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Song based rival assessment in songbirds

Ph.D. Thesis

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Annotation

This study shows the importance of the basic spectro-temporal song parameters in territorial contests between male songbirds. Chiffchaff and willow warbler were selected as model species for their phylogenetic and ecological similarity, strong territorial behaviour, but very different singing styles. We found that bigger males of both species sing lower pitched songs and that males of both species adjust their behaviour according to song pitch of their rival simulated by playback. Thus, it seems that both species use song pitch to acoustically assess the body size of their rivals. We also show that song length and syllable rate are important features of the song, probably signalling short-term motivation to escalate territorial conflict.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

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Pavel Linhart

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List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

I. **Linhart, P**., Slabbekoorn, H., & Fuchs, R. (2012). The communicative significance of song frequency and song length in territorial chiffchaffs. *Behavioral Ecology*, *23*(6), 1338–1347. doi:10.1093/beheco/ars127 (IF$_{2012}$ = 3.216)
Pavel Linhart was largely involved in experimentla design conception, conducted the field experiments, analysed the data, and substantially contributed to writing of the manuscript.

II. **Linhart, P**., Jaška, P., Petrusková, T., Petrusek, A., & Fuchs, R. (2013). Being angry, singing fast? Signalling of aggressive motivation by syllable rate in a songbird with slow song. *Behavioural Processes*, *100*, 139–145. doi:10.1016/j.beproc.2013.06.012 (IF$_{2012}$ = 1.591)
Pavel Linhart was largely involved in experimentla design conception, helped to conduct the field experiments and to analyse the data, and substantially contributed to writing of the manuscript.

III. **Linhart, P**., & Fuchs, R. Song pitch indicates body size and influences males' response to playback in a songbird. *Animal Behaviour*, submitted.
Pavel Linhart was largely involved in experimentla design conception, conducted the field experiments, analysed the data,and substantially contributed to writing of the manuscript.

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Chapter 1

Introduction, thesis overview, and synthesis

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Song based rival assessment in songbirds

Introduction

Bird song has fascinated people for ages. Since the introduction of tape recorders and spectrographic sound analysis some 60 years ago, this fascination further transformed into serious scientific interest. Bird song has remained an important topic in animal behaviour research ever since providing rich opportunities to test various hypotheses on function and evolution of animal signals (Baker, 2001; Slater, 2003). Bird song is important for intra- as well as for inter-specific communication.

On within species level, the two principal basic functions of bird song were identified – to repel rivals and to attract mates. Both, males and females can potentially extract valuable informations from songs about a singing individual. It has been documented, that male song can be a rich source of information for females. Females could judge different qulities of males to select partners that guarantee the best fitness promise for their offspring. For example, males with long songs, high song rate and rich reprtoire pair earlier and have higher reproduction success (see W A Searcy & Yasukawa, 1996 for a review of song preferences by females).

Males, on the other hand, could profit from correct assesment of fighting abilities / RHP (rersource holding potential) of their rivals (Maynard-Smith & Harper, 2004; Parker, 1974). Males involved in aggressive encounters could for example distingusih whether it is worth to escalete conflict in case the chances of winning are high or stakes are high, or whether they should retreat and spare energy for more suitable occassions (Parker, 1974; Smith & Parker, 1976). Assessment of the rival is apparently a very important aspect of ritualized behaviour sequences preceding fights. On the other hand, males must deal with the fact that ritualized behaviours and signals can provide only estimate of their rival's RHP. It is immediately apparent that disentangling signals and direct confrontation leaves space for uncertainity and even for exaggerating of

RHP by signalling males. In turn, mechanisms ensuring honesty of signals should evolve.

Males and females could use similar strategies and follow very same features when assessing singers or each of the sexes could focus on different aspects of the song revealing an important information for a single sex only. In many cases, song carries multiple messages for multiple receivers (Leitão & Riebel, 2003). For example, females should prefer males possesing the resources that allow successfull breeding and power to defend these resources over breeding period (RHP). But the same trait is also of a crucial importance for males during fights over these resources. But in this case, same trait should repell not attract the other males. It is very likely that for such traits with dual function, female preference for a certain trait is implied by the role of the trait in competition between males (Berglund, Bisazza, & Pilastro, 1996) which is subsequently used for female choice. And it is reasonable to assume that it is the intrasexual context that better ensures reliabillity of these signals through social controll of deception in aggressive situations (Berglund et al., 1996). Studying signals in male-male competition can therefore provide important background information for understanding sexual selection.

When preparing the topic of my PhD thesis, I had an impression that the literature was full of examples for female preferences (Andersson, 1994; e.g. W A Searcy & Yasukawa, 1996), many of which should also play important role in male-male competition, but their function in itrasexual context was far less studied (Berglund et al., 1996). Of course, many nice studies focused on signals in male-male competition in birds and songbirds. Field playback experiments with territorial males became and still are very frequently used to study signal function between males. Teritorial response of males to playback of songs was used to investigate many aspects of male interactions like localization and ranging (estimating the distance from the intruder), recognition of territorial neighbours from strangers, functional aspects of geographic variation in songs, strategies for mediating aggressive interactions - song overlapping, song matching, song swiching, etc. However, the question whether songs could carry information about fighting abilities such as strength, endurance, size, etc. was mostly overlooked.

Also, very little information was available on fucntion of basic song parameters that actually might provide information on some aspects of RHP and fighting abilities like song duration, song amplitude, song rythm and song pitch. Some of these traits were studied early on but mostly in relation with species recognition (Dabelsteen & Pedersen, 1985; e.g. Schubert, 1971), not within the context of male-male interactions. Therfore, I decided to focus the topic of my PhD thesis on simple song features and I asked whether the basic song parameters could provide information about fighting ability of the singer and whether the other males do use this information in aggressive interactions. I especially focused on the question whether the song pitch could work as an index of body size in songbirds.

I focused on the two related species of small songbirds: chiffchaffs *Phylloscopus collybita* and willow warblers *P. trochilus*. The selection of these species was both for theoretical and practical reasons. Theoretical reason was that both species are related, share very similar ecology but have very different songs. Very apparent aspect of willow warbler song is a declining pitch and very low pitched ending part of the song. Therefore, I assumed that the pich might have important signalling function in willow warblers. Chiffchaffs on the other hand, have song that are composed of monotonically repeated alternating syllables. Song length and song rythm could therefore be more important for signalling than pitch in chiffchaffs. Both species also had large populations on the former military training area right in the vicinity of the University ensuring reasonable sample sizes for observations and playback experiments.

Factors affecting RHP

Factors that influence the results of the aggressive encounters are of the two types: 1) Inherent factors of contestants, for example, size, aggressiveness, endurance, strength, etc. Especially, size seems to be a crucial factor affecting RHP and fighting ability in many cases (reviewed in Archer, 1988). 2) Factors associated with the fight context affecting motivation and preparation of both contestants for a fight, for example, knowledge of the environment, prior territory ownership, phase of the breeding cycle, etc. (Jonart, Hill, & Badyaev, 2007). Both, inherent and context factors are important (Hyman, Hughes, Searcy, & Nowicki, 2004). In my disertation work I further focus on the inherent factors.

Body size counts among factors that are crucial for the RHP. This is true especially in case of species that continue to grow over the whole life, like some invertebrates, fish, amphibians, and reptiles. In these species, size differences between young and old males can be very large and have a large impact on fight outcomes (Bradbury & Vehrencamp, 1998). But body size is also important for species with determinated growt like birds and mammals (Bradbury & Vehrencamp, 1998). Accordingly, fighting rituals of many bird and mammals involve behaviours allowing size assessment or exagerating size (Parker, 1974). Contests between red deer stags represent a good example of such behaviours. Males first signal to each other with vigorous roaring. Spectral properties of roars and roaring intensity immediately informs involved males about the RHP (size, weight, age) of their rivals (Clutton-Brock & Albon, 1979; D Reby & McComb, 2003). If both males signall similar RHP and the contest cannot be settled in this stage, males escalate conflict and proceed to parallel walk display allowing size and antler assessment for both males (Clutton-Brock, Albon, Gibson, & Guinness, 1979; Clutton-Brock & Albon, 1979). Only if the the RHP assymetry is not apprent from these ritualized behviours only then males will start fighting. The presence of ritualals preceeding real contests probably results in relatively low number of injuries within a population of red deers (Clutton-Brock et al., 1979). Body size is also an important factor in bird contests (Jonart et al., 2007; e.g. W. Searcy, 1979).

