each bird.

Mate Recognition

We conducted paired Student's t-tests to compare the number of calls and average distance from the speaker for males in response to calls from their mate versus a stranger.

RESULTS

Individual Boldness - Repeatability

Both females and males showed repeatability in their behavior in response to alarm call playbacks. The number of calls produced was repeatable for females (Pearson Correlation: r_p= 0.628, p= 0.001; ICC(r)= 0.635; ANOVA: F= 4.476, p< 0.001; Figure 1; Table 1; Table 2; Table 3). The average distance from the speaker was repeatable for females (Pearson Correlation: r_p= 0.726, p< 0.001; ICC(r)= 0.538; ANOVA: F= 3.331, p= 0.002; Figure 2; Table 1; Table 2; Table 3). The number of calls produced was repeatable for males (Pearson Correlation: r_p= 0.457, p= 0.029; ICC(r)= 0.493; ANOVA: F= 2.949, p= 0.006; Figure 3; Table 1; Table 2; Table 3). The average distance from the speaker was repeatable for males (Pearson Correlation: r_p= 0.580, p= 0.003; ICC(r)= 0.464; ANOVA: F= 2.731, p= 0.009; Figure 4; Table 1; Table 2; Table 3).

Boldness within Pairs

Within pairs, males and females had similar responses to alarm call playback. There was a significant positive correlation between the average distance from the speaker for males and females in pairs (Pearson Correlation: r_p= 0.684, p< 0.001; Figure 5; Table 4). However, there was not a significant correlation between the number of calls produced by males and females in pairs (Pearson Correlation: r_p= 0.117, p= 0.538; Figure 6; Table 4). The number of calls produced in response to playback was not significantly different between males and females on average (Paired Student's t-test: p= 0.734, t= 0.343, df= 29; Table 5). The average distance from the speaker was not significantly different between males and females on average (Paired Student's t-test: p= 0.920, t= 0.101, df= 29; Table 5).

Mate Recognition

Male song sparrows responded differently to calls from their mate versus calls from a stranger. Male average distance from the speaker was significantly lower in response to calls from a stranger compared to calls from their mate (Paired Student's t-test: p= 0.028, t= 2.363, df= 21; Figure 7; Table 6). There was not a significant difference between the number of calls produced by males in response to calls from a stranger versus their mate (Paired Student's t-test: p= 0.543, t= 0.619, df= 21; Figure 8; Table 6).

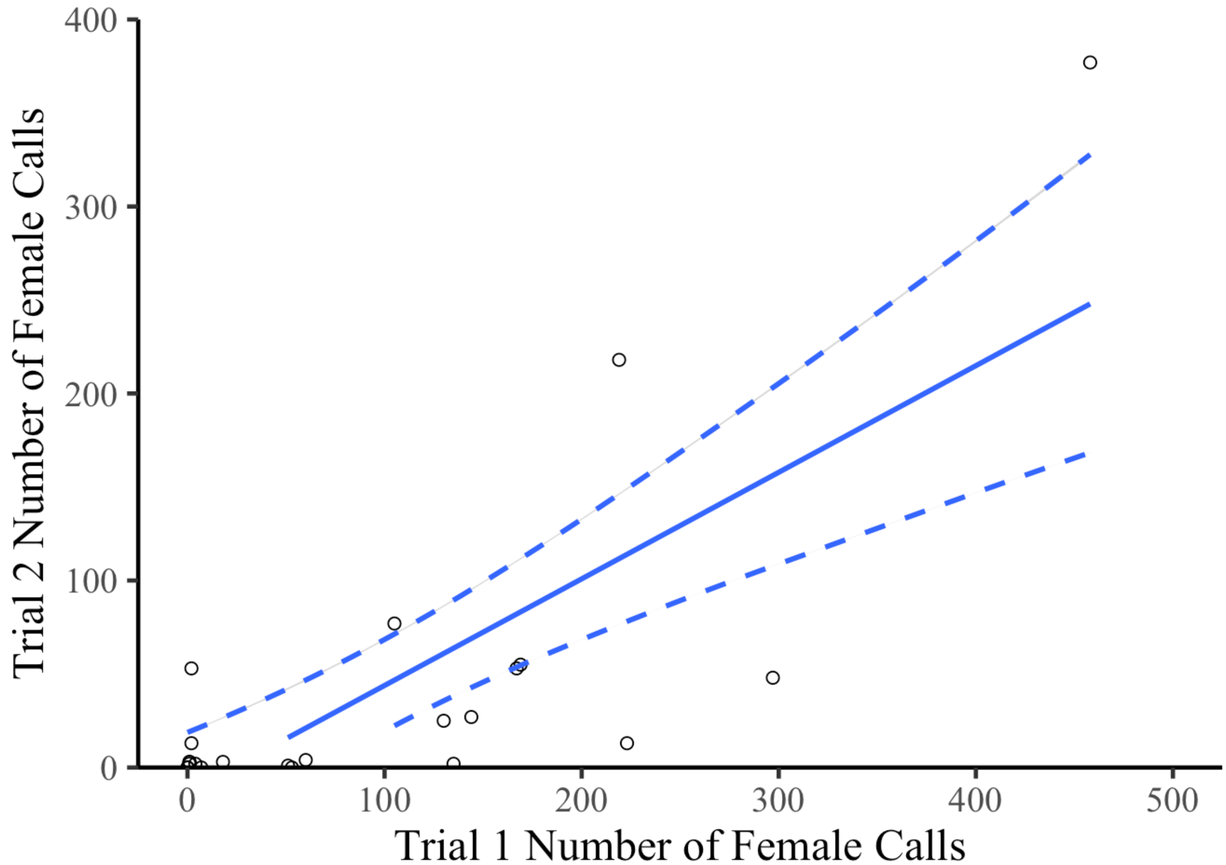

Figure 1. Pearson correlation of trial 1 versus trial 2 number of calls for 23 female song sparrows in response to Carolina wren alarm call playback with least-squares fit and 95% confidence intervals (r_p= 0.628, p= 0.001).

Table 1. Pearson correlation summary for repeatability of log+1 transformed number of calls and log+1 transformed average distance from the speaker (m) for male and female song sparrows in response to Carolina wren alarm call playback.

	R	R^2	*df*	*P*
Female Number of Calls	0.628	0.394	21	0.001
Female Average Distance from the Speaker (m)	0.726	0.527	22	<0.001
Male Number of Calls	0.457	0.209	21	0.029
Male Average Distance from the Speaker (m)	0.580	0.336	22	0.003

Table 2. Intraclass correlation coefficient (ICC) summary for repeatability of number of calls and average distance from the speaker (m) for male and female song sparrows in response to Carolina wren alarm call playback (confidence interval type – Smith, 1956).

