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Liu, Pengfei, Lloyd, Huw, Lou, Yingqiang and Sun, Yuehua (2023) Soft song provokes stronger aggressive responses than broadcast song in the Plain Laughingthrush (Pterorhinus davidi). IBIS: The International Journal of Avian Science, 165 (2). pp. 561-570. ISSN 0019-1019

DOI: https://doi.org/10.1111/ibi.13141

Publisher: Wiley

Version: Accepted Version

Downloaded from: https://e-space.mmu.ac.uk/630861/

Additional Information: This is the peer reviewed version of the following article: Liu, P., Lloyd, H., Lou, Y. and Sun, Y. (2023), Soft song provokes stronger aggressive responses than broadcast song in the Plain Laughingthrush (Pterorhinus davidi). Ibis., which has been published in final form at https://doi.org/10.1111/ibi.13141. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions. This article may not be enhanced, enriched or otherwise transformed into a derivative work, without express permission from Wiley or by statutory rights under applicable legislation. Copyright notices must not be removed, obscured or modified. The article must be linked to Wiley's version of record on Wiley Online Library and any embedding, framing or otherwise making available the article or pages thereof by third parties from platforms, services and websites other than Wiley Online Library must be prohibited.

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Soft song provokes stronger aggressive responses than broadcast song in the Plain Laughingthrush (*Pterorhinus davidi*)

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Many bird species produce low-amplitude acoustic signals, known as soft songs, which are widely believed to function primarily in aggressive interactions. However, there are surprisingly few experimental studies that have examined whether soft songs are reliable signalling strategies for escalating aggressive interactions. Here, we investigate the func-tions of soft song in a colour-marked population of the Plain Laughingthrush *Pterorhinus davidi*, using playback experiments combined with specimen-based simulated territory intrusions. We found that playback of male soft song provoked quicker response and stronger aggressive intent by territorial pairs compared with playback of male broadcast song. Responses by territorial pairs to male soft song playback were significantly stronger compared with responses to broadcast song playback, with a greater number of flights over the speaker, more time spent within 1 m of the speaker and closer approaches to the speaker. Furthermore, closer approach with the production of soft song by focal males was the best predictor of aggressive intent in combined playback and specimen intrusion experiments. Our results suggest that male soft song is used as an aggressive signal in the Plain Laughingthrush, and represents the first evidence of such for a mono-gamous temperate duetting species.

Keywords: aggression, duetting, playback, signalling strategies, simulated territory intrusion, soft song.

Acoustic signalling is widespread in animals and plays a critical role in determining fitness (Catchpole & Slater 2008, Akçay & Beecher 2012, Xia *et al*. 2013). In birds, high-amplitude long-range signals such as broadcast songs function in intra- and inter-sexual interactions such as mate attraction and intra-sexual competition (Catchpole & Slater 2008, Jakubowska & Osiejuk 2018). Many bird species also produce low-amplitude close-range acoustic signals (Dabelsteen *et al*. 1998) that have historically been overlooked (Hof & Hazlett 2010) and remain largely understudied in comparison to broadcast songs (Catchpole & Slater 2008, Jakubowska & Osiejuk 2018). In North America, up to 50% of all songbird species use these low-amplitude vocalizations, also known as soft songs, leading to the suggestion that they may represent an ancestral trait (Reichard & Welklin 2015). Soft songs are used in short-range communications and courtship display (Titus 1998, Vargas-Castro *et al*. 2017), but also play a role in aggressive interactions (Dabelsteen *et al*. 1998, Morton, 2000, Searcy *et al*. 2013), particularly between males (Searcy & Nowicki 2006, Searcy & Beecher 2009). In other instances, soft songs are also produced in the same social contexts as broadcast songs (Reichard & Anderson 2015), and for some passerines they represent a significant predictor of stronger aggressive responses than broadcast songs (e.g. Dabelsteen & Pedersen 1990, Anderson *et al.* 2007, Xia *et al.* 2013, Moran *et al.* 2018).