Another factor that could contribute to RHP might be related to the level of aggressive motivation of the male. Aggressivness and boldnes are considered as a valid interconnected personality traits which are useful in some contexts but with potential trade-offs in other contexts (Sih, Bell, & Johnson, 2004). Clearly, agressiveness and boldness could also play an important role in male-male competition, although they are not contributing to RHP directly in most cases. It has been suggested that larger males are likely to escalate conflicts because they judge themselves as having superior RHP. But there is also evidence that aggressivenes and hence willingnes to escalate conflicts can be associated with increase in growth in sheepshead swordtail, *Xiphophorus birchmanni* (Wilson, Grimmer, & Rosenthal, 2013). Aggressiveness thus might not be merely the function of size and might represents independent factor that can affect overall RHP. In sea anemones, *Actinia equina*, the RHP increases with boldness (measured as short startle response) independently on their size (Rudin & Briffa, 2012). It seems that one of the mechanisms how aggressiveness interfere with RHP assessment of self and the others is that it increases self-assessment of own RHP (Tibbetts, Vernier, & Jinn, 2013). Although we generally see the assymetry in RHP of both contestants as the most important for fight outcome, the self assessment (own-RHP) might be more important than we previously thought and could be more relevant than RHP assymetry for many animals (P. . Taylor & Elwood, 2003).

Body size and vocalization frequency

In previous section, body size has been identified as an important component of RHP. I also presented examples from which it is apparent that assessment of body size is an important part of contests between males. Intuitively, rivals can assess each other by visual means as in case of parallel walk of red deers. However, visual assessment of body size requires that the two contestants approach close enough to see each other well and such proximity might already bring serious risks to both males. Because information about body size is often inherently present in animal vocalizations and allows for remote, acoustic sizing of the signallers, vocalizations became an important part of the initial stages of fights (Bradbury & Vehrencamp, 1998).

Mechanisms behind sound production and transmission predict existence of inverse relationship between sound frequency and body size (inverse size-pitch allometry). Generally, the bigger the vibrating and resonating structure, the longer waves (and hence lower frequencies) can be effectively transmitted into the environment (Bradbury & Vehrencamp, 1998; Fletcher, 2004). Body size can be manifested in different spectral properties of vocalizations depending on whether the body size correlates with sound source (vocal folds), or resonant structures (vocal tract), or both of them. Fundamental frequency (F0, frequency at which vocal folds, or similar sound generating tissues vibrate) and formants (the highlighted parts of the spectrum due to resonance of vocal tract) are the most often cited parameters indicating body size (A. M. Taylor & Reby, 2010). Also, dominant or peak frequency (the frequency carrying maximum energy within spectrum), distribution of energy within a spectrum (q25; q50; q75 - frequency thresholds deviding the spectrum in quartiles with equal energy within spectrum), or absolute minimum frequencies are often measured as body size indicators.

Body size can, but does not neccessarily need to, affect vocalization frequency. The crucial condition, of course, is that size of the structures enabling vocalization (vibrating and resonating structures) is associated with overall body size.

The relationship between body size and vocalization frequency is working very well when comparing higher taxonomic units, mostly species, among themselves. Big species use low voices; small species use high voices. In birds, the size-pitch allometry has been studied and found in 150 tropical species by Ryan and Brenowitz (1985); in songbirds by Wallschlager (1980); also, the expected negative correlation between body size and song spectral features has been found in *Phylloscopus* and *Hippolais* genera (Badyaev & Leaf, 1997) and in *Corvus* and *Nucifraga* genera (Laiolo & Rolando, 2003). However, it is unknown whether the size of heterospecifics is of any importance for birds, althought, in case of hearing unknown sound, individuals could judge potential threat by assessing the likely motivation (aggressive, non-agressive) and the size of the signaller and prepare for the encounter. However, individuals might profit much more from assessing the size of their conspecifics with whom they directly compete rather than unknown heterospecifics. This task becomes more challenging because the size differences between species can be much bigger than size differences between individuals within a single species.

The size-pitch allometry within single species was first studied in frogs. In most cases, there was convincing inverse relationship between body size and vocalization pitch (Arak, 1983; Davies & Halliday, 1978). Frogs belong among animals with indeterminated growth where largest males can be commonly more than twice larger than smallest males. Similarly, other taxa with indeterminated growth produce large differences in body size between individuals and accordingly the size-pitch allometry has been revealed in these taxa as well (Clark & Moore, 1995; Hibbitts, Whiting, & Stuart-Fox, 2007; e.g. Ladich, 1998). On the other hand, birds and mammals determinate the growth after they become adult and generally, the differences in size between individuals are smaller than in frogs or other species with undeterminated growth. Therefore the size pitch allometry could be less apparent or missing in these species (Jones & Siemers, 2011; Patel, Mulder, & Cardoso, 2010).

In mammals, the size-pitch allometry has been documented for examples in red deers (D Reby & McComb, 2003), elephant seals (Sanvito, Galimberti, & Miller, 2007), dogs (T. Riede & Fitch, 1999), and some primates including humans (Munhall & Byrne, 2007). Nevertheless, in humans, who were more thoroughly studied, the results are not consistent. Some studies found very good inverse relationship between frequency of vocalizations (Bruckert, Liénard, Lacroix, Kreutzer, & Leboucher, 2006; Evans, Neave, & Wakelin, 2006; W. Tecumseh Fitch & Giedd, 1999), but some others did not (Collins, 2000; González, 2004). There are two possible explanations: 1) Humans are very vocal species with very pronounced vocal plasticity and the relationship does not need to be aparent in every vocalization produced or in every context; and 2) body size is not the primary cause of variance in vocalization frequency, but there is some other factor influencing both body size, and pitch of vocalizations and the result of study depends on actual combination of underlying factors.

Also in birds, there are studies documenting inverse relationship between body size and vocalization frequency that can be found in literature. The inverse relationship between body size and vocalization frequency parameters has been found in little penguins *Euyptula minor* (Miyazaki & Waas, 2003), common loons *Gavia immer* (Mager, Walcott, & Piper, 2007), scops owl *Otus scops* (Hardouin, Tabel, & Bretagnolle, 2006), and corncrakes *Crex Crex* (Osiejuk & Olech, 2004). It seems that negative size-pitch allometry could be also common in non-songbirds. In songbirds, however, there is far less information and the available studies provide controversial findings.

When I started to work on the PhD. project in 2007 there were four studies reporting relationships between size and song spectral features within a songbird species available to me. The results were split exactly in two halves: two studies suported size-pitch allometry (*Geospiza conirostris* Bowman, 1979; *Zonotrichia capensis* Handford & Lougheed, 1991) and two studies contradicted it (*Geospiza difficilis* Bowman, 1979; *Piranga rubra* Shy, 1983). In the last two species, the authors indeed found relationship between body size and spectral song features, but it was a positive one, therfore, in striking contrast to the predicted inverse relationship. In all cases, authors also agreed that other ecological factors (e.g. habitat, etc.) do have greater impact at the song parameters than body size. From these studies it seemed that songbirds, who are vocally very competent taxon, could escape the size-pitch allometry. Nevertheless, there were also studies indicating that song frequency could still be an important factor in male-male contest in songbirds whether backed up by size-pitch allometry or not. For instance, males of *Psarocolius montezuma* use lower pitched songs during singing duels (Price, Earnshaw, & Webster, 2006).

Do males use the information on aspects of RHP in vocalizations during male-male interactions?

In the previous section, I reviewed many published evidences that size of the individual can be manisfested in its vocalization. In this part of the thesis I will briefly review, whether other males do assess the information encoded in vocalizations and act accordingto this information during the male-male interactions. I will mostly refer to the body size again as a prominent factor affecting RHP.