	ICC	*LowerCI*	*UpperCI*	*N*	*k*	*VarW*	*VarA*
Female Number of Calls	0.635	0.388	0.882	23	2	4202.087	7303.439
Female Average Distance from the Speaker (m)	0.538	0.251	0.825	24	2	3.198	3.727
Male Number of Calls	0.493	0.181	0.806	23	2	6327.500	6164.621
Male Average Distance from the Speaker (m)	0.464	0.147	0.781	24	2	2.562	2.219

Table 3. Analysis of variance (ANOVA) summary for repeatability of number of calls and average distance from the speaker (m) for male and female song sparrows in response to Carolina wren alarm call playback.

	F	df_n	df_d	*P*
Female Number of Calls	4.476	22	23	<0.001
Female Average Distance from the Speaker (m)	3.331	23	24	0.002
Male Number of Calls	2.949	22	23	0.006
Male Average Distance from the Speaker (m)	2.731	23	24	0.009

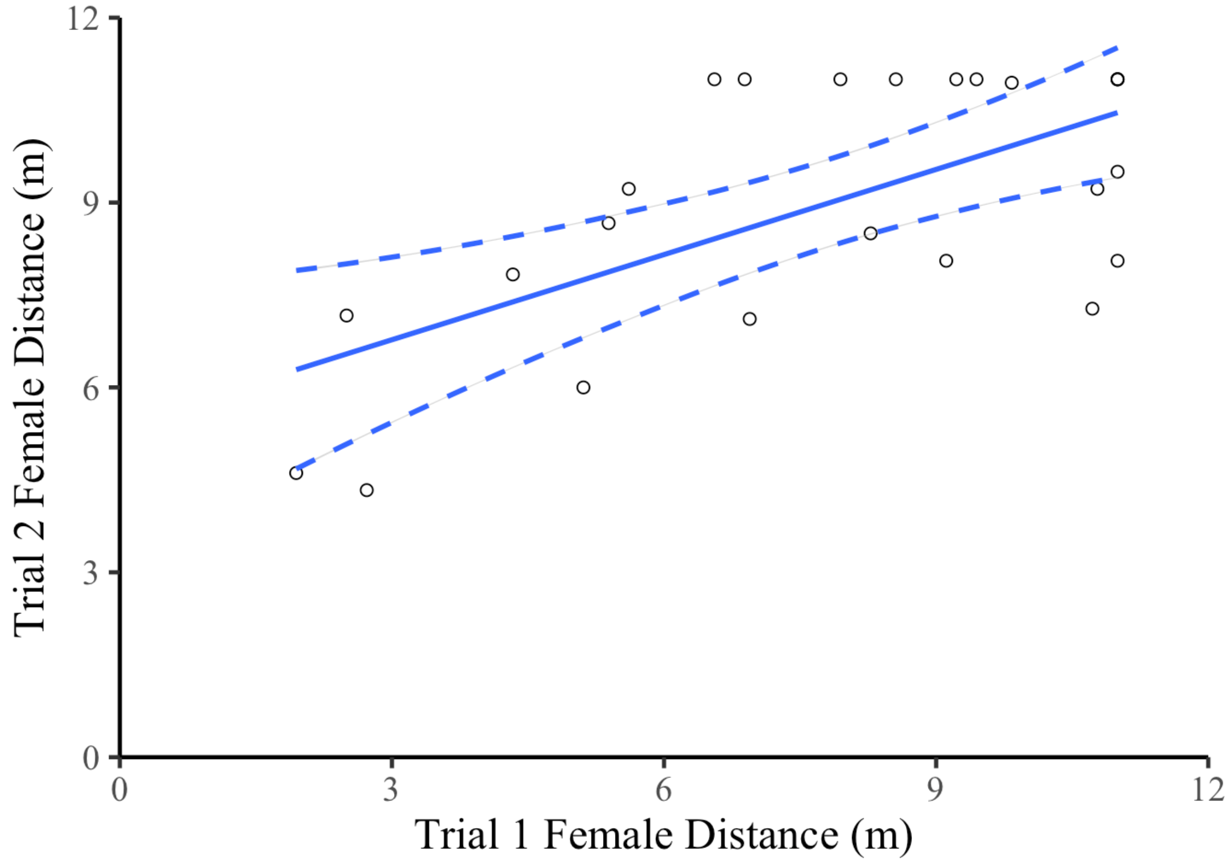

Figure 2. Pearson correlation of trial 1 versus trial 2 average distance from the speaker (m) for 24 female song sparrows in response to Carolina wren alarm call playback with least-squares fit and 95% confidence intervals (r_p= 0.726, p< 0.001).