Soft songs are employed by individuals during aggressive encounters to conceal themselves from rivals and to minimize information transfer about the aggressive interaction with the rival to eavesdroppers (Dabelsteen & Pedersen 1990, Ręk & Osiejuk 2011, Moran *et al.* 2018). For soft songs to serve as a reliable signalling strategy for aggressive interactions (e.g. Laidre & Vehrencamp 2008) they need to fulfil three criteria: first, there must be a clear and significant increase in the use of the signal during aggressive interactions; second, receivers should respond to the signal as if it is an aggressive signal; and third, the signal should invoke the escalation of the aggressive interaction if the receiver does not back down (Searcy & Beecher 2009). Three hypotheses have been proposed to explain the maintenance of soft signal reliability. (1) The receiver-retaliation rule (Euquist 1985) states that soft songs are employed by the intruder to avoid retaliation behaviour by the receiver (Anderson *et al.* 2007), as has been suggested for the Corn Crake *Crex crex* (Ręk & Osiejuk 2011, Ręk 2013). (2) The eavesdropping avoidance hypothesis proposes that soft songs more easily reduce detection risk by predators when males are engaged in aggressive interactions with conspecific competitors (Dabelsteen *et al.* 1998), with some evidence emerging for this in the White-throated Thrush *Turdus assimilis* (Vargas-Castro *et al.* 2017). (3) The readiness hypothesis proposes that individuals preparing to fight are constrained to produce soft vocalizations, although empirical examination for this third theory is still lacking (AkÇay *et al.* 2015).

One method to assess whether soft songs, in comparison with broadcast songs, are significant predictors of escalations in aggressive interactions is to combine experimental playback trials of broadcast and soft song with the placement of a conspecific specimen to provide an opportunity for the focal birds to attack (e.g. Searcy & Nowicki 2006, Ballentine *et al.* 2008, Laidre & Vehrencamp 2008, Ręk & Osiejuk 2011). To our knowledge, no study has yet used experimental trials of this nature to test the function of soft song and its role in predicting aggressive interactions in species of laughingthrush, one of the most species-rich clades in the family Leiothrichidae (Moyle *et al.* 2012, Cibois *et al.* 2018). Laughingthrushes are characterized by their complex songs and social systems, and the females of several species are noted for both their solo songs and their duets with males (Lei & Lu 2006, Liu *et al.* 2022). More interestingly, for one species, the Plain Laughingthrush *Pterorhinus davidi* (Cibois *et al.* 2018), males sing soft songs when unpaired or when they first occupy a new territory (unpublished observations). Hence, for this species at least, soft song could be used in the context of courtship, as has been noted for other passerines (Reichard & Anderson 2015).

Here we explore the functions of soft song in a colour-marked population of the Plain Laughingthrush using a combined playback and specimen intrusion experimental approach. We first describe the broadcast songs and soft songs of the males in this species, and examine behavioural responses to playback experiments of broadcast song and soft song to identify the possible function of soft song. Finally, we use behavioural observations from combined playback trials and simulated territorial intrusions using a mounted specimen to model whether broadcast or soft song best predicts aggressive escalation.

METHODS

Study site and focal species

Our study site is an area of mixed open woodland habitats and fields on the campus of Longdong University (35°07′N, 107°07′E; 1360 m elevation), located in the interior of the Loess Plateau, eastern Gansu province, central China. We have studied a colour-banded population of the Plain Laughingthrush at this site since 2016, gathering biometric and territory data, which have revealed that Plain Laughingthrush is a year-round territorial resident, in which males and females duet and jointly defend a territory. Pair relationships and territory size are relatively stable from year to year.

Playback experiments

Experiments were conducted from 20 February to 10 May 2021, which corresponds to the breeding season of the Plain Laughingthrush (Liu *et al.* 2021). To test whether pairs respond differently to soft songs and broadcast songs, we conducted playback experiments on 25 colour-banded focal pairs; each pair had at least one member banded with a three-colour leg ring combination.