How males assess RHP of their rivals from vocalizations has been mostly studied in frogs. As documented above, the size-pitch allometry in frog vocalizations is common. Further, the bigger male generally wins the contest and hence body size is an essential contributing factor to male's RHP (Arak, 1983; Davies & Halliday, 1978; Wagner, 1989a). Therefore, it is not surprising that males are attantive to vocalizations of other males. In most extreme cases, the playback of low croaking can even cause the other males to draw away from the simulated, big opponent (Arak, 1983; Wagner, 1989b). In other cases, the playback can cause change in calling behaviour in the males receiving playback, for example, males may increase the calling rate and can decrease the pitch of the calling (Bee, Perril, & Owen, 1999). This response is interpreted as a part of a vocal duel in which challenged males are pushing their vocalizations to the limits of their vocal abilities and try to sound as low (and hence appear as big) as possible. Despite the tight correlation between body size and pitch, males generally have some ability to adjust the pitch of their vocalizations. If calling low is demanding, than males would probably use lowest voices only in contexts when it is neccessary like during intrasexual interactions.

In case of *Rana catesbiana*, males surprisingly responded similarly to low and high calls, eventhough there is pitch-size allometry in this species too (Bee, 2002). Therefore, it is apparent, that even in very vocal species possesing the size-pitch allometry, vocalizations do not need to play the crucial role in rival assessment. Definitely, acoustic signals can only contribute to the assessment together with other visual, chemical, and / or tactile stimuli. An excellent example showing the interplay of different aspects of rival assessment is the study by Davies and Halliday (1978). They chosed average-sized, non-paired *Bufo bufo* males. These males were confronted with an established pair (female carrying the male). Males in the pair were either big or small and they were artificially muted. Instead of their own vocalizations, playbacks of high and low calls were used. Single males would fight only with small males presented with high voices playbacks. Big males with either low or high calls played back were challanged only rarely. Also, low call playback seemed to protect the small males from being challanged to some extent. It is likely, that especially species that can modify the pitch of calling must possess other assessment strategies as well to check and get more detailed information about the rival's RHP.

In mammals and birds, the evidence for assessment of rival's body size based on vocalizations was rare in time when I begun working on the thesis. In mammals, red deers roared more often and were more attentive in response to playback of low frequency stimuli (David Reby et al., 2005). Human males rated imaginary men represented by playback of low voices as being physically more dominant and males also tended to increase voice pitch when having conversation with other male whom they perceived as physically dominant (Puts, Gaulin, & Verdolini, 2006).

In birds, males of common loon *Gavia immer* (Mager et al., 2007) and Scops owl *Otus scops* (Hardouin, Reby, Bavoux, Burneleau, & Bretagnolle, 2007) responded diferently to low and high call variants. Common loon males called more in response to low call variants. In contrast, Scops owl males called less in response to playback of low call variant. They further decreased the pitch of calling. Authors also observed approach behaviour and found out that scops owls approached less in response to low stimulus. Different response to the low and high vocalization variants are taken as a general evidence for assessment. It shows that males do perceive the two variants differently.

Other factors affecting vocalization pitch

Vocalization frequency is primarily constrained by the size of vibrating and resonating structures. Generally, we would assume that the size of these structures increases along with increasing body size. However, this does not need to be true.

Humans represent a good example. During the puberty, larynx and the vocal folds go throught a period in which their size grows independently on body size under the influence of increased levels of testosterone. Some studies investigating relationship between body size and vocalization frequency in humans consequently did not find size-pitch relationship, because they focused on analysing fundamental frequency of vocalizations, which reflects vocal fold dimensions and is rather reflecting hormonal state during the puberty and the masculinization level. In contrast, dimensions of vocal tract do correlate with body size. Accordingly, it has been shown that formant spacing (bands of resonant frequencies that are accentuated by vocal tract and depend on vocal tract dimensions) do reflect vocal tract length and hence body size.

In birds, syrinx size can correlate with body size (e.g. Bowman, 1979) and hence vocalizations could indicate their body size. However, in some species correlation between body size and syrinx size is missing and hence frequency of vocalizations is probably determined by other factors too. At least in some species, male calls show a rapid change in vocalization frequency without similar increase of body size, suggesting that frequency of vocalizations is affected significantly by hormonal changes as in case of humans (Klenova, Volodin, & Volodina, 2007; Radford, 2004). Males with high levels of androgens could have lower vocalizations (Cynx, Bean, & Rossman, 2005). Therfore, it is possible that body size does not need to be directly correlated with syrinx size and hence does not need to be a primary factor affecting vocalization frequency in birds. As mentioned above, fundamental frequency is not a single spectral parameter that could be related to size. Dimensions of vocal tract probably correlate with overall body size closer than syrinx size.

Although the importance of the vocal tract has been neglected for a long time in birds, it is now clear that filter (vocal tract) properties affect bird vocalizations substantially (Nowicki, 1987; Tobias Riede, Suthers, Fletcher, & Blevins, 2006). In birds with harsh or harmonically rich vocalizations, formant like structures were found (formants) and their dispersal has been even found to correlate with body size as in mammals (Budka & Osiejuk, 2013). It has been suggested that elongated tracheas of certain bird species could evolve due to sound lower in a similar way like descended larynges in mammals (including humans) could have evolved to better emitt low frequencies (W. T. Fitch, 1999; W. Tecumseh Fitch, 2003).

However, many bird species possess whistle like vocalizations with very high fundamental frequency and steep spectral slope making the formants indetectable and signalling size through formant dispersal inefficient. Despite this limitation, it is clear that vocal tract (esophagus) has very important function in vocal production of these species and their whistle like songs seem to result from close interaction between both syrinx and vocal tract actions (Tobias Riede et al., 2006). However, very little is currently known whether and how size of vocal tract constraints vocalizations of songbirds except that indications have been found that bill size (part of suprasyringeal vocal tract) affect vocalizations pitch (Huber & Podos, 2006).

Other possibilities for RHP assessment from the vocalizations

Despite the fact that vocalization frequency is maybe the most obvious song parameter associated with RHP, it is definitely not the only parameter found to correlate with some aspect contributing to RHP. A number of other song features could be potentially source of the information about RHP or fighting ability. Song length, song amplitude, song rate, syllable rate, or presence of specific costly song structures were also suggested to play a role in assessment of rival's fighting abilities.

From these, song length probably represents a song trait most often associated with male competition qualities in past studies. Lambrechts and Dhondt (1986) were the first who suggested that song length could indicate competition qualities of males. They found that male dominance on feeder correlated with strophe length in Great tit *Parus major*.

A series of studies on hoopoes probably represents the best example available how song length could be associated with aspects of fighting ability in birds. Martin-Vivaldi et al. (2004) carried out playback experiments with hoopoe *Upupa epops*. They compared responses elicited by long and short cooing sequences. Interestingly, authors did not find expected difference in response to the two stimulus treatments. However, they found that the intensity of response increased positively with the average length of the spopntaneous coo sequence of tested males. Males singing longer coo sequences approached closer to loudspeaker and they sang unfinished coo sequences. Males with short spontaneous coo sequences stayed away form loudspeakers and responded mainly by singing. Length of the coo sequences has been further found to correlate with male condition (M Martín-Vivaldi, Palomino, & Soler, 1998) and long coo sequences are also attractive for females (Manuel Martín-Vivaldi, Martínez, Palomino, & Soler, 2002; Manuel Martín-Vivaldi, Palomino, & Soler, 2000).

Vocalization duration could be constrained by body size as bigger lungs would provide larger air volume and hence could sustain phonation longer (W. Tecumseh Fitch, 2003). Besides body size alone longer vocalizations could also reflect large aerobic capacity (W. Tecumseh Fitch, 2003).

Although it is generally not known whether and how song length is affected by the body size or by the size of organs participating in song production, song length has been shown to correlate with many different male qualities like age, condition, parasite load and even cognitive abilities. Older males had longer songs in starlings *Sturnus vulgaris* (Eens, Pinxten, & Verheyen, 1991) and in barn swallows *Hirundo rustica* (Garamszegi, Heylen, Moller, Eens, & de Lope, 2005). However, other studies do not show such relationship including a study on willow warblers (Gil, Cobb, & Slater, 2001). Comparing results from cross-sectional and longitudinal analyses, it seems that songs do not become longer with age but males with longer songs survive (Garamszegi et al., 2005). Song length might also indicate condition as it has been show in hoopoes (M Martín-Vivaldi et al., 1998) but it seems that song length is not affected by condition directly because song length, unlike song rate, does not increase in diet supplemented Australian reed warbler males *Acrocephalus australis* (Berg, Beintema, Welbergen, & Komdeur, 2005) and hence seems not to indicate actual condition. Song length decreases with chewing louse load in barn swallows (Garamszegi et al., 2005) and call length decreases with parasite load in tawn owls (Appleby & Redpath, 1997). Interestingly, song length correlates with certain cognitive abilities namely with spatial learning in starlings (Farrell, Weaver, An, & MacDougall-Shackleton, 2012).