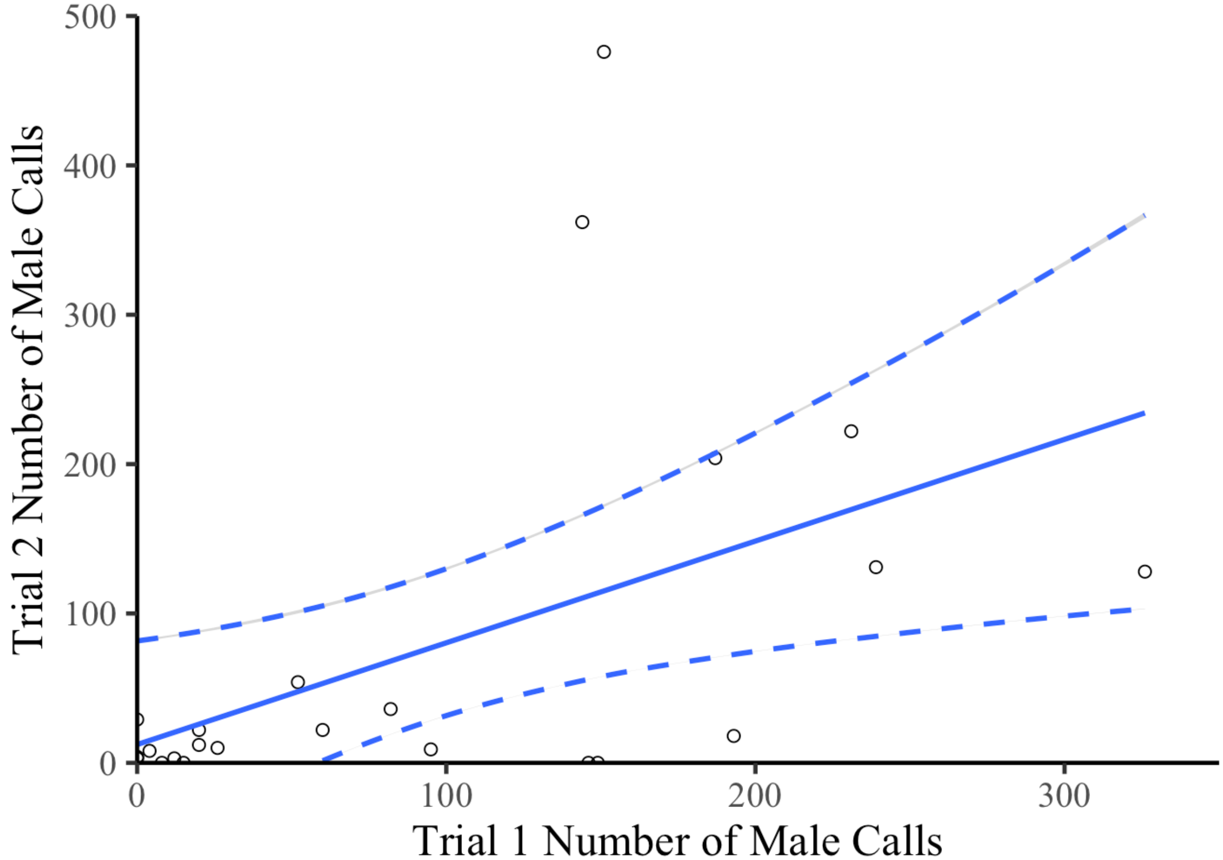

Figure 3. Pearson correlation of number of calls in trial 1 versus trial 2 from 23 male song sparrows in response to Carolina wren alarm call playback with least-squares fit and 95% confidence intervals (r_p= 0.457, p= 0.029).

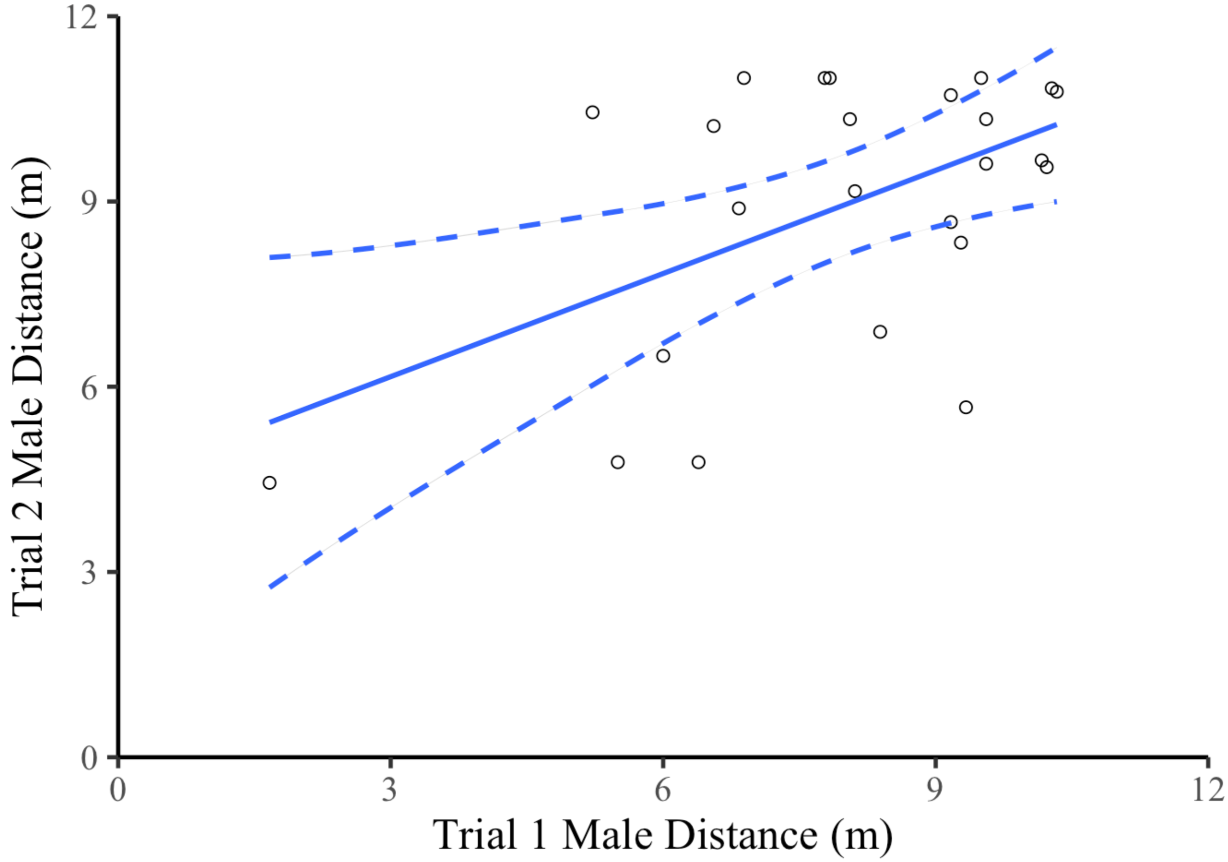

Figure 4. Pearson correlation of trial 1 versus trial 2 average distance from the speaker (m) for 24 male song sparrows in response to Carolina wren alarm call playback with least-squares fit and 95% confidence intervals (r_p= 0.580, p= 0.003).