For each pair, we performed three different playback trials: (1) broadcast song of the male Plain Laughingthrush; (2) soft song of the male Plain Laughingthrush; and (3) as a control, soft song of the male White-browed Laughingthrush *Pterorhinus sannio*, a species that is sympatric with the Plain Laughingthrush in our study site with a small population of fewer than 20 individuals. The five broadcast songs used in the playback experiments were recorded during the 2018 breeding season, but we only used three soft songs recorded from three different males in 2017 and 2019 (one recorded in 2017 and two in 2019) because soft song in this species is rarely observed under natural conditions and is difficult to record. Soft song has a greater number of notes, is both more variable in frequency and longer in duration than broadcast song, and has a different structure (Fig. 1). All song exemplars were from Plain Laughingthrush pairs recorded elsewhere (approximately 20 km from our study site). For the control trials, we used three soft songs from three different male White-browed Laughingthrush individuals that were recorded during the 2020 breeding season. For our experiments, all playback songs were randomly selected, and represented spontaneous songs that were not themselves elicited by playback. Plain Laughingthrushes are very responsive to playback of both familiar and stranger conspecific vocalizations, suggesting that the results of our experiments will not depend on the birds identifying the individual making the recorded vocalization.

Songs were broadcast using an iPod linked to a JBL Clip3 portable speaker (JBL, Los Angeles, CA, USA, https://jblpro.com/en), without employing any lossy audio compression. Each of the three trials per pair were conducted on three consecutive days between 8:00 and 11:00 a.m. local time. Before each playback, we confirmed visually that the target pairs were within their territory, and were less than 5 m from the speaker, so we assumed that they could hear the soft song. Each playback trial was standardized to a 5-min period because soft song duration is longer than broadcast song duration. We structured the playback files so that broadcast playback contained 12 songs per minute, and soft playback contained 2 songs per minute, ensuring that both types of playback presented the same duration of acoustic stimulation. The order of playback trials was randomly selected for each pair and was balanced across the subjects, with the speaker volume measured by a Digital Sound Level Meter (AS804, Smart Sensor, Hong Kong). Amplitude was measured at a 1-m distance between the speaker and the decibel meter and adjusted to 85 and 56 dB for broadcast song and soft song playback, respectively, which approximates the natural amplitude of male broadcast and soft singing in the environment. We positioned the speaker on a nearby woody shrub close to the centre of each pair's territory, and at a height of 1 m above the ground. Adjusted volumes were within the ranges of spontaneous songs under natural conditions. We marked a 1-m radius with coloured marking tape around the speaker, then two observers, partially concealed and positioned approximately 10 m from the speaker, recorded the following variables for each focal pair during each trial: latency time (± 1 s) for either pair member to appear and approach the speaker; the sex of the first pair member to approach the speaker; the closest distance (± 0.1 m) that individuals approached the speaker; number of flights over the speaker, time spent within 1 m range of the speaker by the focal male and number of broadcast songs by males, females or male–female duets; and whether broadcast or soft song were produced. A result of the typical long duration of the soft song vocalization in the species was that it was not possible to count the number of notes in each soft song bout. Overall, we obtained response data from 22 of the 25 focal pairs.

Playback with specimen intrusion experiments

To determine if male soft song would provoke a more significantly strong aggressive response by the territory owners, we conducted playback in combination with specimen intrusion experiments within the focal pair's territory – these pairs were the same as those used in the previous playback experiments. Intrusion experiments with specimen mounts were conducted at least 3 days after the completion of the previous playback experiment. Five specimens of male Plain Laughingthrushes from the specimen collection at Longdong University were used for the experiments. In each trial, we randomly selected a specimen which was subsequently fixed into a metal cage (height 50 cm, width 35 cm, length 35 cm) to avoid possible damage from aggressive behaviour by focal pairs. Songs were broadcast using the same iPod-linked

