Maximum frequency of songs reflects body size among male dusky warblers *Phylloscopus fuscatus* (Passeriformes: Phylloscopidae)

J. P. LIU1,2,3, L. K. MA2, Z. Q. ZHANG1, D. H. GU1, J. J. WANG1, J. J. LI3, L. J. GAO3, & J. H. HOU*1

1College of Life Science, Hebei University, PR China, 2College of Life Science, Hainan Normal University, PR China, and 3College of Life Science, Hebei Agricultural University, PR China

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Abstract

Song plays a vital role in communication in songbirds, primarily for territorial defense and mate attraction. Larger animals, having larger vocal organs, produce low sound frequencies more efficiently. Accordingly, the frequency of vocalizations is often negatively correlated with body size across species, and also among individuals of many species, including several non-songbirds. However, little is known about whether song frequency reveals information about body size among males. We tested for the predicted positive relationship between body size and song traits in male dusky warblers (*Phylloscopus fuscatus*). In many animal species, some vocalizations are difficult to produce and can therefore indicate quality of signalers. Male dusky warblers produce songs with trilled sequences that are limited in their timing and frequency structure by physiological constraints on vocal performance. We investigated whether there was a relationship between body size and trilled vocalizations in the dusky warbler. We recorded songs of free-living male dusky warblers at dawn, and captured birds with mist nets for morphometric measurements (tarsus length, as an indicator of body size). We conducted correlation analyses between tarsus length and a composite measure of songs. In dusky warblers, body size was not significantly related to the average frequency of their overall songs, low frequency, bandwidth frequency, average note number of songs, or average song duration. In contrast, the maximum frequency of song was found to correlate positively with tarsus length. For trilled vocalizations, we found most trill traits were not significantly related to body size, and only the maximum frequency of trills was positively related to body size. Additionally, the maximal value of frequency bandwidth decreased with increasing trill rates. Overall, these results suggest that in male dusky warblers, the maximum frequency of the entire song is a reliable indicator of body size.

Keywords: Bird song, body size, dusky warbler, maximum frequency, trill traits

Introduction

Bird song is one of the most widely used means of communication in both oscine and suboscine birds. Songbirds use acoustic signals to elicit courtship and defend territories from rival conspecifics (Catchpole Slater 2008). Therefore, birdsong, as a sexually selected multidimensional signal, can reveal singer information to some extent. It is well known that advertising songs can indicate body size (Hall et al. 2013; Price & Crawford 2013). In many studies, all song characteristics reveal signaler quality through traits such as repertoire size (Soma & Garamszegi 2011; Hesler et al. 2012; Kagawa & Soma 2013), song timing (Poesel et al. 2006), song amplitude (Brumm 2009; Schuchmann & Siemers 2010), song frequency, song bandwidth or song rate (Potvin 2013; Linhart & Fuchs 2015; Mason & Burns 2015).

However, in other studies, many song characteristics failed to predict the body size of songbirds (Brumm 2009; Geberzahn et al. 2009; Miyashita et al. 2016). In these studies, song bandwidths, song rate, average frequency, song amplitude and repertoire size of the total song showed no relationship to body size. Therefore, females are unlikely to assess the size of male birds solely from individual parts of the song, but rather use the total song in their assessment of males. Perhaps some fine-scaled variation in sound production can reflect body size.
Songs of dusky warblers have been studied extensively. Males usually reduce the intensity of singing once they are mated with a female. Mated dusky warblers sing as much before as they do after pairing (Forstmeier & Balsby 2002). The reason that mated dusky warblers continue to sing is to assure within-pair paternity and gain the opportunity for extra-pair copulations. In another study fine-scaled variation in sound amplitude was correlated with both male success in obtaining extra-pair copulations and male longevity (Forstmeier et al. 2002). This indicates that females judge male quality by subtle differences in their performance during the production of song notes, rather than by the quantity or versatility of the song. However, the question remains whether songs themselves or fine-scaled variation within songs correlate with body size.