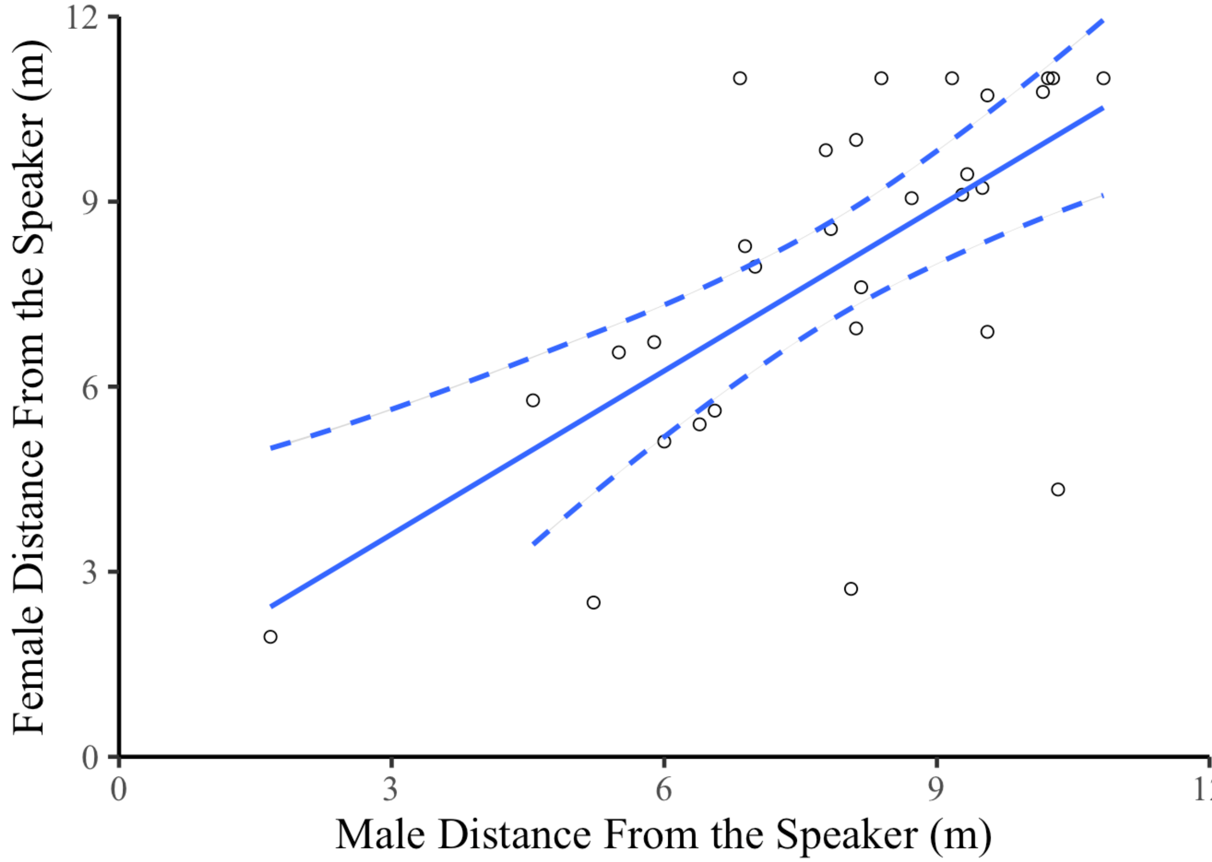

Figure 5. Pearson correlation for male versus female average distance from the speaker (m) for 30 song sparrow pairs in response to Carolina wren alarm call playback with least-squares fit and 95% confidence intervals (r_p= 0.684, p< 0.001).

Table 4. Pearson correlation summary for male versus female log+1 transformed number of calls produced and log+1 transformed average distance from the speaker (m) for 30 song sparrow pairs in response to Carolina wren alarm call playback.

	R	R^2	*df*	*P*
Male v. Female Number of Calls	0.117	0.014	28	0.538
Male v. Female Avg. Distance from the Speaker (m)	0.684	0.468	28	<0.001

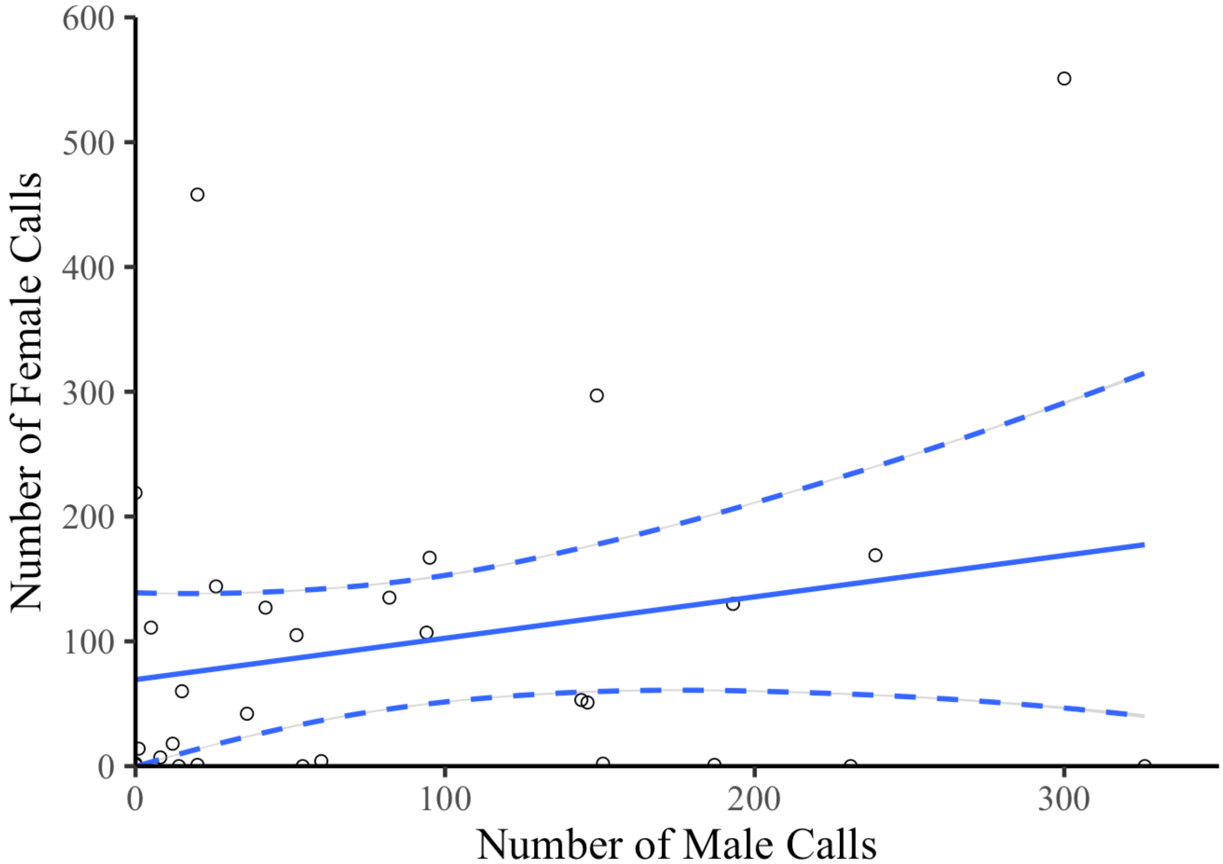

Figure 6. Pearson correlation for male versus female number of calls produced for 30 song sparrow pairs in response to Carolina wren alarm call playback with least-squares fit and 95% confidence intervals (r_p= 0.117, p= 0.538).

Table 5. Paired Student's t-test summary for male versus female number of calls and average distance from the speaker (m) in response to Carolina wren alarm call playback.

