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Male and female Australian magpie-larks respond differently to variation in song frequency (pitch)

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Abstract

Male signals that advertise dominance are often also attractive to females, but female signals advertising dominance can make them less attractive to males. For example, in species where males are larger than females, low-frequency male vocalisations are expected to be selected for by intra-sexual selection, inter-sexual selection, and selection to identify sex, but these selective forces may act antagonistically on female vocalisations. Consistent with this, low-pitch human voices can be perceived as more dominant in both sexes and more attractive to the opposite sex in males, but less attractive to the opposite sex in females. Research on factors influencing the frequency of animal vocalisations has largely focused on males, and little is known about the drivers of variation in vocal pitch between sexes in animals. We tested for sex differences in a species where both sexes sing, the Australian magpie-lark (*Grallina cyanleuca*), by simulating a territorial intruder with experimentally manipulated high- or low-pitched playback songs. Both sexes sang significantly sooner and alarm called more in response to low-frequency playback, regardless of the sex of the 'intruder', suggesting that both sexes perceived low-frequency songs as more threatening. However, there were sex differences in how males and females changed their song frequencies in response to playback. Males sang at a lower frequency to low-pitched male than female playback. In contrast, females sang songs with a significantly higher frequency in response to low- than high-pitched male playback. Our results highlight that selection may act differently on song frequency in male and female birds.

Keywords: sex-specific response, selection, birdsong, female song, Australian magpie-lark

Introduction

Sexual selection is considered the primary social driver of variation in the frequency (pitch) of vocalisations (Mikula et al., 2021; Price, Earnshaw, & Webster, 2006; Puts et al., 2016). Since the pitch of vocalisations may convey information about body size or dominance, low-

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frequency male vocalisations are expected to be favoured by intra-sexual selection, inter-sexual selection, and selection to identify sex (Puts et al., 2016). However, these selective forces may act contrastingly on female vocalisations: intra-sexual selection could favour low-frequency vocalisations, while inter-sexual selection and selection to identify sex are expected to select for high-frequency vocalisations (Feinberg, DeBruine, Jones, & Perrett, 2008; Jones, Feinberg, DeBruine, Little, & Vukovic, 2010). Consistent with this, low-pitched voices are perceived as more dominant in both sexes in humans (Borkowska & Pawlowski, 2011) and elicit more aggressive responses in males during conflict (Zhang & Reid, 2017). Yet human voices are more attractive to the opposite sex if they are low frequency in males, but high frequency in females (Apicella & Feinberg, 2009); females raise their voice pitch when speaking to males they find attractive (Fraccaro et al., 2011). Research on factors influencing the frequency of non-human animal vocalisations typically focuses on males, and little is known about whether these factors differ between the sexes.

The pitch of vocalisations may honestly signal body size, sex, individual quality and aggression. Physiological limitations on sound production mean that larger body sizes are associated with lower-frequency calls across animal species, from mice to elephants (Bradbury & Vehrencamp, 1998; Hauser, 1993; Hoskin, James, & Grigg, 2009; Ryan & Brenowitz, 1985), and in some cases within species (Bee, Perrill, & Owen, 1999; Hardouin, Reby, Bavoux, Burneleau, & Bretagnolle, 2007; Linhart, Slabbekoorn, & Fuchs, 2012). Sex differences in size are also associated with sex differences in vocalisation frequency in some species (Logue et al., 2007; Mikula et al., 2021; Patel, Mulder, & Cardoso, 2010). Since larger individuals are more likely to win physical contests (Archer 1988) and larger males are often more attractive to females (Hunt, Breuker, Sadowski, & Moore, 2009), the pitch of vocalisations correlated with body size have potential signalling value in inter- and intra-sexual contexts (Mikula et al., 2021). Further, within bird species, a negative relationship between body size and frequency may only be revealed within particular contexts, such as during territorial disputes (Galeotti, Saino, Sacchi, & Moller, 1997; Geberzahn, Goymann, Muck, & ten Cate, 2009; Howard & Young, 1998; Price et al., 2006), and not in spontaneous song (Cardoso, Mota, & Depraz, 2007).