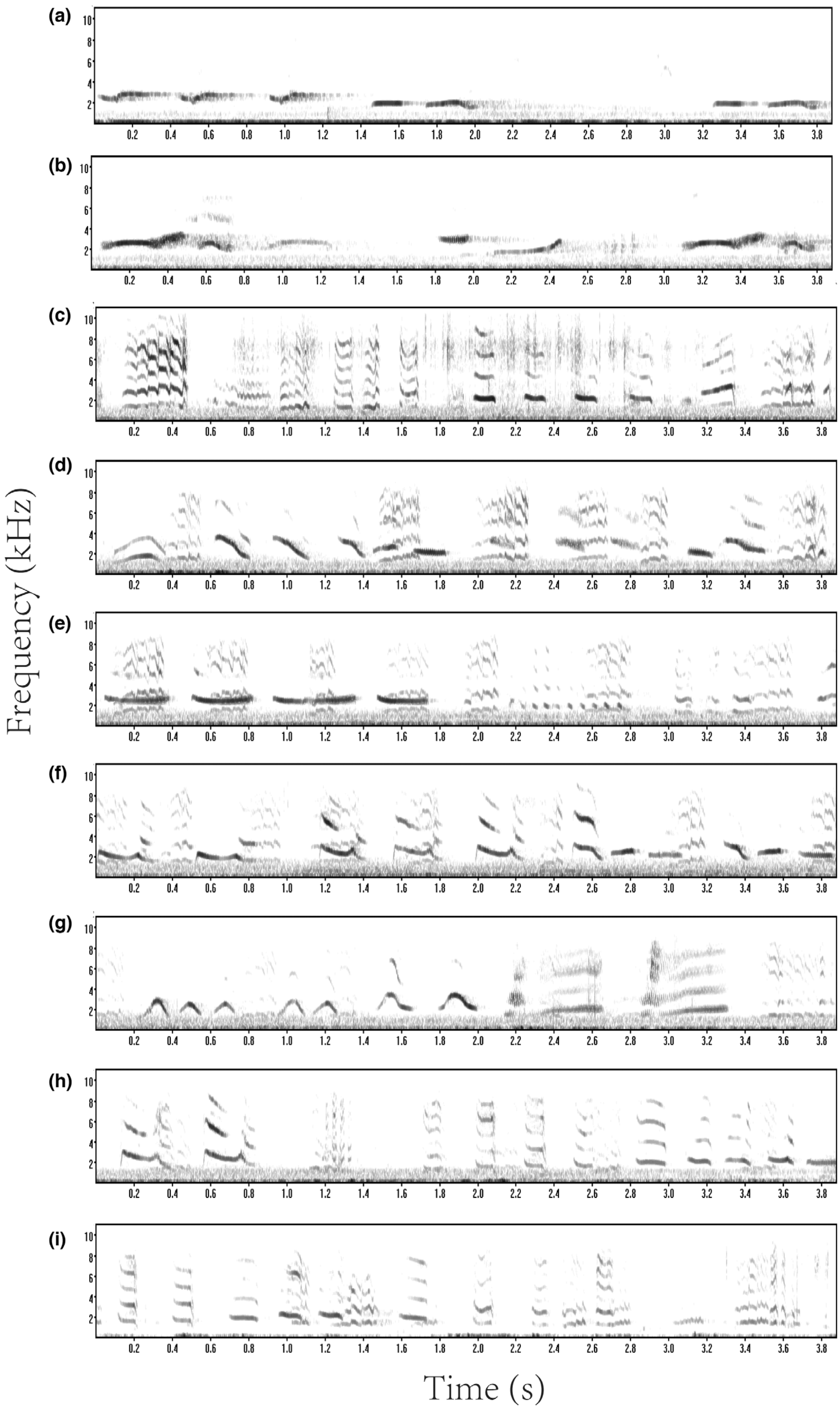

Figure 1. Sonograms of broadcast song (a, b) in two different males and soft song (c–i) of one bout in one male Plain Laughingthrush *Pterorhinus davidi*. The soft song has a greater number of notes, is more variable in frequency and is longer in duration than broadcast song.

JBL Clip3 louder portable speaker placed beside the cage at a height of 1 m above the ground near the centre of the focal pair's territory. We conducted playback of broadcast song or soft song combined with specimen intrusion for 5 min, with both experiments separated by a 5-min period with no playback. Given that the purpose was to determine if soft song provoked possible attack, broadcast song was always played first in each experiment, followed by soft song, with random selection of individual songs used in the playback. Speaker volume was adjusted to 85 and 56 dB for broadcast song and soft song, respectively, as described above. Two observers then spent 5 min recording the same variables as for the previous playback experiments, but with the additional variables of recording when a pair member perched on the specimen cage, and whether any apparent aggressive intent occurred. In this experiment, we obtained data from 17 focal pairs.

Statistical analysis

We used Avisoft-SASLab Pro 5.2.08 (Avisoft Bioacoustics, Berlin, Germany) to describe the spectrograms of male broadcast and soft songs. Variables were set as follows: sampling rate 22 050 Hz because the maximum frequency of both broadcast song and soft song are less than 11 025 Hz, 16-bit, frequency resolution 43 Hz, temporal resolution 2.9 ms, FFT length 512 points, window FlatTop, overlap 87.5%.