The dusky warbler produces songs with trilled sequences (Forstmeier & Balsby 2002; Ivanitskii et al. 2012). Ivanitskii et al. (2012) found that 90% of the songs of dusky warblers contained only one trill. In dusky warblers, trilled sequences can be a part of a song, or a complete song in themselves, and they are limited in their timing and frequency structure by the physical constraints of vocal performance. We tested whether trill traits are signals of body size in male dusky warbler.

Podos (2001) suggested that trill rate and frequency bandwidth could be used by birds in mate recognition. Sexually selected signals are reliable in revealing singer quality. These factors led us to focus our attention on trilled sequences within dusky warbler songs. In this study, we first investigated the relationship between trill rate and frequency bandwidth. This was done to assess whether this relationship in dusky warblers was similar to that observed in other species. Second, we investigated the hypothesis that trill traits are related to body size, and that larger males within a species sing songs of higher quality. Higher quality songs have larger bandwidth and faster pitch. Finally, we tested whether trill traits indicate body size.

Materials and methods

Study species

The dusky warbler is a single-brooded, long-distance migratory passerine with a cryptic, sexually monomorphic plumage lacking any obvious visual sexual decoration (Forstmeier et al. 2002). We conducted an investigation of a population of individual male dusky warblers in Saihanba Forest Farm, Hebei Province (42°02′–42°36′N, 116°51′–117°39′E) during July 2015. The dusky warbler lives at high population densities, in bushlands of varying coverage and height, on the banks of the Luan River. During the breeding season males do not guard fertile females, but singing does intensify when their partners are fertile (Forstmeier & Balsby 2002). They spend most of the daytime singing. Dusky warblers sing two distinct kinds of song: S- and V-song. There are differences between S- and V-song: (1) V-song is, on average, produced at a higher rate per minute compared to S-song; (2) in V-song, pauses between strophes may contain series of short calls, and these calls were present in 50% of 107 V-song recordings, but were never heard in S-song; (3) V-song strophes, on average, are longer than S-song strophes, and contain more and a more variable number of syllables; (4) V-song regularly shows syllable-type switches within strophes, that do not occur in S-song; and (5) V-song comprises large individual repertoires (Forstmeier & Balsby 2002). Because different song categories have different structures and functions, it seems important to account for song category in the analysis. For this reason, we only analyzed V-songs.

Song analyses

We recorded male songs between 5:00 a.m. and 10:00 a.m., at a distance not exceeding 10 m around the singing bird in good weather conditions (no wind, no rain). We used a TASCAM DR-680 recorder and a Sennheiser ME67 microphone with a Rycote Softie windshield. Songs were recorded at a 44.1 kHz sampling rate and 16-bit depth. Spectrograms were generated using Raven Pro sound analysis software (version 1.4; Cornell Laboratory of Ornithology, Ithaca, NY). Within Raven, we used a Hann spectrogram window, a DFT size of 512 samples, a hop size of 3.4 ms, a sampling frequency of 44.1 kHz, and a time resolution of 11.6 ms. Measurements were taken by visually cross-referencing the spectrogram and wave-form displays to determine when vocalizations began and ended. Raw measurements included the start and end time as well as the low, high and maximum frequency of each trill and song. Maximum frequency was the frequency at which maximum power occurs in the song. The number of the notes in a trill was visually counted in Raven (Figure 1). For analysis, (1) we defined a trill as a series of notes or note groups repeated in succession, and trill rate as the number of notes per second in a trill; (2) frequency bandwidth was calculated as the difference
between the upper and lower frequency, and duration was the difference between start time and end time (Figure 1); and (3) trill quality was calculated as the product of trill bandwidth and trill rate.

**Body size measurements**

To investigate the relationship between body size and song pitch, 33 males were recorded and measured, between 15 and 20 July 2015. We recorded males for 10 minutes and then lured them into a mist net with playback. We measured tarsus length (± 0.1 mm) 3 times at each capture, and used the average length as an indicator of body size. We used tarsus length as our measure of body size for the following three reasons: (1) it is observed to predict male quality and female choice in some birds (Kempenaers et al. 1992; Möller & Ninni 1998; Hall et al. 2013); (2) it has an observed relationship with call frequency (Price et al. 2006; Brumm 2009; Geberzahn et al. 2009; Linhart & Fuchs 2015); and (3) it is constant in adults.