Territory holders may interpret differences in the song frequency of intruders as an index of threat. For instance, male black-capped chickadees (*Poecile atricapillus*), willow warblers (*Phylloscopus trochilus*) and chiffchaffs (*Phylloscopus collybita*), were more cautious in their response to a low- than high-frequency male song, suggesting they perceived

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a higher threat level (Linhart & Fuchs, 2015; Linhart et al., 2012; Shackleton & Ratcliffe, 1994). Females in the sex-role-reversed African black coucal (*Centropus grillii*) responded more cautiously to low-frequency female playback (Geberzahn, Goymann, & ten Cate, 2010). Female serins (*Serinus serinus)* responded to frequency-shifted male conspecific playback by approaching closer to 'high' frequency male playback, whereas males did not discriminate based on frequency differences (Cardoso et al., 2007). However, no study to our knowledge has examined within-subject changes in response to manipulated relatively low- versus high-frequency as an index for threat level. Furthermore, no study to our knowledge has examined how female songbirds with standard sex roles respond to frequency differences in an aggressive context, even though females use male song frequency to assess male quality during mate choice (Catchpole, Mann, & Slater, 2008; Riebel, Odom, Langmore, & Hall, 2019). Whether females and males respond to variation in female song frequency is unknown.

Here, we used playback experiments to investigate sex differences in responses to variation in song frequency during simulated territorial interactions in Australian magpie-larks (*Grallina cyanoleuca*), a highly territorial species where both sexes defend all-purpose territories year-round (Hall & Magrath, 2000). We examined male and female responses to playback of high-frequency and low-frequency versions of conspecific song. We used both male and female magpie-lark playback stimuli to test for sex-specific responses. Specifically, we aimed to test whether (i) magpie-larks respond more aggressively to lower-frequency conspecific songs, (ii) magpie-larks adjust the frequency of their songs during a territorial dispute, and (iii) responses to variation in song frequency are sex-specific.

Methods

Study Species

Australian magpie-larks, hereafter, magpie-larks, are medium-sized passerine birds that inhabit woodlands, gardens, and parks throughout tropical and temperate Australia. They form long-term monogamous breeding partnerships with low rates of divorce and extra-pair paternity (Hall, 1999). Both sexes sing solo songs and duet with their partner to defend territories. While partner response to territorial threats are broadly correlated (Hall, 2000; Ręk & Magrath, 2016), there are also sex differences (Hall, 2000; Mulder et al., 2003). Males are slightly (but not visibly) larger than females, and song types are shared between the sexes, with no acoustic sexual dimorphism to human ears (Hall, 2006). However, sexually

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dimorphic facial plumage patterns permit the sexes to be easily distinguished in the field (Hall, 1999).

Recording and playback creation

To obtain songs for playback stimuli, we recorded spontaneous solo song from 14 male magpie-larks and 13 female magpie-larks from 11-29 March 2013 between 07:00 and 10:00 hours from seven parks (mean ± s.e. = 4.14 ± 0.80 focal recordings per park, see supplementary material Table S1 for stimulus recording frequencies). Magpie-larks were not banded, however as magpie-larks are territorial year-round and we only visited each site once, we were confident each recording was from a different magpie-larks. Songs were recorded with a Sennheiser ME67 shotgun microphone and Marantz Professional solid-state recorder PMD660 at a sampling rate of 44.1 kHz. Birds were recorded from no more than 20 m away, and in good weather conditions (no wind or rain). Songs used to create playback stimuli were chosen as the first high quality (high signal-to-noise ratio) song within the focal recording. We created songs following Hall (2000) by repeating a song syllable five times at one-second intervals to match a natural song. Song syllables were isolated, filtered and normalised to -1.0 dB using Audacity v.2.03. Playback files were created by repeating the song six times, with the song sounding at 00:00, 00:20, 00:45, 01:15, 02:00 and 03:00, followed by two minutes of silence creating a five-minute recording (corresponding to the trial length). We created 27 stimuli recordings from 27 different focal birds (to avoid pseudoreplication). We used solo's instead of duets for playback as a simulated solo intruder may be more likely to evoke sex-specific responses due to the additional threat of mate usurping on top of territorial threat (Hall & Magrath, 2000).

Magpie-larks respond with aggressive approach and song to conspecific playback, where frequency is unmanipulated (Hall, 2000; Ręk & Magrath, 2016), but it is unknown if they respond to playback with a manipulated frequency. We only used manipulated versions for playback to avoid the possibility that differences in response could be due to birds perceiving an original (unmanipulated) version differently to a manipulated version (Nelson, 1988; Slabbekoorn & Ten Cate, 1998). To achieve this, we used the "change pitch" function in Audacity to shift the song frequency down or up by 75Hz, creating a 150Hz difference between the 'low' and 'high' frequency versions (Figure S1; see supplementary material for audio). As such, the song type used for playback was controlled for within each trial. Our recording of spontaneous song showed a difference of 911Hz between the naturally lowest and highest pitched version of the song (Table S1), so a difference of 150Hz was well within