During playback experiments, all focal pairs showed no response to the soft song playback of the sympatric White-browed Laughingthrush, and consequently we excluded this stimulus in all subsequent analyses. The majority of response variables were correlated with each other, so we used principal component analysis (PCA) to reduce the number of variables and to remove any multicollinearity, using the *psych* package (Revelle & Revelle 2015) in R software (R Core Team 2015). Six variables were included in the PCA: latency time to respond, male flights over the speaker, time spent within 1-m range of the speaker, first responding sex and the closest approach distance to the speaker. We used a Kaiser–Meyer–Olkin and Bartlett's test of sphericity to extract eigenvalue factors > 1 and used Varimax rotation to assist in the interpretation of the factor solutions. The retained composite with a PCA loading value > 0.60 was used for characterization in our analysis (Stevens 2002). We used linear mixed models (LMMs) with pair identity as a random effect to test whether the experiment time, date, broadcast song vs. soft song, playback stimulus identity and playback order (all set as independent variables) affected the responses represented by the PCA components (dependent variables). Variables in the model were selected using a backwards selection procedure based on P values. Wilcoxon paired-sample tests were used to compare the response differences (LMM dependent variables) between soft song and broadcast song. For the combined playback and specimen intrusion experiments, we assessed whether the playback-stimulus-provoked focal males attacked the cage with the mount or not by using Fisher's exact test. All LMMs were fitted with *lmer* functions from the *lme4* package (Bates *et al.* 2014). The significance of fixed effects in the models were calculated using Wald χ^2 tests. All statistical analyses were conducted using R software (R Core Team 2015). All results are expressed as mean ± standard deviation, with two-tailed probabilities and significance level set as a P value < 0.05.

RESULTS

Description of soft and broadcast songs

Male soft song and broadcast song types were easily distinguishable from each other (Supporting information, Audio S1 and S2), with obvious structural and temporal differences evident in the sonograms (Fig. 1). Male broadcast song was louder and audible from further distances, with far more distinct intervals between phrases. Male Plain Laughingthrush soft song had more syllables and a more variable frequency bandwidth than broadcast song, and was audible only at a closer range to the receiver. The duration of a typical soft song was too long to consistently count the number of notes in each bout, and as the sample size was extremely small ($n = 3$), we did not statistically compare differences in acoustic structure between soft song and broadcast song. However, it was readily apparent from the sonograms (Fig. 1) that soft songs were typically of longer duration, quicker pace and higher pitch than broadcast song, as well as consisting of more notes.

Broadcast vs. soft song playback experiments

The PCA generated two components that explained 75.9% of the variation in the playback response (Table 1). The first component explained 40.1% of the variance, and corresponded with latency time to respond, and time spent within a 1-m range of the speaker; the second principal component, which explained 35.8% of the variance, corresponded to male flights over the speaker and closest distance approached to the speaker. Therefore, we used the latency time to respond, time spent within a 1-m range of the speaker, male flights over the speaker and closest distance approached to the speaker as the response behaviours in subsequent analyses. Initial model results showed that playback order, playback stimulus identity, date, time and pair ID all had a non-significant effect on the behavioural responses, and so were removed from the models using a backward elimination process, leaving playback stimuli (broadcast song vs. soft song) as having the only significant relationship with latency time to respond ($\chi^2 = 3.808$, $P < 0.001$), male flights over the speaker ($\chi^2 = 3.189$, $P = 0.001$), closest distance approached to the speaker ($\chi^2 = 6.995$, $P = 0.008$) and time spent within 1-m range of the speaker ($\chi^2 = 3.162$, $P = 0.002$). Wilcoxon paired-sample tests revealed that males exhibited a significantly slower response to playback of male broadcast song compared with soft song ($Z = -4.783$, $P < 0.001$, $n = 18$; Fig. 2a). Playback of soft song also resulted in significantly more flights over the speaker ($Z = -4.126$, $P < 0.001$, $n = 18$; Fig. 2b) and focal males spent more time within 1-m range of the speaker compared with playback of male broadcast song ($Z = -4.070$, $P < 0.001$, $n = 20$; Fig. 2c); in addition, the approach of focal males to the speaker was at a significantly closer distance in response to soft song than broadcast song ($Z = -3.419$, $P < 0.001$, $n = 20$; Fig. 2d). Broadcast song invoked responses of only broadcast song production in six of the 22 focal male pair members, of only broadcast song of two of the 22 focal female pair members, and male-initiated duetting in one pair, with all vocal responses preceded by approaching the speaker. A male-dominated approach was noted for 13 of the focal pairs. The playback of soft song invoked responses of soft song production in 12 of 22 focal male pair members and again was preceded by approaching the speaker, with only the approach evident in 10 focal pairs, and no male responded to soft song with broadcast song.