Song length is also important in male-male interactions as suggested by several lines of evidence. First, males manipulate the song length during vocal contests and fights. However, studies with conflicting results were published. If song length indicates song quality than males would be expected to sing longer songs when challenged. This has been found in dark-eyed juncos *Junco hyemalis* (G C Cardoso, Atwell, Ketterson, & Price, 2009). On the other hand, some species, like the white-crowned sparrow *Zonotrichia leucophrys,* shorten the songs when challanged (Nelson & Poesel, 2012), and other species were reported to give short songs when highly aroused or prior to attack (bobolink: Capp & Searcy, 1991; willow warbler: Jarvi, Radesater, & Jakobsson, 1980) suggesting that short song could be signal of upcoming escalated aggression.

There is also a number of studies showing that song length is important for receivers. In this case, the results prevalently agree on males responding more intensely (closer approach, more flights, attacks, singing) to playback of long songs (Adhikerana & Slater, 1993; Balsby & Dabelsteen, 2001; Lattin & Ritchison, 2009; Nelson & Poesel, 2012; Ríos-Chelén & Garcia, 2007). In few studies, playback song length did not seem to have significant effect on males’ responses (Langemann, Tavares, Peake, & McGregor, 2000; McGregor & Horn, 1992). In these cases song length still could be important though the different mechanism as males with longer songs may respond more intensely (M Martín-Vivaldi et al., 2004; McGregor & Horn, 1992).

From the overview above it is clear that despite solid body of studies suggesting a role of song length in male-male competition, good examples linking the response in playback experiments with specific male qualities important in male-male competitions are almost missing. Most studies showing different response to long and short songs did not link the song duration with some aspect of male quality and in studies that found correlations of song length with male qualities, it is not clear whether and how these qualities affect male-male competition. Especially, very few studies addressed the primary factors theoretically constraining vocalization duration like body size.

Regarding the nature of the chiffchaff song that consists of a series of rythmically repeated syllables, it seemed possible that syllable rate (number of song notes per time interval) could also play role in signalling. Nevertheless, very few studies have been published on the signalling role of syllable rate. As high syllable rates require precise motor coordination, it has been suggested that syllable rate could be an index of male qualities (Podos, 1996; Suthers, Goller, & Pytte, 1999; Vallet & Kreutzer, 1995). Nevertheless, syllable rate has been rarely studied in context of male-male competition or female choice. At the time when I started to work on the PhD thesis, Cardoso et al. (2007) published a work in which they suggest that in serin *Serinus serinus* the very high syllable rate could evolve due to function in male-male competition. Females seemed to be intimidated by songs with increased syllable rates.

Some works did not directly assessed the importance of syllable rate but they considered it as a part of vocal performance. Podos suggested that there is a trade off between syllable rate and bandwidth - production of syllables with wide bandwidths require significant adjustments of vocal tract (low sound = closed bill; high sound = wide opened bill) which becomes more and more challenging with increasing rate of syllable repetitions in fast trills. Podos suggested that there is negative correlation between syllable rate and frequency bandwidth and upper limit exists that determines maximum syllable rate for a particular syllable bandwidth. The concept has been applied within species and vocal deviation (distance from the syllable rate per bandwidth singing) has been introduced as a vocal performance measure. It has been shown that females prefer males with high song performance and that other males respond differently to songs differing in their song performance.

Despite the apparent usability of vocal performance, this measure has its disadvantages. It is composed from the two traits and hence it is not always clear whether both measures are neccessary to explain results as contributions of syllable rate and bandwidth are unclear. Cardoso et al.

(2007) suggested that bandwidth could play a minor role in signalling process as longer syllables (naturally repated in slower rate than short syllables) can accumulate more frequency modulations (and hence larger bandwidths) just due to the fact that they are longer. Moreover, it is well known that bandwidth can be very much affected by the degradation during sound transmission through the environment. Hence, it might be difficult for males and females take the bandwidth into account when assessing the singer as bandwidth would vary with distance from the signaller. On the other hand, syllable rate is very robust to degradations and could be more suitable as a signal of male quality.

Thesis overview and synthesis

The main aim of the thesis was to test whether there is a relationship between the song frequency and body size of the males in two songbird species - the chiffchaff and willow warbler - and whether males could use this acoustic information about body size of their rivals during territorial contests. I expected that there will be inverse size-pitch allometry in willow warblers because their songs have conspicuous decreasing pitch pattern and the minimum frequencies can get very low (cca 1800 Hz) relative to their body size. On the other hand, I expected that chiffchaffs could use different strategy to assess their rivals because song duration and song rythm are more conspicuous features of their songs. The possible role of song length and syllable rate was therefore tested in chiffchaffs.

The thesis consists of three chapters. Chapters follow in a chronological order in which the data were collected and do not correspond to order in which they were published. Despite the fact that I had good idea about the questions of my PhD thesis the methodical approach developed over the course of the three studies reflecting increasing experience in bioacoustics field in which I started without significant previous experience.

First chapter deals with size pitch allometry in willow warblers and reports the first results on size-pitch allometry in willow warbler and results of playback experiments. I planned to get correlative data to show possible relationships between song pitch and body size as well as playback experiments that would show how willow warblers perceive the pitch in the intruder's songs. We show that there indeed is inverse relationship between song pitch and tarsus length (a proxy for body size). Moreover, the relationship seeems to be quite strong compared to the other birds and songbirds (Patel et al., 2010). We also found that response strength in playback experiments correlates with assymetry in song pitch between intruder and resident male. Males approached closer the lower were their songs compared to the songs of our playback stimuli. Assuming song pitch reflects body size, the bigger was the perceived size difference between resident male and simulated intruder in favor of resident male, the bolder response we got (closer approach). The results fit very well in hypothesis that body size is reflected in songs and perceived by males.

The second chapter basically replicates similar results on chiffchaff. Surprisingly, we also found size-pitch allometry in songs of chiffchaffs. In case of chiffchaffs, the design of playback experiments was much more complicated. As we doubted about signalling role of song pitch in chiffchaffs we decided to test two song parameters: song length and song frequency at once. We also did not wanted to rely on simple correlation of response strength and song features of stimuli and/or resident males as in case of willow warblers. Therefore, we prepared four experimental treatments in a factorial setting and tested whether song duration (experimentally elongated and shortened songs), song frequency (experimentally increased and decreased frequency) and their combinations have an impact on response strength. Interestingly we found that it might be the interaction between song length and song frequency that matters to resident males. They responded intensively (attacks) to elongated songs. Approach response was intriguing. Males approached close only in response to elongated high pitched songs. They kept further off loudspeaker during playback of elongated low pitched songs, which might indicate that decreased pitch hindered approach response. Further, we considered and compared different other models that implement relative differences (assymetries) in song length and song frequency of resident males and stimuli and we found out that response based on relative differences was more appropriate for song pitch but was not appropriate for song length. In other words males did seem to care whether the stimulus sung songs that were higher or lower pitched than their own songs but they did not do so in case of song length. We conlcuded that chiffchaffs responded to variation in song pitch as it would signal body size and hence fighting ability, because low pitched song hindered approach and response to pitch seemed to be driven by assymetries in song pitch as it would be expected in case of size. Apparently, song length is important song feature in context of male aggressive interactions. However, we were not able to link song length with body size or any other factor influencing fighting ability. We concluded that it might signal aggressive motivation.

Both chapters brought interesting results. First, the two studies bring important evidence that even at the within species level, body size can be reflected in songs of songbirds. Very few studies have been published on the topic but recently the interest increases. The size pitch allometry has been reported from few other songbird species during the course of my PhD (Hall, Kingma, & Peters, 2013; Turcokova, Osiejuk, Pavel, Glapan, & Petruskova, 2010) but not in some other species (Goncalo C. Cardoso et al., 2008). Our two studies also belong to very few examples examining the signalling function of song pitch as body size indicator in male-male competition. Recent review pointed out that birds represent a uniqe opportunity to study diversification of signal function, because in many species of birds high pitched vocalizations are more relevent in context of sexual selection (signal dominance or are prefered by females) than low pitched vocalizations as it would be expected (Goncalo C. Cardoso, 2012). It seems that supressed competition between males and female preference for high pitched songs in some species could relax inverse size-pitch allometry (Goncalo C. Cardoso, 2012). However, more studies on signalling function of song pitch are needed to make conclusions on causes for evolution of such diversification in signalling role of song pitch, especially for songbirds, as this topic still remains understudied.