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the natural range for magpie-larks and as birds have highly sensitive frequency resolution this difference is likely sufficient for magpie-larks to perceive (Dooling, Leek, Gleich, & Dent, 2002; Fishbein, Prior, Brown, Ball, & Dooling, 2021). Note that shifting the entire song up and down changed the fundamental frequency of the songs, but not formant spacing (Fitch & Kelley, 2000). We used this simple method to focus on the effect of changes in fundamental frequency alone, which allowed us to make conclusions about the function rather than the mechanisms of frequency variation in song. We did not examine the role of song type on receiver response or how song type affected frequency this experimental design did not allow us to thoroughly test these questions.

Playback experiments

Playback experiments were conducted on 27 pairs resulting in 108 trials (two playback per pair, male and female tested within each trial) at parks around Melbourne, Australia (n = 12 parks, mean ± s.e. = 2.42 ± 0.57 pairs per park), from 30 March to 29 April 2013 between 07:30 and 11:00 hours. Magpie-larks tested were of wild origin, as such age, personality type, body size and condition were unknown. However, as pairs were chosen randomly and all tested were included for analysis, including any that didn't respond, we did not expect bias in our sampling. Tested magpie-larks had not been exposed to playback or other experiments before testing and we expected no influence of social learning between pairs. We visited each site only once to conduct playback experiments, so we were confident each trial was conducted on different magpie-lark pairs (as magpie-larks are highly territorial). Each trial consisted of 5 minutes of playback, 5 minutes of silence, and 5 minutes of playback. During each playback period, we broadcast a set of high- and low-frequency playback from either a male or female bird (male: n = 14; female: n = 13), with each playback a unique recording. The order of presentation was balanced (high then low: n = 14; low then high: n = 13). To ensure the playback stimuli were from unfamiliar birds, pairs were presented stimuli from a different park (mean distance between playback source and experimental location ± s.e. = 6.13 ± 0.70 km). The files were stored on an iPhone 5 and played through a Moshi Bassburger speaker (frequency response 240 Hz – 16 kHz) hung in a tree close to a pair. Two observers took notes on flyovers, approach and song, identifying the sex of responders by their facial plumage. A third observer recorded the audio response using the recording equipment as above and made voice comments of observations. As it was rare for birds to not approach or sing in response to playback (8% of cases), we assigned the maximum value of five minutes (trial duration) for latency in these cases.

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Audio files were analysed with Audacity v.2.03 to obtain song rate by manually counting the number of songs within each playback period. Song rate was calculated as the number of solos and initiated duets per individual per minute and we recorded the frequency of each song and alarm call. We used the 'plot spectrum' function in Audacity to measure the frequency of the peak amplitude by visually placing the cursor on the lower of the two frequency bands ('low-peak frequency') that characterise magpie-lark song (see Figure S1 and Hall, 2006) to represent the fundamental frequency of the song. This function automatically snaps to the frequency with the highest decibel reading making for accurate recordings. Only song data (not alarm calls) were used to test frequency response to playback. We measured frequency for each element of every known-sex song that did not overlap with another song. We focused on low-peak frequency rather than overall peak frequency to examine whether the whole song shifted in frequency, avoiding the possible confound of large frequency changes due to shifts in energy between the upper and lower frequency band of songs (Westneat, Long, Hoese, & Nowicki, 1993). We did not filter out noise as the signal-to-noise ratio was high and songs were well above the frequency range of any underlying anthropogenic noise, such as traffic.

Statistical Analysis

We used mixed models to test whether playback frequency type (high or low), playback sex, receiver sex and treatment order affected responses to playback. All response variables were log-transformed to meet assumptions of residual normality. As we predicted that there might be a sex-specific response, we tested for a 3-way interaction between the sex of the receiver, the sex of the playback and frequency of the playback for all models. In the case of a significant interaction, we used Tukey's Honest Significant Difference (HSD) as a planned post-hoc test and corrected the results with false discovery rate detection for multiple tests (Verhoeven, Simonsen, & McIntyre, 2005). Non-significant interactions were dropped. We included bird identity nested within pair identity as random factors to control for repeated measures and assess the variance attributed to the pairs and individuals. For the analysis of response song frequency, we included song nested within bird and pair identity as another random effect, to control for repeated measures of syllables within songs. We calculated confidence intervals for the model parameter's effect sizes using model simulation bootstrapping methods with 100,000 simulations (Faraway, 2016; Gelman & Hill, 2007). Variables with confidence intervals that did not overlap zero were considered to have a significant effect (Cumming & Finch, 2005). We tested for correlations among the response

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variables within individuals, and between males and females within pairs, using Spearman correlations, and corrected p-values for multiple tests using false discovery rate (Verhoeven et al., 2005). Statistical analysis was conducted in R-3.2.4 using the packages 'nlme', 'lmerTest', 'arm' and 'MLMLglmm' (Bates, Machler, Bolker, & Walker, 2015, the R Foundation for Statistical Computing, Vienna, Austria, www.r-project.org).