**Statistical analyses**

We used quantile regression to explore how changes in syllable repetition rate affected the ninetieth
percentile of the frequency bandwidth distribution. We used quantile regression rather than standard linear regression because we were mainly interested in the effects of song features at the tail of the frequency distributions. This analysis was conducted using the quantreg package in R software. For this, 1313 trilled sequences from 33 male dusky warblers were analyzed, with an average of 40 trills per individual. We calculated average values of all songs for each individual, and used Pearson’s correlation to test for a relationship between tarsus length and song traits. Multiple comparisons were required to consider the probability of false positives, so we applied Benjamini–Hochberg procedure to control the false discovery error rate and to correct P values.

### Results

**Relationship between trill rate and frequency bandwidth**

Quantile regression analysis showed that frequency bandwidth correlated negatively with trill rate (Figure 2). Quantile regression for the sample was statistically significant at \( p < 0.05 \), and had a negative slope (\( y = -121.46x + 3672.66, R^2 = 0.05, P = 0.00020 \)).

**Sound traits and body size**

We conducted correlation analyses between tarsus length and a composite measure of songs (Table I). Tarsus length showed no significant relationship with low frequency of songs (Pearson correlation: \( r = -0.188, P = 0.30, P_{critical} = 0.033 \)), bandwidth frequency of songs (Pearson correlation: \( r = -0.164, P = 0.36, P_{critical} = 0.038 \)), average note number of songs (Pearson correlation: \( r = 0.245, P = 0.17, P_{critical} = 0.025 \)), and average song duration (Pearson correlation: \( r = 0.206, P = 0.25, P_{critical} = 0.029 \)). The only significant relationship of tarsus length was with song maximum frequency, which showed a positive correlation (Pearson correlation: \( r = 0.524, P = 0.002, P_{critical} = 0.004 \)).

Data in Table I show that tarsus length also was positively related to average maximum frequency of trills (Pearson correlation: \( r = 0.471, P = 0.006, P_{critical} = 0.008 \)). In this study, for all other trilled vocalization features, we found no significant correlations with tarsus length (Pearson correlation test; low frequency of trills: \( r = -0.411, P = 0.017, P_{critical} = 0.016 \); low frequency of trills: \( r = 0.415, P = 0.016, P_{critical} = 0.013 \); low frequency of trills: \( r = 0.524, P = 0.002, P_{critical} = 0.004 \); low frequency of trills: \( r = 0.245, P = 0.169, P_{critical} = 0.025 \); low frequency of trills: \( r = 0.206, P = 0.251, P_{critical} = 0.029 \)).

**Discussion**

Maximum song frequency was the best predictor of body size, not only in the trill part of the song, but also in the measurement of the entire song. Therefore, we conclude that maximum frequency of whole songs was the best indicator of body size in male warblers. However, an inverse relationship between body size and vocalization pitch has been described in a number of species (Smith & Harper 2004; Koetz et al. 2007; Potvin 2013; Linhart & Fuchs 2015; Mason & Burns 2015). In these studies, low frequency was the best predictor of body size,
which is contrary to our results. The maximum frequency of entire songs was the best predictor of body size in male dusky warblers, likely because the maximum frequency of songs was closer to the upper limit of its potential frequency range. Therefore, this species may use maximum frequency as a relevant sexual signal, and female dusky warblers may prefer males with larger body size that sing at a higher maximum frequency.

Forstmeier & Balsby (2002) revealed that males sing to guard territory and to announce their quality for copulation. Male dusky warbler songs were classified into two distinct types: S-song and V-song. S-song was a territorial signal to mark claimed territory. V-song appeared to attract females for copulation. However, it was not known whether V-song quality was correlated with body size, or how song was correlated with male body size. In another study, Forstmeier et al. (2002) showed that a male’s ability to maintain a high sound amplitude during singing could reflect individual quality, and that amplitude in songs was correlated with male success in obtaining extra-pair copulations. However, it was not known whether the size of extra-pair fathers was bigger than the male they cuckolded.