	T	*MeanDiff*	df	*CI.high*	*CI.low*	*P*
Male v. Female Number of Calls	0.343	9.133	29	63.602	-45.335	0.734
Male v. Female Avg. Distance from the Speaker (m)	0.101	0.039	29	0.825	-0.747	0.920

Table 6. Paired Student's t-test summary for number of calls and average distance from the speaker (m) in response to alarm calls from a mate versus stranger for 22 male song sparrows.

	T	*MeanDiff*	df	*CI.high*	*CI.low*	*P*
Number of Calls in Response to Stranger v. Mate	0.619	7.136	21	31.125	-16.852	0.534
Avg. Distance from the Speaker (m) in Response to Stranger v. Mate	2.363	1.557	21	2.927	0.187	0.028

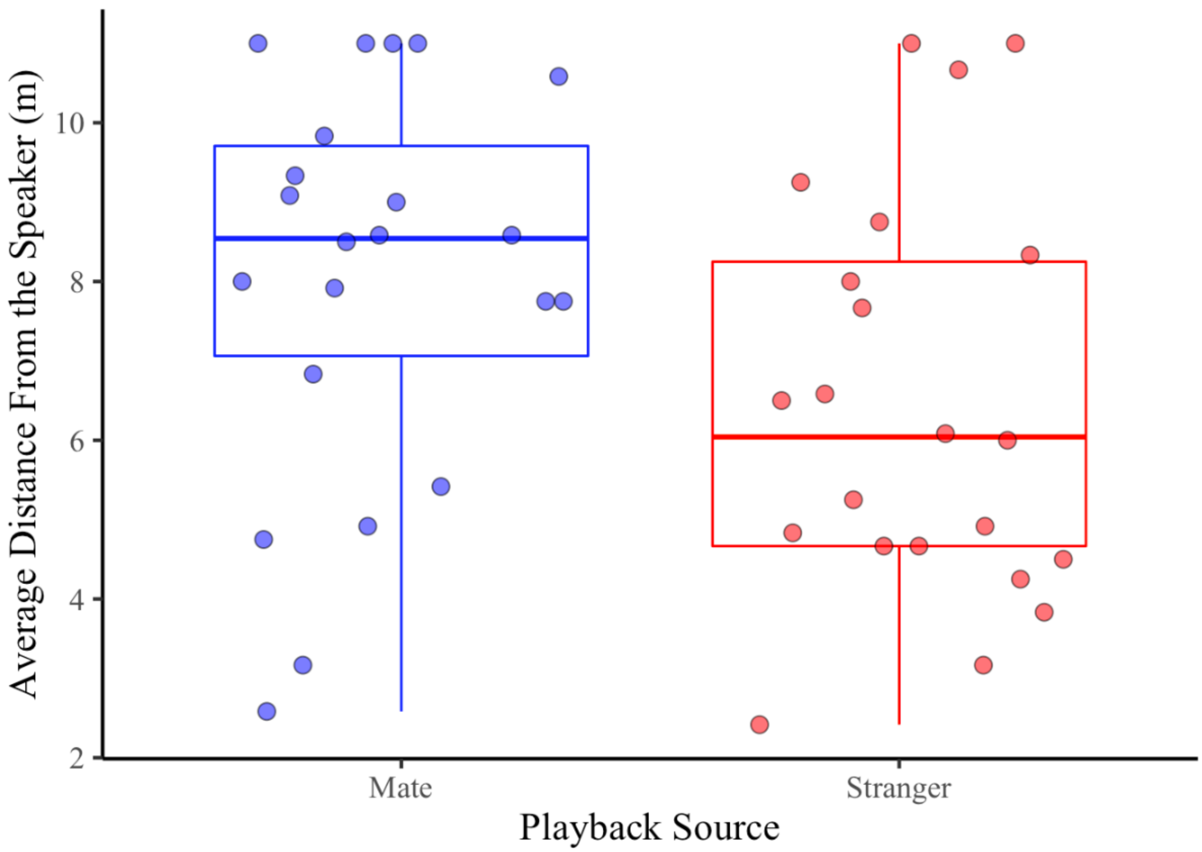

Figure 7. Boxplot of mean average distance from the speaker (m) in response to alarm calls from a mate versus stranger for 22 male song sparrows (p= 0.028, t= 2.363, df= 21).