Ethical note

Methods were approved by the University of Melbourne Animal Ethics Committee 1312772.1

Results

Experimentally manipulated playback songs elicited strong territorial responses from magpie-larks (104 of 108 trials elicited approach and/or song from one or both pair members). Pairs had significantly shorter latencies to sing and higher alarm call rates in response to low-frequency playback compared to high-frequency playback (Table 1, Figure 1). Both playback types attracted similar approach times and numbers of flyovers and songs (Table 1, Figure 1). No interactions were statistically significant and were therefore dropped from these models. Overall, male song rates were significantly higher than female song rates, but the sexes responded similarly strongly in terms of flyovers, alarm call rates and latencies to approach and sing (Table 1). Within individuals, short latencies to approach and sing, and high numbers of songs and flyovers were correlated, whereas number of alarm calls and low-peak frequency of songs were independent of other measures of territorial response yet positively correlated to each other (Table 2). Within a pair, male and female flyovers and song and approach latency were correlated, but low-peak frequency, song and alarm call rate were not (Table 3).

The frequency of songs sung in response to playback varied and depended on a significant 3-way interaction between sex, playback sex, and playback frequency type (Table 4, Figure 2, Table S2). The planned post-hoc test, a Tukey's Honest Significant Difference (HSD) test, showed that females did not adjust their song frequency to frequency differences in a simulated female intruder ($p > 0.05$). However, females sang at a significantly higher pitch in response to low-male playback when compared to high-male playback (mean difference = 116.40 Hz, $p = < 0.001$, Table 4, Figure 2). Males sang at a significantly lower pitch to low-male playback, compared to both female playbacks (high-female, mean

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differences = 84.94 Hz, p = < 0.01; low-female, mean differences = 81.67 Hz, p = 0.02) and there was no difference between their response frequency to high-male playback and both female playback frequencies (p > 0.05) while the difference between frequency in response to male playback was not significant.

Discussion

Our findings are consistent with the view that the selective forces influencing the frequency of vocalisations may act differently on males and females (Feinberg et al., 2008; Jones et al., 2010). Magpie-larks adjusted their territorial responses based on the song frequency and sex of simulated intruders. Both males and females alarm called more and sang sooner in response to low-frequency songs, suggesting these were perceived as stronger territorial threats than high-frequency conspecific songs. Intensity of a territorial response (song rate, flyovers, and approach and song latency) was correlated within individuals and between pair members as well (expect without song rate in pairs). However, the frequency of songs sung in response to playback was not reduced in parallel with these aggressive behaviours. Magpie-larks instead adjusted song frequency depending on the sex and song frequency of simulated intruders. Males sang at lower frequencies in response to low-male playback than females yet did not adjust responses to frequency differences in female playback. In contrast, females shifted song pitch to sing at a higher frequency to low-frequency male playback only. As magpie-lark pairs were chosen randomly; we expect our findings to be representative of general magpie-lark behaviour.

Low-frequency songs appeared to be perceived as more threatening territorial signals than high-frequency songs, consistent with the possibility that they might signal higher threat. Lower frequency vocalisations indicate dominance in humans (Borkowska & Pawlowski, 2011; Zhang & Reid, 2017), non-human primates (Hauser, 1993), other mammals (Vannoni & McElligott, 2008) and birds (Geberzahn et al., 2010; Linhart & Fuchs, 2015). Both male and female magpie-larks approached and sang significantly faster in response to low-frequency playback compared to high-frequency playback. Subsequent responses (number of songs and flyovers) to the two treatments were similarly intense. Variation in song frequency appears to influence territorial responses to same-sex song in other species, based on experiments testing manipulated and natural variation in song frequency. Females in the sex-role-reversed African black coucal approached later but remained further away in response to lowered frequency experimental songs than unmanipulated songs (Geberzahn et al., 2010).