For the two song types, we studied only the V-song, because V-song or advertising song was used to attract females for copulation. Therefore, this song type is more likely to be correlated with body size. Ivanitskii et al. (2012) made a detailed comparison of syntax in the advertising song of dusky and Radde’s (Ph. schwarzi) warblers, and he found that in Southern Siberia 90% of songs produced by male dusky warblers contained only one trill. That was also the case in our study, because most of the songs of this species contained only one trill, and the trill could be a single song. However, many songs consisted of two parts: a trilled part and a non-trilled part. We expect that trill parts (perhaps difficult to produce) are more likely to reflect body size. Here we have shown that the trilled part was closely related to body size. For the parameters of advertising song of dusky warblers, our study differed from that of Ivanitskii et al. (2012). In our study, advertising songs had a duration of 0.13–3.30 s and a frequency range of 1.22–9.53 kHz, while in the study of Ivanitskii et al. (2012) advertising songs had a duration of 0.7–1.5 s and a frequency range of 1.2–7.6 kHz. Differences in sample size may lead to the difference in song duration and frequency range between the two studies. Ivanitskii et al. (2012) analyzed 24 songs of eight males, while we analyzed 1313 songs of 33 males. The range of time characteristics and frequency characteristics was larger than 11.6 ms and 44.1 Hz. Thus we consider that spectrograph measurement error will have little impact on our conclusions.

Frequency bandwidth was negatively correlated with trill rate. These findings are in agreement with previous studies. There are physiological limits during trilled vocalization production. For example, in hand clapping, hands can be clapped either quickly or loudly, but beyond a certain point one may not produce claps both quickly and loudly. The ability to perform physically challenging songs predicts a male’s quality. Perhaps in the songs of male dusky warblers, trilled parts are more difficult to produce than other variables that are closely related to body size. We found the maximum frequency of trills was positively correlated with body size, and this means that maximum frequency of trills could also be a reliable indicator of body size.

Trill rate, bandwidth and their product were not correlated with tarsus length. This result seems to be contrary to the “trill performance” hypothesis (at least if size reflects quality). One possible explanation is that the sample size was insufficient for detecting a relationship. Perhaps upon enlarging the sample size, there will be an opposite result. Another explanation may be that trill characteristics were used in territorial defense. Thus, advertising songs would not show a relationship with body size, while in territorial songs trill characteristics may be related to body size. Additionally, perhaps trill characteristics reflect body mass or male age. For example, Sprau et al. (2013) found that trill rate, bandwidth, and their product did not significantly predict body size, but predicted age in male nightingales Luscinia megarhynchos. Some studies discovered that frequency bandwidth or trill rate was negatively related to body mass (Tubaro & Mahler 1998; Michael et al. 2012). Lastly, bird song is comprised of multiple components, each of which can be limited by specific constraints. Although body size has been reported to be an important factor in songbird contests, it is still unknown whether tarsus length – used as an indicator of body size in this study – is an appropriate indicator of such constraints. Different studies have used different indicators to describe body size, such as weight, wing length, beak size, or a combination of these body size measures (Searcy 1979; Koivula et al. 1993; Jonart et al. 2007). We hypothesize that different methods of measurement may lead to different results. Additionally, large individuals do not ensure high song quality. Songs of the same individual are not concentrated in one area, and this also suggests that
large individuals only signal that they have the ability to sing high quality songs, and not that they will sing songs of high quality all the time.

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Author contributions

The experiments were conceived and designed by Liu Jianping and Hou Jianhua, and performed by Liu Jianping, Zhang Zhenqun, Gao Lijie, Gu Dehai, Ma Laikun and Li Junjie. The data were analyzed by Liu Jianping, Zhang Zhenqun, Gu Dehai, Ma Laikun and Wang Jiaojiao. Liu Jianping wrote the manuscript.

Ethical standards

Our research was approved by Hebei provincial wildlife administrative institution in Hebei province. For our study, we captured birds in mist nets and then recorded morphometric measurements (tarsus length). No birds were injured and, after measuring body traits, birds were released as soon as possible. Our study did not involve anesthesia, euthanasia or any kind of animal sacrifice. The safety and well-being of the birds was the primary concern at all times.

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