In the last chapter, we investigate signalling role of the third prominent basic parameter (besides the pitch and song length) of chiffchaff song - syllable rate. During previous studies we noticed that syllable rate can increase in aggressive context which we tested and proved in this last study. We again did playback experiments and presented three variants of syllable rate. In comparison with the approach in the second chapter, we manipulated syllable rate (fast, slow, and control = non-manipulated treatments) to extreme values present in the spontanous songs in our population. In previous study we manipulated the song parameters in order of standard deviation up or down. Manipulation in order of one standard deviation gives more natural setting but also brings problems, for instance it probably decreases the effect of the treatment and complicates analysis and interpretation of results. Due to variation in parameters of stimulus songs and tested males it can happen that some birds will get songs of increased duration but which are actually same or even shorter than resident's songs. For these reasons we changed our approach and manipulated the syllable rate more dramatically.

Males were generally able to substantially increase their syllable rate in aggressive context of simulated territorial intrusion. They apparently differentiated between fast and slow songs as they increased syllable rate in response to fast playback but not in response to slow playback. And males that attacked loudspeaker during playback had faster spontaneous syllable rate. These facts lead us to conlcude that syllable rate plays a role in male-male interactions and it conforms the criteria for agressive signal (William A. Searcy & Beecher, 2009).

The studies on signallling role of syllable rate were almost missing until recently. Recently, there is an increase of interest in signalling function of syllable rate. As in chiffchaffs, the other studies found increase of syllable rate in aggressive context and different response to fast and slow song variants. However, these studies were conducted on species with very fast syllable rates that likely underwent sexual selection for this trait. Our study shows that syllable rate could be more generall signall of aggressive motivation. The possible reasons still need to be explored.

The thesis focused on signalling function of simple song parameters. It brought some interesting results. I show that very simple spectral and temporal song parameters can have important roles in acoustic communication of birds. Studying of these parameters has drawbacks and adventages over more detailed studies of strategic use of repertoires. One important advenatge is that they can be easily compared between species and comparative studies can shed more light on evolution processes.

From my research and also from research of other people it is possible to conclude that size-pitch allometry can be found in songbirds. Due to their unusual vocal plasticity, determinated growth and small size differences between individuals the mere existence of size-pitch allometry is unexpected and puzzling in songbirds. It is stil not very clear whether the allometry really results from constraints imposed by sound production principles or emerges through interplay of some other key factors.

References

Adhikerana, A. S., & Slater, P. J. B. (1993). Singing interactions in coal tits, *Parus ater*: an experimental approach. *Animal Behaviour*, *46*(6), 1205–1211. doi:10.1006/anbe.1993.1311

Andersson, M. B. (1994). *Sexual Selection*. Princeton University Press.

Appleby, B. M., & Redpath, S. M. (1997). Indicators of male quality in the hoots of Tawny Owls (*Strix aluco*). *Journal of Raptor Research*, *31*, 65–70.

Arak, A. (1983). Sexual selection by male–male competition in natterjack toad choruses. *Nature*, *306*(5940), 261–262. doi:10.1038/306261a0

Archer, J. (1988). *The Behavioural Biology of Aggression*. CUP Archive.

Badyaev, A. V., & Leaf, E. S. (1997). Habitat associations of song characteristics in *Phylloscopus* and *Hippolais* warblers. *Auk*, *114*(1), 40–46.

Baker, M. C. (2001). Bird Song Research: The Past 100 Years. *Bird Behavior*, *14*(1), 3–50.

Balsby, T. J. S., & Dabelsteen, T. (2001). The meaning of song repertoire size and song length to male whitethroats *Sylvia communis*. *Behavioural Processes*, *56*(2), 75–84.

Bee, M. A. (2002). Territorial male bullfrogs (Rana catesbeiana) do not assess fighting ability based on size-related variation in acoustic signals. *Behavioral Ecology*, *13*(1), 109–124. doi:10.1093/beheco/13.1.109

Bee, M. A., Perril, S. A., & Owen, P. C. (1999). Size assessment in simulated territorial encounters between male green frogs (Rana clamitans), *45*(3), 177–184. doi:10.1007/s002650050551

Berg, M. L., Beintema, N. H., Welbergen, J. A., & Komdeur, J. (2005). Singing as a handicap: the effects of food availability and weather on song output in the Australian reed warbler Acrocephalus australis. *Journal of Avian Biology*, *36*(2), 102–109. doi:10.1111/j.0908-8857.2005.03285.x

Berglund, A., Bisazza, A., & Pilastro, A. (1996). Armaments and ornaments: an evolutionary explanation of traits of dual Utility. *Biological Journal of the Linnean Society*, *58*(4), 385–399. doi:10.1111/j.1095-8312.1996.tb01442.x

Bowman, R. I. (1979). Adaptive morphology of song dialects in Darwin's finches. *Journal of Ornithology*, *120*(4), 353–389. doi:10.1007/BF01642911

Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of Animal Communication* (First edition.). Sunderland: Sinauer Associates, Inc.

Bruckert, L., Liénard, J.-S., Lacroix, A., Kreutzer, M., & Leboucher, G. (2006). Women use voice parameters to assess men's characteristics. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1582), 83–89. doi:10.1098/rspb.2005.3265

Budka, M., & Osiejuk, T. S. (2013). Formant Frequencies are Acoustic Cues to Caller Discrimination and are a Weak Indicator of the Body Size of Corncrake Males. *Ethology*, *119*(11), 960–969. doi:10.1111/eth.12141

Capp, M. S., & Searcy, W. A. (1991). Acoustical communication of aggressive intentions by territorial male bobolinks. *Behavioral Ecology*, *2*(4), 319–326. doi:10.1093/beheco/2.4.319

Cardoso, G. C. (2012). Paradoxical calls: the opposite signaling role of sound frequency across bird species. *Behavioral Ecology*, *23*(2), 237–241. doi:10.1093/beheco/arr200

Cardoso, G. C., Atwell, J. W., Ketterson, E. D., & Price, T. D. (2007). Inferring performance in the songs of dark-eyed juncos (Junco hyemalis). *Behavioral Ecology*, *18*(6), 1051–1057. doi:10.1093/beheco/arm078

Cardoso, G. C., Atwell, J. W., Ketterson, E. D., & Price, T. D. (2009). Song types, song performance, and the use of repertoires in dark-eyed juncos (*Junco hyemalis*). *Behavioral Ecology*, *20*(4), 901–907. doi:10.1093/beheco/arp079

Cardoso, G. C., Mamede, A. T., Atwell, J. W., Mota, P. G., Ketterson, E. D., & Price, T. D. (2008). Song Frequency Does Not Reflect Differences in Body Size among Males in Two Oscine Species. *Ethology*, *114*(11), 1084–1093. doi:10.1111/j.1439-0310.2008.01552.x

Cardoso, G. C., Mota, P. G., & Depraz, V. (2007). Female and male serins (Serinus serinus) respond differently to derived song traits. *Behavioral Ecology and Sociobiology*, *61*(9), 1425–1436. doi:10.1007/s00265-007-0375-5

Clark, D. C., & Moore, A. J. (1995). Variation and repeatability of male agonistic hiss characteristics and their relationship to social rank in Gromphadorhina portentosa. *Animal Behaviour*, *50*(3), 719–729. doi:10.1016/0003-3472(95)80132-4

Clutton-Brock, T. H., & Albon, S. D. (1979). The Roaring of Red Deer and the Evolution of Honest Advertisement. *Behaviour*, *69*(3-4), 145–170.