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Male willow warblers likewise stayed further away but increased activity in response to lower frequency same-sex songs, suggesting a cautious but aggravated response to the 'more threatening' playback (Linhart & Fuchs, 2015). Lower frequency songs may elicit more aggressive responses because they represent a stronger territorial threat: the lowest frequency an animal can sing is correlated with body size (Geberzahn et al., 2009; Hall, Kingma, & Peters, 2013), and larger individuals are more threatening rivals (Archer, 1988). While we cannot directly test for a relationship between body size and vocalisation frequency in magpie-larks with our data, previous research shows that males magpie-larks are bigger than females (Hall, 2006), and our findings suggest that vocalisation frequency may potentially be linked to body size in this species. It has been suggested that frequency allometry might be less apparent in oscine songbirds because of vocal learning (Osiejuk, Ratynska, Cygan, & Dale, 2005; Patel et al., 2010). Our results do not support this, which may be attributed to the very simple stereotyped songs of magpie-larks (Hall, 2006).

Despite perceiving songs shifted to a lower frequency as more threatening, magpie-larks did not dynamically adjust song frequency in line with their overall aggressive territorial response, and instead used it to signal a sex-specific response. Within a pair, song and approach latency and flyovers were correlated, consistent with cooperative defence in this species (Hall, 2000; Ręk & Magrath, 2016). Frequency, song rate and alarm rate, however, were not correlated with the pairs response. Magpie-larks defend territories year-round and use both solos and duets for cooperative defence (Hall, 2000). Coalition quality in timing of both vocal and physical displays offers a measure of pair cohesion, which is important for territory defence: more co-ordinated vocal and physical displays are perceived as more threatening to conspecifics (Hall & Magrath, 2007; Ręk, 2018). Despite their generally co-ordinated response to simulated territory intrusions, our findings show that frequency of song response may be more flexible to allow partners to send different messages depending on the sex of the intruder. Previous research has shown a sex-specific response to magpie-lark playback (Hall, 2000; Hall & Magrath, 2000; Mulder et al., 2003), yet how magpie-larks can discriminate between sex from call alone is undetermined (Mulder et al., 2003).

Male magpie-larks used lower-frequency songs in response to low-frequency intruder song (similar to their more aggressive territorial response) for same-sex playback only. This suggests that selection for low-frequency song by males might be stronger in the context of intra- than inter-sexual interactions, or that males represent a stronger territorial threat than

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females. Our findings are consistent with those in human and other anthropoid males, where vocalisation formant dispersion can be better explained by intra-sexual selection rather than inter-sexual selection (Puts et al., 2016). Costs of physical contests may favour using low-frequency signals to allow competitors to assess each other and avoid fights (Collins, 2004; Puts et al., 2016). In addition, male magpie-larks initiated significantly more songs than females overall, which is consistent with previous studies (Hall, 2000; Ręk, 2018) and suggests a stronger role in territory defence, as in many territorial songbirds (Reber et al., 2016; Searcy & Beecher, 2009). Male magpie-larks may also represent a stronger territorial threat than females, since previous studies (but not the current study) found higher song initiation rates to male than female playback by male magpie-larks (Hall, 2000; Hall & Magrath, 2000; Mulder et al., 2003). Sex-specific responses have often been recorded in song rate (Appleby, Yamaguchi, Johnson, & MacDonald, 1999; Arcese, Stoddard, & Hiebert, 1988; Cain & Langmore, 2015; van den Heuvel, Cherry, & Klump, 2014), but the current study is one of the first, to our knowledge, to show frequency changes as a sex-specific response (see Kirschel et al., 2019).

Consistent with our proposal that intra- and inter-sexual selection might act antagonistically on female vocal frequency, we found the effects of simulated intruder sex and song frequency on the response song frequencies of female magpie-larks differed from those of males. Females did not adjust their song frequency in response to frequency differences from same-sex intruders, but females sang at a higher frequency in response to low-frequency male playback (in contrast to their more aggressive territorial response to low frequency playback overall). Mean song frequencies of spontaneous song were lower in males than females, though not significantly. Females singing higher-frequency song may magnify otherwise small sex differences to advertise that they are female to reduce the likelihood of costly physical conflict. This allows them to still participate in co-operative defence, as duets are more threatening than solos in this species (Hall, 2000; Hall & Magrath, 2007). Male magpie-larks are usually larger than females and are generally more aggressive which may drive females to do this (Hall, 1999). A similar finding was found in another species, the Mexican antthrush, *Formicarius moniliger* (Kirschel et al., 2019): while females were less likely to respond to male playback compared to female playback, when they did respond to male playback they sang at a higher frequency. They hypothesised this may be to reduce the likelihood of physical conflict with rival of a large body size.