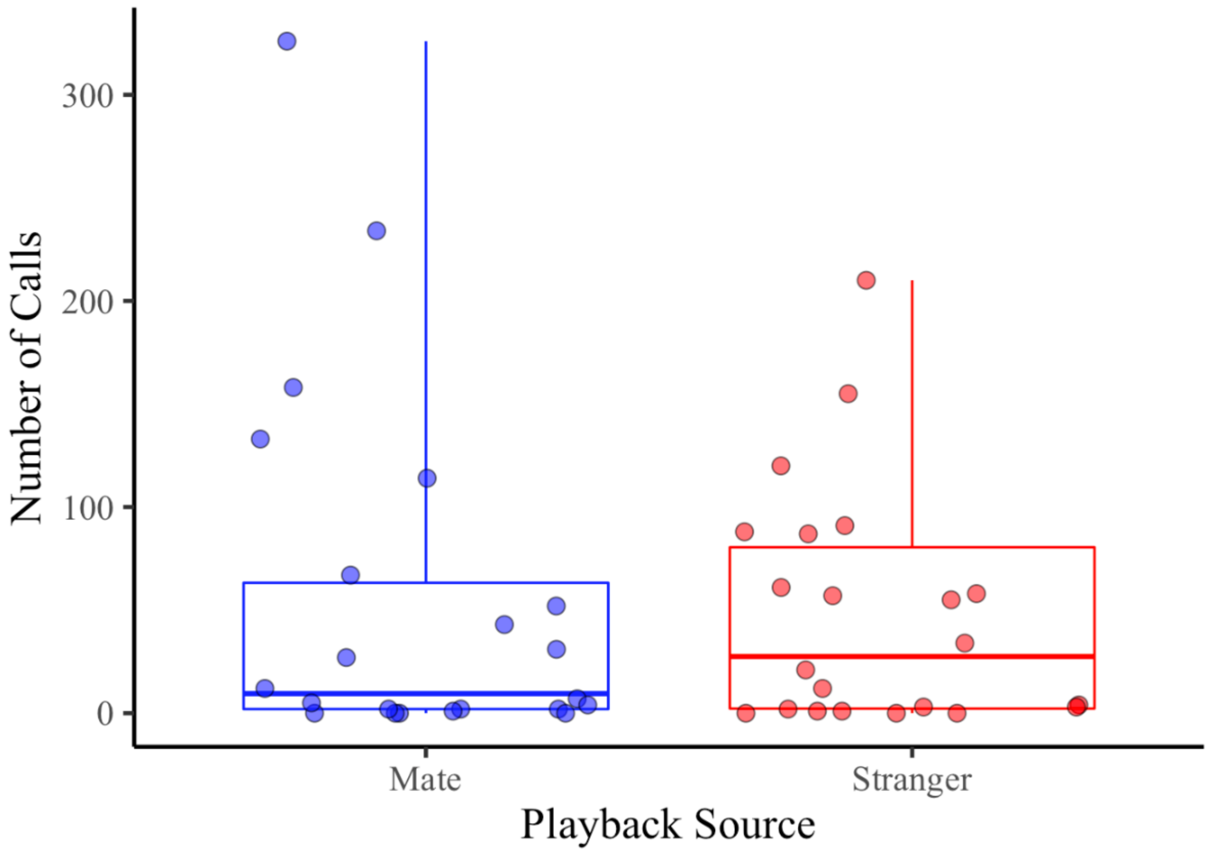

Figure 8. Boxplot of mean number of calls produced in response to alarm calls from a mate versus stranger for 22 male song sparrows ($p = 0.543$, $t = 0.619$, $df = 21$).

DISCUSSION

In this study, we examined patterns of boldness in pairs of wild song sparrows during the breeding season. We measured boldness as average distance from the speaker and number of alarm calls produced in response to heterospecific alarm call playback. Three main findings have emerged: 1) consistent with previous findings for males, female boldness is repeatable; 2) within pairs, females and males have similar levels of boldness; 3) males can recognize their mate based on their alarm calls.

Individual Boldness

Our results indicate that boldness is individually repeatable and variable among individuals for both female and male song sparrows. This was the case for both measures of boldness in the study; the number of calls produced and the average distance from the playback speaker in response to a heterospecific alarm call. These results are consistent with previous research findings for male song sparrows (Hyman et al. 2013; Myers & Hyman, 2016). The only other study that has measured female song sparrow boldness was unable to determine repeatability due to small sample size in two different experiments (n=5, n=8; Higgins et al., 2022). While our sample sizes were larger (n_{calls}=23, $n_{distance}$=24), intraclass correlation coefficient (ICC) values, a measure of repeatability, may still be imprecise. Repeatability measurements could be low due to sample size, the duration of the study, or habituation (Runyan & Blumstein, 2004; Carrete & Tella, 2009; Fossett & Hyman, 2021). With two measurements per individual and a sample size of 23, an ICC score above 0.8 would be needed to estimate ICC with 95% confidence intervals (Wolak et al., 2012). The ICC values for our study ranged between 0.464 to 0.635 and had lower confidence intervals. However, in our study, the confidence interval ranges for all the ICC values were above zero. We also produced Pearson correlations and ran ANOVAs as additional measures of repeatability. Pearson correlation r values ranged between 0.457 and 0.726 (and correlations were significant) and ANOVAs determined that the within trial variance was significantly greater than the between trial variance for both males and females, and across both measures of boldness. Using all three measures of repeatability, we determined that boldness, as measured by number of calls and average distance from the speaker in response to heterospecific playback, was repeatable for both male and female song sparrows.

With the knowledge that boldness is a repeatable trait, we can make comparisons between individuals with different personalities. Individual personality may have fitness implications for songbirds and other species. Many studies suggest that aggression, neophobia and boldness have fitness consequences across taxa (Dingemanse et al., 2004; Dingemanse et al., 2005; Smith & Blumstein, 2008; Higgins et al., 2022). A meta-analysis of fitness consequences of personality found that aggression in non-human animals has a positive effect on both reproductive success and survival (Smith & Blumstein, 2008). The same meta-analysis found that explorative individuals tend to have higher survival, and bolder individuals have increased reproductive success, but lower survival (Smith & Blumstein, 2008). In song sparrows, male aggression is correlated with one measure of offspring quality, chick growth rate (Krippel et al., 2017), and aggressive males tend to also be bolder (Evans et al., 2010; Myers & Hyman, 2016; Scales et al., 2011). However, the fitness benefits of individual variation in behavior could be dependent on the species, population, or environment in question. A study of song sparrows found that more neophobic individuals were more likely to survive to hold a territory in the next breeding season than less neophobic birds (Higgins et al., 2022), but in a study of great tits

(*Parsus major*) the fitness benefits of neophobia level varied with sex and environmental conditions (Dingemanse et al., 2004). Our study establishes that boldness is variable among individuals and individually repeatable for male and female song sparrows. However, how their boldness may be influenced by territory quality and fluctuating environmental conditions and the fitness consequences associated with varying levels of individual boldness in song sparrows are not known.

Boldness within Pairs

Since boldness is repeatable for males and females, comparisons within song sparrow pairs can be drawn. The average distance from the speaker for males and for females was positively correlated. In other words, one mate's boldness was predictive of the other mate's boldness. This could be evidence for assortative mating or intentional coordination of behavior. In both scenarios, this pair similarity in boldness suggests that there are reproductive benefits to pair coordination and cooperation.

There are a number of studies suggesting that birds mate assortatively for personality. Collins et al. (2019) found that kittiwakes mated assortatively for boldness. Pogany et al. (2018) found that in captive zebra finches, females preferred males with similar neophobia scores to their own. Similarly, another study on captive zebra finches found that explorative females preferred explorative males over non-explorative males (Schuett et al., 2011b).

Other studies have examined patterns of personality within pairs and how those patterns influence reproductive success. Several studies on eastern bluebirds found evidence for assortative mating based on aggression and higher reproductive success for pairs that mated assortatively (Harris & Siefferman, 2014; Burtka & Grindstaff, 2015). Within pairs, male and female bluebirds tend to have similar levels of aggression towards intruders and pairs with two highly aggressive members, or two very non-aggressive members have the highest reproductive success (Harris & Siefferman, 2014). In a different measure of aggression, nest defense, bluebird mates with similar strategies had more fledglings than those with opposing nest defense strategies (Burtka & Grindstaff, 2015). Similarly, in a captive zebra finch population, mates with similar personalities (aggression and neophobia) produced chicks of better condition than pairs with dissimilar mates (Schuett et al., 2011a). In one study on great tits, mates with similar levels of neophobia had the most reproductive success, but birds tended to mate disassortatively (Dingemanse et al., 2004). This could indicate that offspring with intermediate phenotypes for explorative behavior have increased fitness, particularly in fluctuating environmental conditions. In a different population, pairs in which both mates were fast explorers, or both mates were slow explorers, had chicks with the best body condition (Both et al., 2005).