Clutton-Brock, T. H., Albon, S. D., Gibson, R. M., & Guinness, F. E. (1979). The logical stag: Adaptive aspects of fighting in red deer (Cervus elaphus L.). *Animal Behaviour*, *27, Part 1*, 211–225. doi:10.1016/0003-3472(79)90141-6

Collins, S. A. (2000). Men's voices and women's choices. *Animal Behaviour*, *60*(6), 773–780. doi:10.1006/anbe.2000.1523

Cynx, J., Bean, N. J., & Rossman, I. (2005). Testosterone implants alter the frequency range of zebra finch songs. *Hormones and Behavior*, *47*(4), 446–451. doi:10.1016/j.yhbeh.2004.11.018

Dabelsteen, T., & Pedersen, S. B. (1985). Correspondence between Messages in the Full Song of the Blackbird Turdus merula and Meanings to Territorial Males, as Inferred from Responses to Computerized Modifications of Natural Song. *Zeitschrift Für Tierpsychologie*, *69*(2), 149–165. doi:10.1111/j.1439-0310.1985.tb00142.x

Davies, N. B., & Halliday, T. R. (1978). Deep croaks and fighting assessment in toads *Bufo bufo*. *Nature*, *274*(5672), 683–685. doi:10.1038/274683a0

Eens, M., Pinxten, R., & Verheyen, R. F. (1991). Male song as a cue for mate choice in the European starling. *Behaviour*, *116*, 210–238. doi:10.1163/156853991X00049

Evans, S., Neave, N., & Wakelin, D. (2006). Relationships between vocal characteristics and body size and shape in human males: An evolutionary explanation for a deep male voice. *Biological Psychology*, *72*(2), 160–163. doi:10.1016/j.biopsycho.2005.09.003

Farrell, T. M., Weaver, K., An, Y.-S., & MacDougall-Shackleton, S. A. (2012). Song bout length is indicative of spatial learning in European starlings. *Behavioral Ecology*, *23*(1), 101–111. doi:10.1093/beheco/arr162

Fitch, W. T. (1999). Acoustic exaggeration of size in birds via tracheal elongation: comparative and theoretical analyses. *Journal of Zoology*, *248*(1), 31–48. doi:10.1111/j.1469-7998.1999.tb01020.x

Fitch, W. T. (2003). Unpacking "honesty": vertebrate vocal production and the evolution of acoustic signals. In R. R. Fay & A. N. Popper (Ed.), *Acoustic communication* (s. 65–137). New York: Springer-Verlag.

Fitch, W. T., & Giedd, J. (1999). Morphology and development of the human vocal tract: A study using magnetic resonance imaging. *The Journal of the Acoustical Society of America*, *106*(3), 1511–1522. doi:10.1121/1.427148

Fletcher, N. H. (2004). A simple frequency-scaling rule for animal communication. *The Journal of the Acoustical Society of America*, *115*(5 Pt 1), 2334–2338.

Garamszegi, L., Heylen, D., Moller, A., Eens, M., & de Lope, F. (2005). Age-dependent health status and song characteristics in the barn swallow. *Behavioral Ecology*, *16*(3), 580–591. doi:10.1093/beheco/ari029

Gil, D., Cobb, J., & Slater, P. (2001). Song characteristics are age dependent in the willow warbler, Phylloscopus trochilus. *Animal Behaviour*, *62*, 689–694.

González, J. (2004). Formant frequencies and body size of speaker: a weak relationship in adult humans. *Journal of Phonetics*, (2), 277–287. doi:10.1016/S0095-4470(03)00049-4

Hall, M. L., Kingma, S. A., & Peters, A. (2013). Male Songbird Indicates Body Size with Low-Pitched Advertising Songs. *PLoS ONE*, *8*(2), e56717. doi:10.1371/journal.pone.0056717

Handford, P., & Lougheed, S. C. (1991). Variation in duration and frequency characters in the song of the rufous-collared sparrow, *Zonotrichia capensis*, with respect to habitat, trill dialects, and bodysize. *Condor*, *93*(3), 644–658. doi:10.2307/1368196

Hardouin, L. A., Reby, D., Bavoux, C., Burneleau, G., & Bretagnolle, V. (2007). Communication of male quality in owl hoots. *The American Naturalist*, *169*(4), 552–562. doi:10.1086/512136

Hardouin, L. A., Tabel, P., & Bretagnolle, V. (2006). Neighbour–stranger discrimination in the little owl, Athene noctua. *Animal Behaviour*, *72*(1), 105–112. doi:10.1016/j.anbehav.2005.09.020

Hibbitts, T. J., Whiting, M. J., & Stuart-Fox, D. M. (2007). Shouting the odds: vocalization signals status in a lizard. *Behavioral Ecology and Sociobiology*, *61*(8), 1169–1176. doi:10.1007/s00265-006-0330-x

Huber, S. K., & Podos, J. (2006). Beak morphology and song features covary in a population of Darwin's finches (Geospiza fortis). *Biological Journal of the Linnean Society*, *88*(3), 489–498. doi:10.1111/j.1095-8312.2006.00638.x

Hyman, J., Hughes, M., Searcy, W. A., & Nowicki, S. (2004). Individual variation in the strength of territory defense in male song sparrows: Correlates of age, territory tenure, and neighbor aggressiveness. *Behaviour*, *141*, 15–27. doi:10.1163/156853904772746574

Jarvi, T., Radesater, T., & Jakobsson, S. (1980). The song of the willow warbler *Phylloscopus trochilus* with special reference to singing behavior in agonistic situations. *Ornis Scandinavica*, *11*(3), 236–242. doi:10.2307/3676129

Jonart, L. M., Hill, G. E., & Badyaev, A. V. (2007). Fighting ability and motivation: determinants of dominance and contest strategies in females of a passerine bird. *Animal Behaviour*, *74*(6), 1675–1681. doi:10.1016/j.anbehav.2007.03.012

Jones, G., & Siemers, B. M. (2011). The communicative potential of bat echolocation pulses. *Journal of Comparative Physiology A*, *197*(5), 447–457. doi:10.1007/s00359-010-0565-x

Klenova, A. V., Volodin, I. A., & Volodina, E. V. (2007). The vocal development of the Red-crowned Crane Grus japonensis. *Ornithological Science*, *6*(2), 107–119. doi:10.2326/1347-0558(2007)6[107:TVDOTR]2.0.CO;2

Ladich, F. (1998). Sound Characteristics and Outcome of Contests in Male Croaking Gouramis (Teleostei). *Ethology*, *104*(6), 517–529. doi:10.1111/j.1439-0310.1998.tb00087.x

Laiolo, P., & Rolando, A. (2003). The evolution of vocalisations in the genus Corvus: effects of phylogeny, morphology and habitat. *Evolutionary Ecology*, *17*(2), 111–123. doi:10.1023/A:1023003110969

Lambrechts, M., & Dhondt, A. A. (1986). Male quality, reproduction, and survival in the Great tit (*Parus major*). *Behavioral Ecology and Sociobiology*, *19*(1), 57–63.

Langemann, U., Tavares, J. P., Peake, T. M., & McGregor, P. K. (2000). Response of great tits to escalating patterns of playback. *Behaviour*, *137*, 451–471. doi:10.1163/156853900502178

Lattin, C., & Ritchison, G. (2009). Intra- and intersexual functions of singing by male blue grosbeaks: the role of within-song variation. *The Wilson Journal of Ornithology*, *121*(4), 714–721. doi:10.1676/09-026.1

Leitão, A., & Riebel, K. (2003). Are good ornaments bad armaments? Male chaffinch perception of songs with varying flourish length. *Animal Behaviour*, *66*(1), 161–167. doi:10.1006/anbe.2003.2167

Mager, J. N., Walcott, C., & Piper, W. H. (2007). Male common loons, Gavia immer, communicate body mass and condition through dominant frequencies of territorial yodels. *Animal Behaviour*, *73*, 683–690. doi:10.1016/j.anbehav.2006.10.009

Martín-Vivaldi, M., Martínez, J. G., Palomino, J. J., & Soler, M. (2002). Extrapair paternity in the Hoopoe Upupa epops: an exploration of the influence of interactions between breeding pairs, non-pair males and strophe length. *Ibis*, *144*(2), 236–247. doi:10.1046/j.1474-919X.2002.00044.x

Martín-Vivaldi, M., Palomino, J. J., & Soler, M. (1998). Song structure in the Hoopoe (*Upupa epops*) - Strophe length reflects male condition. *Journal Fur Ornithologie*, *139*(3), 287–296. doi:10.1007/BF01653339

Martín-Vivaldi, M., Palomino, J. J., & Soler, M. (2000). Attraction of Hoopoe Upupa epops females and males by means of song playback in the field: influence of strophe length. *Journal of Avian Biology*, *31*(3), 351–359. doi:10.1034/j.1600-048X.2000.310311.x

Martín-Vivaldi, M., Palomino, J. J., & Soler, M. (2004). Strophe length in spontaneous songs predicts male response to playback in the hoopoe *Upupa epops*. *Ethology*, *110*(5), 351–362.