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Alternatively, female magpie-larks may also be signalling attraction to males with lower-frequency songs. In humans, males prefer females with high pitch voices (Feinberg et al., 2008; Jones et al., 2010), and females are more likely to raise the pitch of their voice when speaking to a male they find attractive (Fraccaro et al., 2011). In avian research, there is an almost even amount of evidence for whether females prefer relatively high- or low-frequency male songs during sexual selection by females (Cardoso, 2012), with our results supporting the latter. However, given the low rate of extra-pair paternity (3% of young) in magpie-larks (Hall, 2000) this is not a likely explanation for our results.

Further research could examine whether the frequency shifts are driven by song type switching. Magpie-larks have multiple song types (Hall, 2006), and in other songbirds species male and female can adjust frequency with changes in context through either dynamically shifting song frequency or switching to a song type with a different frequency (Dabelsteen & Pedersen, 1990; Galeotti et al., 1997; Geberzahn et al., 2009).

While magpie-larks used song rate, latency to approach and sing and flyover for a coordinated territorial response by the pair, each sex used song frequency for sex-specific signalling. Male magpie-larks sang lower frequency songs in response to low-frequency same than opposite-sex conspecifics, suggesting that intra-sexual selection may be a stronger driver of variation in male vocal frequency than inter-sexual selection. In contrast, females were responsive to variation in male song frequency, singing higher frequency songs in response to male intruders singing low-frequency songs. Females responding to low-frequency male song may have amplified sex differences in frequency to signal their sex to males that were potentially larger or more dominant, either to reduce the risk of attack, or to signal attraction. This research highlights the potentially different effects of selection on male and female signals in acoustic communication.

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Tables and table legends

Table 1 Factors affecting territorial response behaviours in response to high and low, male and female magpie-lark playback. N = 108 trials to 27 pairs of 54 birds. Estimated effect sizes are shown for fixed effects with 95% confidence intervals and random effects show the variance attributed to bird ID and the pair ID.

	Approach latency	**Song latency**	**Flyover rate**	**Song rate**	**Alarm call rate**
Fixed effects	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Intercept	3.36 (2.76, 5.08)	3.37 (2.42, 4.32)	1.12 (0.77, 1.52)	0.28 (0.15, 0.43)	-0.01 (-0.08, 0.08)
Playback freq (low)	-0.38 (-0.88, 0.16)	**-0.59 (-1.03, -0.08)**	-0.11 (-0.30, 0.06)	-0.06 (-0.15, 0.04)	**0.03 (0.00, 0.06)**
Playback sex (male)	0.10 (-1.16, 1.30)	0.31 (-061, 1.37)	-0.04 (-0.38, 0.41)	0.06 (-0.08, 0.19)	0.05 (-0.03, 0.14)
Sex (male)	-0.22 (-0.75, 0.29)	-0.18 (-0.64, 0.31)	0.13 (-0.06, 0.29)	**0.11 (0.02, 0.20)**	-0.00 (-0.07, 0.06)
Treatment order (low-high)	-0.87 (-2.03, 0.43)	0.05 (-0.94, 1.05)	0.10 (-0.30, 0.50)	-0.06 (-0.20, -0.07)	0.04 (-0.04, 0.13)
Random effects	**σ^2 (95% CI)**	**σ^2 (95% CI)**	**σ^2 (95% CI)**	**σ^2 (95% CI)**	**σ^2 (95% CI)**
Pair	3.06 (1.67, 4.94)	1.81 (0.99, 2.96)	0.31 (0.17, 0.50)	0.02 (0.01, 0.03)	0.00 (0.00, 0.01)
Pair : Bird	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.01 (0.01, 0.02)

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Table 2. Spearman correlations within individual for behavioural responses N = 108. * p <.05, ** p < .01, *** p < .001. N = 108. Results were corrected with false discovery rate detection for multiple tests (Verhoeven et al., 2005)

Behaviour	Mean	Range	Correlations				
			1.	2.	3.	4.	5.
1. Approach latency (s)	95.00	(0-300)					
2. Song latency (s)	78.09	(1-300)	0.70***				
3. Flyovers	3.01	(0-14)	-0.62***	-0.47***			
4. Song rate	2.13	(0-14)	-0.37***	-0.42***	0.45***		
5. Alarm call rate	0.40	(0-6)	-0.18	-0.15	0.13	-0.02	
6. Low-peak frequency	2656.91	(1830-3525)	-0.04	0.08	0.05	-0.13	0.34*

Table 3. Spearman correlations within pair between male and female behavioural responses (N = 27). * p <.05, ** p < .01, *** p < .001. N = 108. Results were corrected with false discovery rate detection for multiple tests (Verhoeven et al., 2005).