Predictors of attack in male Plain Laughingthrush

In 17 playback trials of soft song in combination with specimen intrusion experiments, 12 males responded with soft song, four males approached the speaker without singing, one male responded with broadcast song, and all vocal responses were preceded by approaching the speaker and specimen. Of the 17 males, six sang soft songs and perched on the cage and attempted to attack the specimen, and five males retreated back from the specimen. In two territories, the pair never appeared in view of the observers and no vocal response occurred. In response to broadcast song playback, seven males approached the speaker and then produced broadcast song, six males approached the speaker without singing, only one male produced soft song before retreating, and one male produced soft song and perched on the cage and attempted to attack the specimen. For two focal territories, the pair failed to respond physically or vocally. A Fisher's exact test indicated that playback of soft song was a significant predictor of an escalation to attack by Laughingthrushes ($P = 0.001$).

Table 1. The results of principal component analysis of response behaviours comprising male flights over the speaker, closest distance approached to the speaker, latency time to respond, time spent within 1 m range of the speaker and first responding sex. Factors with an eigenvalue of at least 1 were extracted, and varimax rotation was used to interpret the factor solutions.

	Principal component	
Variables	1	2
Male flights over the speaker	0.043	0.796
Closest distance approached	−0.117	−0.811
Latency time to respond	−0.887	−0.156
Time spent within 1 m range of the speaker	0.987	0.150
First sex to respond	0.202	0.573
Eigenvalue	2.471	1.325
% variance explained	40.109	35.798