Maynard-Smith, J., & Harper, D. (2004). *Animal Signals* (1St Edition edition.). New York: Oxford University Press.

McGregor, P. K., & Horn, A. G. (1992). Strophe length and response to playback in great tits. *Animal Behaviour*, *43*(4), 667–676. doi:10.1016/S0003-3472(05)81025-5

Miyazaki, M., & Waas, J. R. (2003). Acoustic properties of male advertisement and their impact on female responsiveness in little penguins Eudyptula minor. *Journal of Avian Biology*, *34*(3), 229–232. doi:10.1034/j.1600-048X.2003.03099.x

Munhall, K. G., & Byrne, S. K. (2007). Animal Communication: Big Talkers and Small Talk. *Current Biology*, *17*(7), R247–R249. doi:10.1016/j.cub.2007.02.007

Nelson, D. A., & Poesel, A. (2012). Responses to Variation in Song Length by Male White-Crowned Sparrows. *Ethology*, *118*(1), 24–32. doi:10.1111/j.1439-0310.2011.01979.x

Nowicki, S. (1987). Vocal tract resonances in oscine bird sound production: evidence from birdsongs in a helium atmosphere. *Nature*, *325*(6099), 53–55. doi:10.1038/325053a0

Osiejuk, T. S., & Olech, B. (2004). Amplitude spectra of corncrake calls: what do they signalise? *Animal Biology*, *54*(2), 207–220. doi:10.1163/1570756041445218

Parker, G. A. (1974). Assessment strategy and the evolution of fighting behaviour. *Journal of Theoretical Biology*, *47*(1), 223–243. doi:10.1016/0022-5193(74)90111-8

Patel, R., Mulder, R. A., & Cardoso, G. C. (2010). What Makes Vocalisation Frequency an Unreliable Signal of Body Size in Birds? A Study on Black Swans. *Ethology*, *116*(6), 554–563. doi:10.1111/j.1439-0310.2010.01769.x

Podos, J. (1996). Motor constraints on vocal development in a songbird. *Animal Behaviour*, *51*, 1061–1070.

Price, J. J., Earnshaw, S. M., & Webster, M. S. (2006). Montezuma oropendolas modify a component of song constrained by body size during vocal contests. *Animal Behaviour*, *71*, 799–807. doi:10.1016/j.anbehav.2005.05.025

Puts, D. A., Gaulin, S. J. C., & Verdolini, K. (2006). Dominance and the evolution of sexual dimorphism in human voice pitch. *Evolution and Human Behavior*, *27*(4), 283–296. doi:10.1016/j.evolhumbehav.2005.11.003

Radford, A. N. (2004). Voice breaking in males results in sexual dimorphism of green woodhoopoe calls. *Behaviour*, *141*, 555–569. doi:10.1163/1568539041166726

Reby, D., & McComb, K. (2003). Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. *Animal Behaviour*, *65*, 519–530. doi:10.1006/anbe.2003.2078

Reby, D., McComb, K., Cargnelutti, B., Darwin, C., Fitch, W. T., & Clutton-Brock, T. (2005). Red deer stags use formants as assessment cues during intrasexual agonistic interactions. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1566), 941–947. doi:10.1098/rspb.2004.2954

Riede, T., & Fitch, T. (1999). Vocal tract length and acoustics of vocalization in the domestic dog (Canis familiaris). *The Journal of Experimental Biology*, *202*(Pt 20), 2859–2867.

Riede, T., Suthers, R. A., Fletcher, N. H., & Blevins, W. E. (2006). Songbirds tune their vocal tract to the fundamental frequency of their song. *Proceedings of the National Academy of Sciences*, *103*(14), 5543–5548. doi:10.1073/pnas.0601262103

Ríos-Chelén, A. A., & Garcia, C. M. (2007). Responses of a sub-oscine bird during playback: effects of different song variants and breeding period. *Behavioural Processes*, *74*(3), 319–325. doi:10.1016/j.beproc.2006.11.007

Rudin, F. S., & Briffa, M. (2012). Is boldness a resource-holding potential trait? Fighting prowess and changes in startle response in the sea anemone, Actinia equina. *Proceedings of the Royal Society B-Biological Sciences*, *279*(1735), 1904–1910. doi:10.1098/rspb.2011.2418

Ryan, M. J., & Brenowitz, E. A. (1985). The role of body size, phylogeny, and ambient noise in the evolution of bird song. *American Naturalist*, *126*(1), 87–100.

Sanvito, S., Galimberti, F., & Miller, E. H. (2007). Vocal signalling of male southern elephant seals is honest but imprecise. *Animal Behaviour*, *73*(2), 287–299. doi:10.1016/j.anbehav.2006.08.005

Searcy, W. (1979). Morphological Correlates of Dominance in Captive Male Red-Winged Blackbirds. *Condor*, *81*(4), 417–420. doi:10.2307/1366971

Searcy, W. A., & Beecher, M. D. (2009). Song as an aggressive signal in songbirds. *Animal Behaviour*, *78*(6), 1281–1292. doi:10.1016/j.anbehav.2009.08.011

Searcy, W. A., & Yasukawa, K. (1996). Song and female choice. In D. E. Kroodsma & E. H. Miller (Ed.), *Ecology and Evolution of Acoustic Communication in Birds*. Ithaca, N.Y.: Comstock Publishing.

Shy, E. (1983). The relation of geographica variation in song to habitat characteristics and body size in north-american tanagers (Thraupinae, *Piranga*). *Behavioral Ecology and Sociobiology*, *12*(1), 71–76. doi:10.1007/BF00296935

Schubert, G. (1971). Experimentelle Untersuchungen Über Die Artkennzeichnenden Parameter Im Gesang Des Zilpzalps, Phylloscopus C. Collybita (Vieillot). *Behaviour*, *38*(3), 289–314. doi:10.1163/156853971X00113

Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, *19*(7), 372–378.

Slater, P. J. B. (2003). Fifty years of bird song research: a case study in animal behaviour. *Animal Behaviour*, *65*(4), 633–639. doi:10.1006/anbe.2003.2051

Smith, J. M., & Parker, G. A. (1976). The logic of asymmetric contests. *Animal Behaviour*, *24*(1), 159–175. doi:10.1016/S0003-3472(76)80110-8

Suthers, R. A., Goller, F., & Pytte, C. (1999). The neuromuscular control of birdsong. *Philosophical Transactions of the Royal Society of London B Biological Sciences*, *354*(1385), 927–939.

Taylor, A. M., & Reby, D. (2010). The contribution of source–filter theory to mammal vocal communication research. *Journal of Zoology*, *280*(3), 221–236. doi:10.1111/j.1469-7998.2009.00661.x

Taylor, P. ., & Elwood, R. . (2003). The mismeasure of animal contests. *Animal Behaviour*, *65*(6), 1195–1202. doi:10.1006/anbe.2003.2169

Tibbetts, E. A., Vernier, C., & Jinn, J. (2013). Juvenile hormone influences precontest assessment behaviour in Polistes dominulus paper wasps. *Animal Behaviour*, *85*(6), 1177–1181. doi:10.1016/j.anbehav.2013.03.003

Turcokova, L., Osiejuk, T. S., Pavel, V., Glapan, J., & Petruskova, T. (2010). Song divergence of two Bluethroat subspecies (Luscinia s. svecica and L. s. cyanecula). *Ornis Fennica*, *87*(4), 168–179.