	Approach latency	Song latency	Flyovers	Song rate	Alarm call rate	Low-peak frequency
Correlation	0.78***	0.79***	0.80***	0.13	0.26	-0.22

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Table 4. Factors affecting the low-peak frequency (pitch) of songs sung in response to high and low, male and female magpie-lark playback (N = 1152). Estimated effect sizes for fixed effects are shown with 95% confidence intervals, and variance attributed to the random effects pair ID (N = 26), bird ID (N = 51), and song ID (N = 277).

	Response frequency	
Fixed effects	**β**	**(95% CI)**
Intercept	2723.74	(2604.93, 2811.71)
Playback frequency (low)	-5.94	(-69.98, 71.89)
Playback sex (male)	-87.07	(-222.68, 37.80)
Sex (male)	-102.21	(-247.15, 26.77)
Treatment order (low/high)	-8.51	(-100.38, 76.33)
Sex : Playback sex	82.96	(-103.71, 264.89)
Sex : Playback frequency	0.52	(-97.57, 100.91)
Playback sex : Playback frequency	**119.98**	**(20.93, 214.96)**
Sex : Playback sex : Playback frequency	**-146.89**	**(-287.36, -16.49)**
Random effects	**σ^2**	**(95% CI)**
Pair	0.00	(0.00, 0.00)
Pair : Bird	**21,791.40**	**(15,292.15, 30714.75)**
Pair : Bird : Song	**12,775.70**	**(11,188.65, 14,766.30)**

Figure legends

Figure 1. Responses of male and female magpie-larks during high- and low-frequency playback trials (N = 108): a) time taken to approach the speaker, b) time taken to start singing, c) number of flyovers, d) song rate, e) alarm call rate. Boxplots show the median, inter-quartile ranges and outliers.

Figure 2. Frequency of a) female song and b) male song in response to playback stimuli (n = 1152 songs by n = 51 birds). Boxplots show the median, inter-quartile ranges and outliers.

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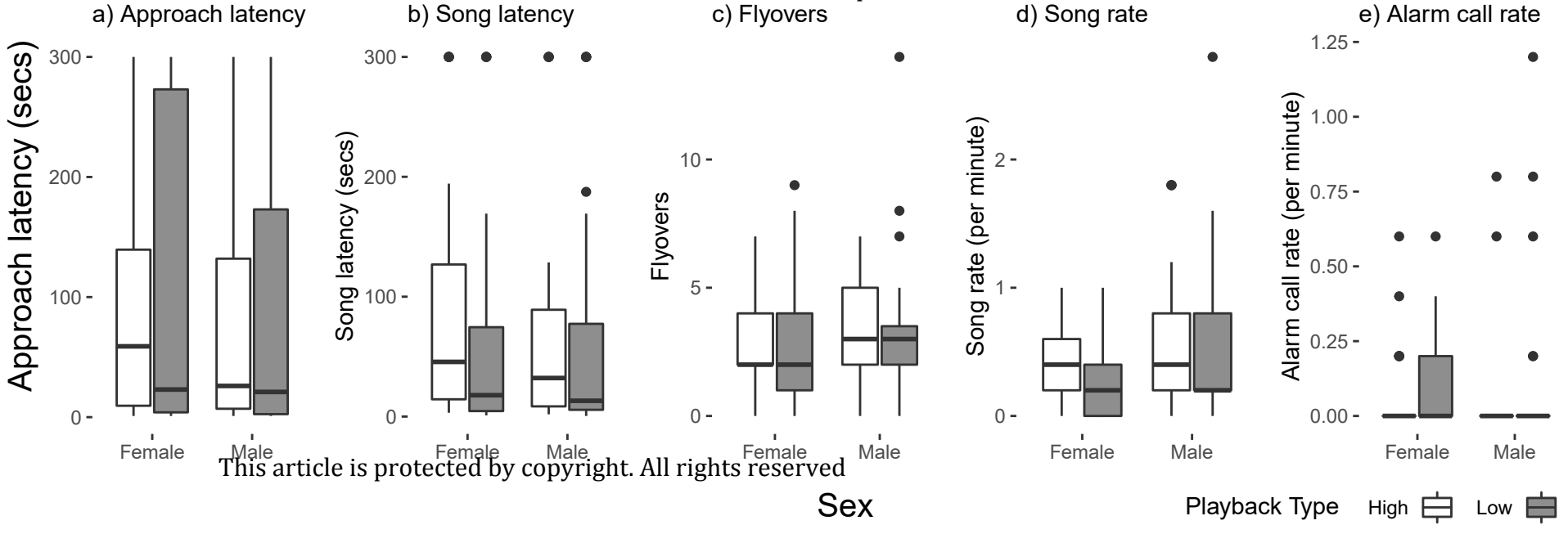