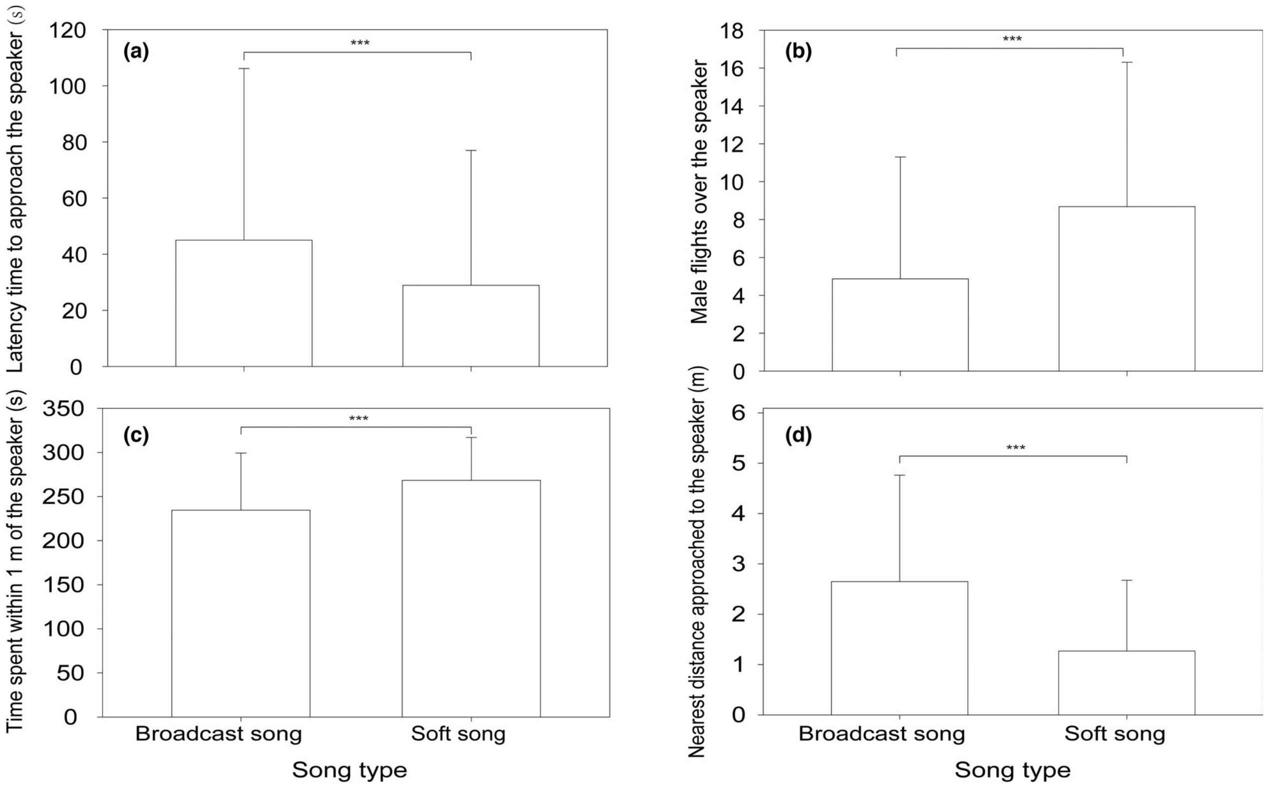

Figure 2. Comparisons of latency time to approach the speaker (a), number of flights over the speaker (b), time spent within 1 m of the speaker (c) and nearest distance approached to the speaker (d) by male Plain Laughingthrushes *Pterorhinus davidi* in response to playback of male broadcast and soft song.

DISCUSSION

Our combined playback and specimen-based simulated intrusion experiments show that soft song is a better predictor of aggressive intent than broadcast song in male Plain Laughingthrushes. Approaches with soft song and specimen stimuli were significant predictors of attack. This reaffirms previous suggestions that soft song is the primary acoustic signal that can reliably predict aggressive escalation in passerines (e.g. Searcy & Nowicki 2006, Ballentine *et al.* 2008, Searcy & Beecher 2009). Closest approach distance has also been identified as a significant behavioural component that can predict aggressive intent (e.g. Hof & Hazlett 2010) and although widely used as a proxy for measuring aggression in avian playback studies (e.g. Vehrencamp 2001, Vehrencamp *et al.* 2007), our findings suggest that closest approach distance needs to be assessed in conjunction with associated vocal responses by the target species.

We found that playback of male soft song provoked a significantly stronger aggressive intent response than broadcast songs in the Plain Laughingthrush, perhaps because the responder perceives the intruder to be closer, as the signal was played at a much lower amplitude. This finding is consistent with other studies of other passerines including Eurasian Blackbird *Turdus merula* (Dabelsteen & Pedersen 1990), Brownish-flanked Bush Warbler *Horornis fortipes* (Xia *et al.* 2013), Savannah Sparrow *Passerculus sandwichensis* (Moran *et al.* 2018), Dark-eyed Junco *Junco hyemalis* (Reichard *et al.* 2011) and Song Sparrow *Melospiza melodia* (Anderson *et al.* 2012, Templeton *et al.* 2012). However, in the Ortolan Bunting *Emberiza hortulana*, soft song is used by males in an aggressive context, although it does not appear to represent a true aggressive signal (Jakubowska & Osiejuk 2018). In our study, combined playback of soft song and specimen intrusion provoked a faster approach, more vocal responses and more attacks by territory holders. Hence, in our experiments, soft song fulfilled all three key criteria to function as a reliable signalling strategy for aggressive escalation.

The soft song stimuli provoked production of soft song by territorial males. Other responses included more flights over the speaker and subsequent closer approach to the speaker. In five cases, only the males appeared and approached the speaker, both to playback of broadcast and soft song. In these instances, it is possible that the females of these pairs did not respond because they may have been incubating.

In our Plain Laughingthrush population, broadcast song playback elicited approach behaviour, production of broadcast song and flights over the speaker, but while these responses were similar to that of responses to soft song, latency time was longer, there were far fewer flights over the speaker and approach distances were significantly greater than for soft song playback trials. Male Plain Laughingthrush broadcast song is typically used to advertise breeding status, and to attract females, but is also used in some male–male competitions (Liu *et al.* 2022). Furthermore, males also coordinate their broadcast songs with their mated females to form male–female duets that play a significant role in joint territorial defence (Liu *et al.* submitted). Given the multi-functionality of their broadcast songs, it is therefore reasonable to suggest that broadcast song is not an aggressive signal in this species.