While our study does not involve mate choice and therefore cannot be considered direct evidence for assortative mating, it does confirm a pattern found across many bird species, that mates tend to have similar personalities to one another. This pair similarity may result from assortative mating, or it could occur when birds modify their behavior to match their mate's. A study on white-shouldered fairywrens (*Malurus alboscapulatus*) found that male aggression was consistent while female aggression varied based on context, particularly their male mate's level of aggression (Jones et al., 2022). Females may modify their behavior based upon their mate's level of aggression in order to minimize risk and conflict. This coordination could improve the pair's success at defending their territory and help sustain their pair bond (Jones et al., 2022). For song sparrows, a similar pattern may be true since in our study, mates had similar average distances from the speaker in response to heterospecific alarm calls. This may indicate that one member of the pair is showing consistent response to potential predation while the other member matches their mate's behavior.

While we found that a pair's average distance from the playback speaker was positively correlated, the number of calls produced was not significantly correlated between males and females within a pair. On average, there was not a significant difference between the number of calls produced by the male versus female. However, female song sparrows showed higher repeatability than males for both measures of boldness. Additionally, the number of calls produced and average distance from the speaker were significantly correlated for females, but not for males (Figure A1; Figure A2, Table A2). These findings may indicate that males and females are using their calls differently. For some songbird species, male and female birds may use vocalizations for different purposes. In eastern bluebirds, female song is structurally equivalent to male song, but is used to communicate with a mate, rather than as a territorial display (Rose, 2020).

The two measures of playback response, distance from the speaker and number of alarm calls, were correlated for female song sparrows, suggesting that both variables are measuring female boldness similarly. However, for males, the response measures were not correlated. The number of alarm calls produced may not be measuring male boldness or may be significantly influenced by other factors in addition to boldness. Additionally, fitness trade-offs between survival and reproductive success related to alarm calling may differ between males and females. Reproduction is more costly for females, so to make their energy investment worthwhile, they may be more likely to put their survival at risk to protect their young, particularly once offspring have fledged. Therefore, males and females in a pair may differ in their likelihood to produce alarm calls.

Mate Recognition

Males moved significantly closer to the speaker in response to stranger's calls compared to their mate's calls. This indicates that male song sparrows can distinguish between alarm calls from strangers and their mate and thus recognize their mate's alarm calls. On the other hand, there was not a significant difference in the number of calls produced by males in response to calls from a mate versus stranger. This may provide further evidence that the number of calls produced by male song sparrows is not strictly related to boldness and functions differently than physical approach. Males could interpret alarm calls from a stranger as territorial intrusions rather than an indication of the presence of a predator. Although we used alarm calls from a female stranger who would be less likely to be considered an intruder, we do not know if song sparrows can distinguish between calls from males versus females.

While many studies suggest that songbirds can recognize their mate's vocalizations, in most of these studies, birds responded more strongly to their mate than to a stranger or neighbor (Miller, 1979; Robertson, 1996; O'Loghlen & Beecher, 1999; Vignal et al., 2004; Vignal et al., 2008; Perez et al., 2015). Female songbirds have been shown to prefer and respond more strongly to their mate's song (Miller, 1979; O'Loghlen & Beecher, 1999) and to their mate's contact calls (Robertson, 1996; Vignal et al., 2004; Vignal et al., 2008). Recognition of a mate's vocalizations, including contact calls and song, plays an important role in maintaining pair bonds (Miller, 1979; Robertson, 1996). If individuals are more likely to trust signals from a familiar bird, whose vocalizations they have had more exposure to (O'Loghlen & Beecher, 1999), then the prediction would be that there would be a stronger response to a mate's contact calls versus a stranger's.

In our study, male song sparrows responded more strongly to a stranger's calls. However, these alarm calls were played on the male's territory during the breeding season, and males may perceive alarm calls from a conspecific stranger as a territorial intrusion (Myers & Hyman, 2016) which could lead to a stronger response as a male approaches the source of calls in search of an intruder. Regardless, the difference in response to a mate versus a stranger coincides with the findings of many other studies showing that male songbirds are able to discriminate between vocalizations from different individuals and recognize their mate's. Furthermore, vocal recognition of a mate may facilitate coordination of pair response to potential predators.

Conclusions

Boldness is individually repeatable for male and female song sparrows and varies among individuals. Within pairs, male and female song sparrows have similar levels of boldness. There is likely a fitness benefit to this pair similarity, although the cause of this similarity remains unclear. Male song sparrows are able to recognize their mate from their alarm calls. This may facilitate coordination of behavioral response to potential predators within pairs and aid in maintaining bonds between mates. Future studies should examine the impacts of female and pair boldness on reproductive success and individual fitness. Additionally, boldness should be measured across multiple breeding and non-breeding seasons and compared to environmental factors to assess the impacts of boldness on fitness in fluctuating environmental conditions.

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APPENDIX

Table A1. Paired Student's t-test summary for number of calls and average distance from the speaker (m) in trial 1 versus trial 2 in response to Carolina wren alarm call playback.

	T	*MeanDiff*	df	*CI.high*	*CI.low*	*P*
Female Number of Calls	3.530	55.130	22	87.516	22.745	0.002
Female Avg. Distance from the Speaker (m)	-2.564	-1.192	23	-0.230	-2.154	0.017
Male Number of Calls	0.747	17.696	22	66.816	-31.425	0.463
Male Avg. Distance from the Speaker (m)	-2.222	-0.951	23	-0.066	-1.837	0.036