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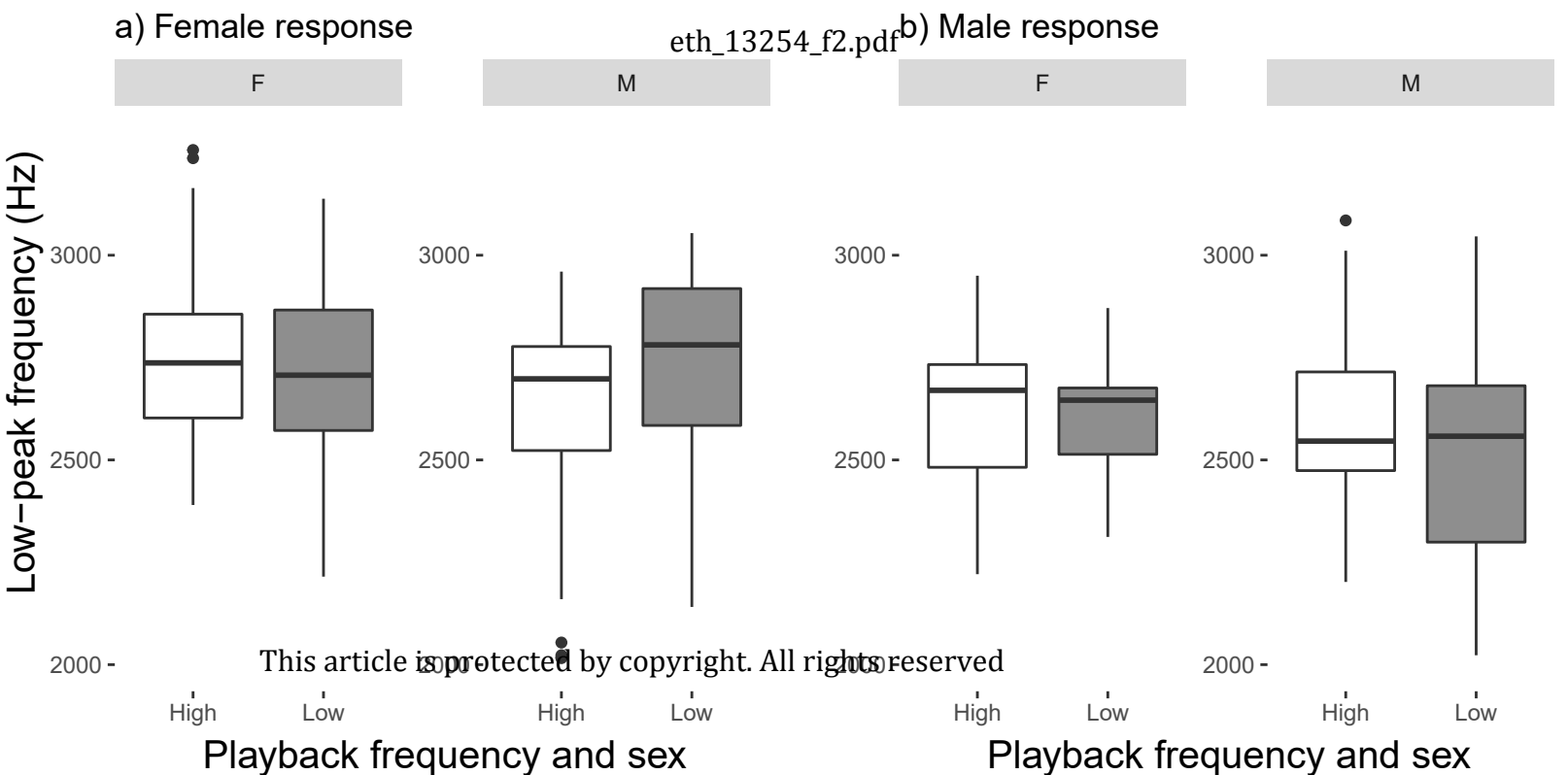

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