In this study, playback of the Plain Laughingthrush soft songs provoked stronger aggressive responses by territorial males than broadcast songs, a finding that is consistent with studies of Eurasian Blackbird (Dabelsteen & Pedersen 1990), Song Sparrow (Anderson *et al.* 2007, Templeton *et al.* 2012), Brownish-flanked Bush Warbler (Xia *et al.* 2013) and Savannah Sparrow (Moran *et al.* 2018). Our study also provides some evidence to support the receiver retaliation hypothesis (Euquist 1985, Anderson *et al.* 2012, Akçay *et al.* 2015) as we found that playback of male soft song resulted in significantly stronger aggressive responses than Plain Laughingthrush broadcast songs, and receivers were more inclined to attack the specimen, or retreat from it. Passerine soft songs are typically 'quiet versions' of broadcast songs (Anderson *et al.* 2008, Ręk & Osiejuk 2011), but in the Plain Laughingthrush, there are some structural differences evident between broadcast songs and soft songs (e.g. soft songs are more complex and have longer bouts), so the latter is not simply the quiet version of the former (Anderson *et al.* 2008). Further research (e.g. using passive acoustic recorders at known territorial boundaries) would generate greater sample sizes of soft song recordings that would enable a more detailed analysis of the Plain Laughingthrush soft song structure to determine whether the sound transmission properties of soft song syllables are consistent with predictions of the eavesdropping hypothesis (Dabelsteen *et al.* 1998).

Soft song could be used when individuals intrude into another male's territory, with the low-amplitude vocalizations enabling a male to intrude and mate with the owner's mate, or to conceal difficulty in repelling a rival male from neighbouring males (Searcy & Nowicki 2006, Reichard & Welklin 2015). There is some evidence to suggest that passerine soft vocalizations play a function similar to loud vocalizations, such as to advertise territory ownership and attract mates (Akçay *et al.* 2015). In this study, female Plain Laughingthrushes never responded to soft song playback by approaching first or approaching at a closer distance to the speaker, whereas on two occasions, she responded vocally by emitting a broadcast song. Thus, it is unlikely that the soft song functions as a courtship signal for mate attraction in a similar manner to broadcast songs. In addition, we observed unpaired males singing soft song following occupation of a new territory, and further research is needed to determine whether soft song functions to advertise territorial ownership.

Playback of male soft song provoked a stronger aggressive response than broadcast song, providing evidence that soft song in Plain Laughingthrush is an aggressive signal and provoked aggressive escalation, which is consistent with other non-passerine and passerine species in which only males are known to sing. Our work is the first to investigate the function of soft song in a temperate, monogamous duetting species, and extends the recognition of aggressive territorial signals, and supports the suggestion that soft song is a more widespread signal of aggression in birds.

We thank two anonymous reviewers for their valuable comments. We are grateful for help from Yongzhi Wang and Baoyue Zhang with fieldwork.

AUTHOR CONTRIBUTIONS

Pengfei Liu: Conceptualization (lead); formal analysis (equal); investigation (lead); software (equal); writing – review and editing (equal). **Huw Lloyd:** Visualization (equal); writing – review and editing (equal). **Yingqiang Lou:** Investigation (equal); software (equal). **Yue-Hua Sun:** Funding acquisition (equal); supervision (equal); writing – review and editing (equal).

CONFLICT OF INTEREST

The authors declare that they have no conflicts associated with this study.

ETHICAL NOTE

All birds were ringed under licence from the China Bird Banding Center, approved by the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences (Permission No. 20150069). All fieldwork procedures in this study complied fully with the local and national rules concerning the care and use of animal subjects. Playback trials were conducted only during days of good weather to minimize potential adverse impacts on individuals.

FUNDING

This work was supported by the National Natural Science Foundation of China (grant number 32070452).

Data Availability Statement

The data relating to this research are available on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1.

Audio S1. An audio recording of soft song in a male Plain Laughingthrush *Pterorhinus davidi,* recorded in the study site by Pengfei Liu.

Audio S2. An audio recording of broadcast song of a male Plain Laughingthrush *Pterorhinus davidi,* recorded in the study site by Pengfei Liu.