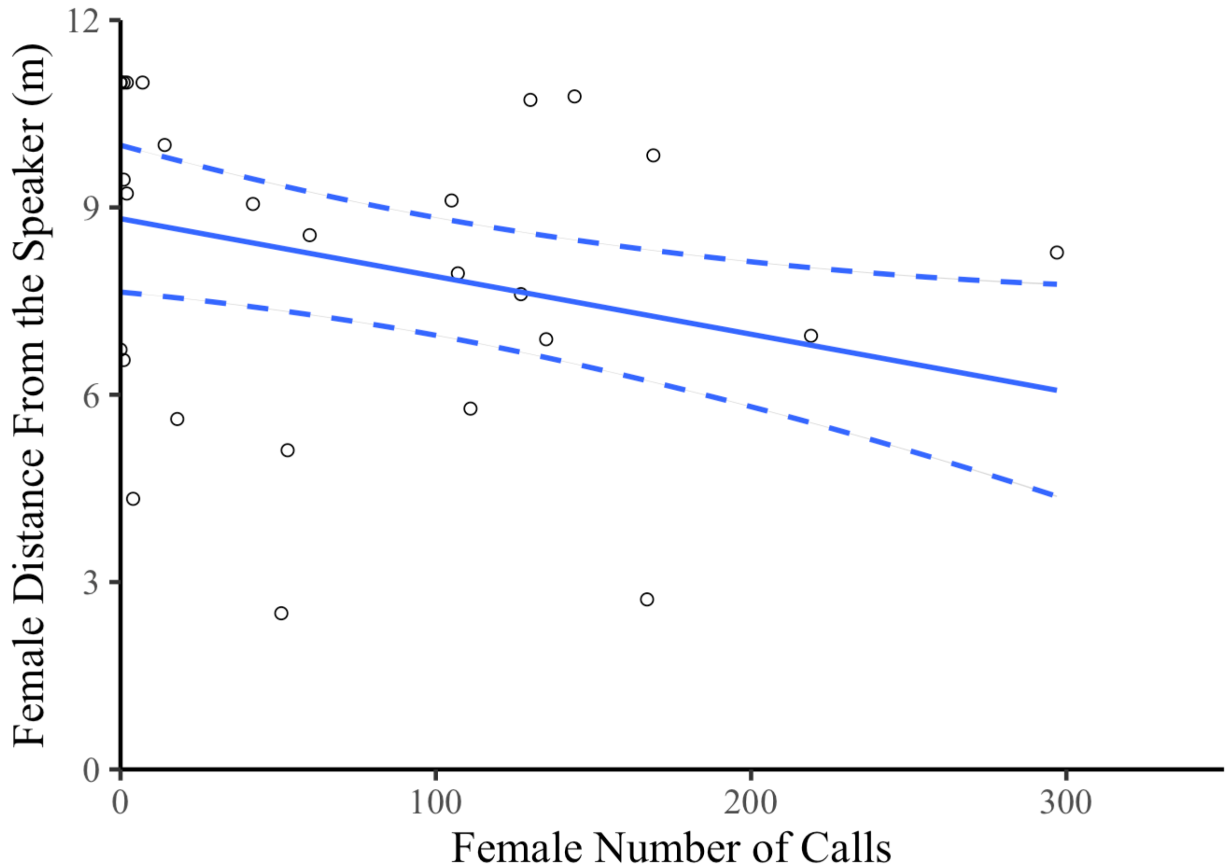

Figure A1. Pearson correlation of number of calls versus average distance from the speaker (m) for 30 female song sparrows in response to Carolina wren alarm call playback with least-squares fit and 95% confidence intervals.

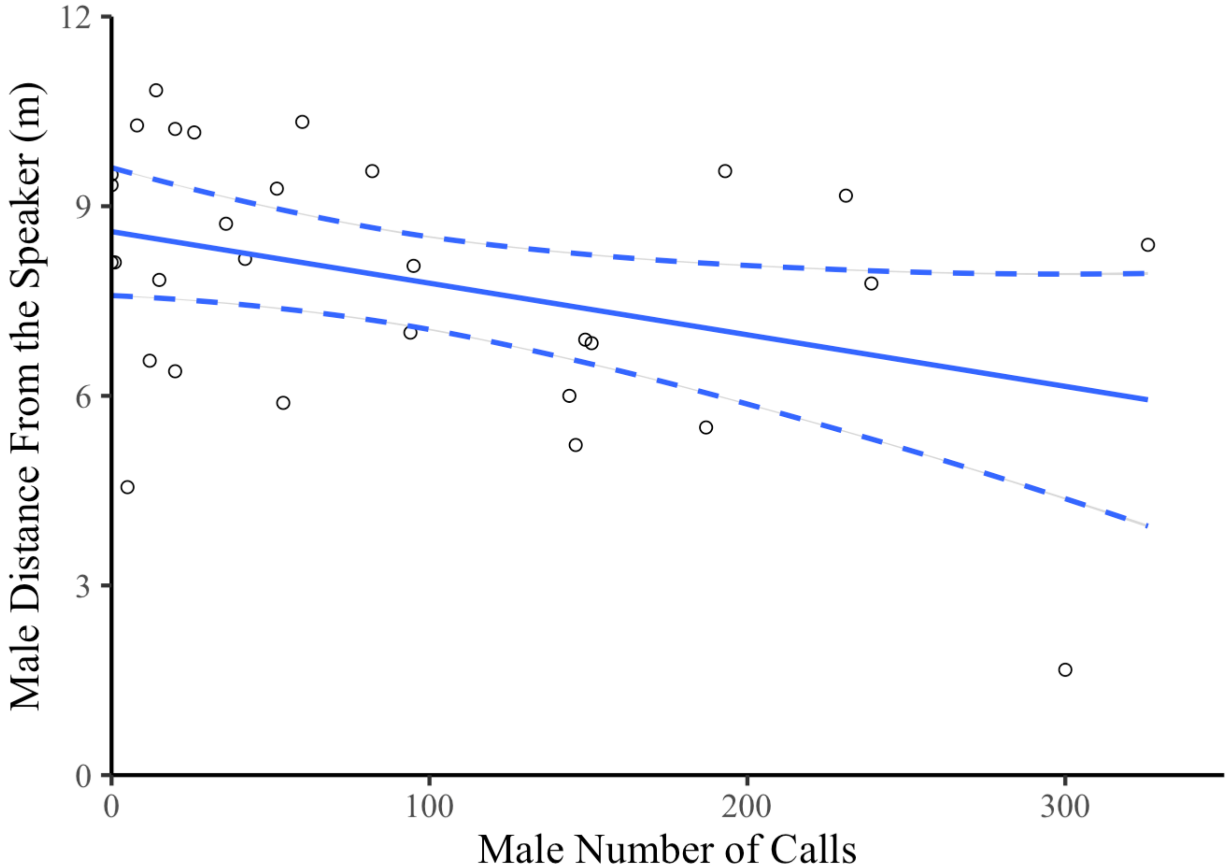

Figure A2. Pearson correlation of number of calls versus average distance from the speaker (m) for 30 male song sparrows in response to Carolina wren alarm call playback with least-squares fit and 95% confidence intervals.

Table A2. Pearson correlation summary for log+1 transformed number of calls versus log+1 transformed average distance from the speaker (m) for 30 male and 30 female song sparrows in response to Carolina wren alarm call playback.

	R	R^2	*df*	*P*
Female Number of Calls v. Avg. Distance from the Speaker (m)	-0.412	0.170	28	0.024
Male Number of Calls v. Avg. Distance from the Speaker (m)	-0.295	0.087	28	0.114