Vallet, E., & Kreutzer, M. (1995). Female canaries are sexually responsive to special song phrases. *Animal Behaviour*, *49*(6), 1603–1610. doi:10.1016/0003-3472(95)90082-9

Wagner, W. E. (1989a). Fighting, assessment, and frequency alteration in Blanchard's cricket frog. *Behavioral Ecology and Sociobiology*, *25*(6), 429–436. doi:10.1007/BF00300189

Wagner, W. E. (1989b). Graded aggressive signals in Blanchard's cricket frog: vocal responses to opponent proximity and size. *Animal Behaviour*, *38*(6), 1025–1038. doi:10.1016/S0003-3472(89)80141-1

Wällschlager, D. (1980). Correlation of song frequency and body weight in passerine birds. *Experientia*, *36*(4), 412–412. doi:10.1007/BF01975119

Wilson, A. J., Grimmer, A., & Rosenthal, G. G. (2013). Causes and consequences of contest outcome: aggressiveness, dominance and growth in the sheepshead swordtail, Xiphophorus birchmanni. *Behavioral Ecology and Sociobiology*, *67*(7), 1151–1161. doi:10.1007/s00265-013-1540-7

Chapter 2

Song pitch indicates body size and influences males’ response to playback in a songbird

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Song pitch indicates body size and influences males' response to playback in a songbird

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Abstract

The inverse relationship between body size and vocalization frequency has been described in a number of species. Nevertheless, the existence of the size-pitch allometry within any song bird species is surprising due to small size variation between males, determinated growth and exceptional vocal prowess of the taxon. It was documented only for a few songbird species so far and consequences of the allometry remain unclear in this taxonomic group. In this study, we provide the evidence that song spectral features may be related to body size even in very small songbird species – the willow warbler (*Phylloscopus trochilus*). Moreover, territorial males showed bolder response the lower-pitched was their song in respect to playback stimulus. Our results suggest that willow warbler males may use song frequency to assess the body size of their rivals (i.e. fighting ability) in male-male competition for resources.

Keywords: allometry, body size, song pitch, fighting ability, rival assessment, male-male competition, intersexual selection

Introduction

Body size is a key feature of an organism affecting many aspects of its biology (Brown & West, 2000). Size of other conspecifics can be of critical importance in certain situations. For contesting males, size asymmetry between contestants often predicts the outcome of a fight (Arnott & Elwood, 2009). Also, females may have preferences for larger males (Andersson, 1994). Thus, both males and females should possess strategies allowing size assessment of other individuals.

Vocalizations can provide information about caller's body size for listeners due to the size-pitch allometry. With increasing body size, lower frequencies can be effectively produced and coupled to the medium (Bradbury & Vehrencamp, 1998). A rich body of empirical evidence exists for inverse relationship between body size (or body mass) and spectral properties of vocalizations among taxa.

Size-pitch allometry is also apparent within species, especially in species with high body size variation between adult individuals and playback experiments showed that males and females are attentive to the body size related acoustic features in those species (Maynard-Smith & Harper, 2004).

Size-pitch allometry might be less common in small animals with determined growth such as birds. Especially, small size variation combined with vocal abilities in songbirds should hardly lead to size-frequency allometry (Patel, Mulder, & Cardoso, 2010). Nevertheless, inverse size-pitch relationship has been found in some songbird species (Hall, Kingma, & Peters, 2013; Linhart, Slabbekoorn, & Fuchs, 2012). The effect of pitch variation on responses of potential receivers remains poorly studied (Gonçalo C Cardoso, 2012), especially in intra-sexual contexts (but see e.g. G C Cardoso, Mota, & Depraz, 2007; Linhart, Slabbekoorn, et al., 2012).

In this study, we investigated the existence of size-pitch allometry and its role in male-male competition in a small (c.a. 9g) passerine species: a willow warbler *Phylloscopus trochilus*. We tested two hypotheses: 1) Spectral features of spontaneous songs show inverse relationship with tarsus length as a proxy for body size. 2) Aggressive response of males in playback experiment increases the lower pitched is the males’ song in respect to playback stimulus song

Material and Methods

To investigate the relationship between body size and song pitch, we recorded and measured 18 males between 18th April and 2nd May 2008. This period corresponded to territory establishment and pairing of willow warblers. The detailed information about the population and locality has been given elsewhere (Linhart, Fuchs, Poláková, & Slabbekoorn, 2012). Males were recorded under same suitable weather conditions (no wind, no rain) between 6:00 and 11:30 a. m. We recorded males for 5 minutes and subsequently lured them into mist net with playback. Males were immediately weighted (0.1g precision; average weight ± SD = 9.4 ± 0.48g) and their tarsus was measured as proxy for body size (0.1mm precision; average tarsus length ± SD = 20.8 ± 0.45mm). Males were banded with standard aluminium ring and colour rings and released in good condition within 5 minutes.

To investigate the potential role of size-pitch allometry in males' contests, playback experiments with 30 male willow warblers were carried out during egg laying and chick rearing phase between 11th May and 7th June 2008. One minute of spontaneous singing from 10 males recorded in 2007 and not present at the study site in 2008 were used as playback stimuli; each one for three different birds. Stimuli contained 5 – 7 songs that were repeated in loop over the course of the experiment (5 minutes). Average song rate of stimuli was slightly higher than that of tested males’ (6 songs / min and 5.5 songs / min respectively). Song pitch parameters and duration of stimuli songs and songs of tested males were comparable with the exception of minimum peak F which was lower in tested males (Table 1). Males were observed and recorded for five minutes prior to the experiment. Subsequently, stimulus was played back for 5 minutes. Loudspeaker was placed in the middle of the territory close to the male’s favoured song post. During playback we noted a number of different behaviours (see Table 2). We did not evaluate singing behaviour. Only males not previously captured and banded were used for testing because capturing may influence behaviour of willow warbler males (Linhart, Fuchs, et al., 2012). Neighbouring males were never tested within the same day.

In both experiments, Recordings were made within 15 meters from the singing male. We used Marantz PMD660 recorder and Sennheiser ME67 microphone with Rycote Softie windshield. Songs were recorded with 44 100 Hz sampling rate and 16-bit depth, but were down-sampled to 22 050 Hz prior to the analysis. First 15 songs in each recording were analyzed. Measurements (see Figure 1) were taken manually from the spectrograms (FFT length = 1024, Hamming window, Bandwidth = 28Hz, 1/bandwidth = 36ms) and power spectra derived from entire songs (peak F) at -55 dB relative to maximum amplitude within each song. The variables were averaged for each individual. Peak F min and Min F min represent absolute minimum values among the 15 measurements. Songs were analysed in Avisoft SASLab Pro (Avisoft Bioacoustics, Berlin).

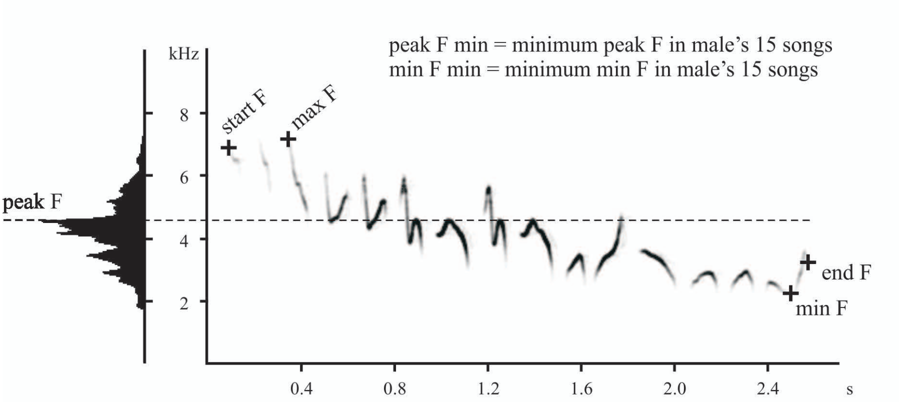

Figure 1. Power spectrum and spectrogram of a typical willow warbler song and parameters measured.

Because the acoustic variables as well as response variables were correlated, we used Principle component analysis (PCA) with varimax rotation to get reduced number of uncorrelated variables. Stimulus songs (*N* = 10) , songs of captured males (*N* = 18) and males from playback experiments (*N* = 30) were all used in a single PCA. Behavioural response variables (from 30 males) were used in the second PCA. We retained principal components with eigenvalues >1 and interpreted them on the basis of variables with loadings >0.7.

To test whether tarsus length predicted song PCs and whether male's relative song pitch predicts playback response we used simple and multiple linear regression models. Relative male's song pitch was defined as stimulus song pitch (stimulus song PC) subtracted from male's spontaneous song pitch (tested male song PC). Multiple regression models accounting for possibly confounding variables (date, daytime, weight) did not change the results substantially (see Table S1 and Table S2) and hence only simple linear model results are presented. We used R for statistical analysis (R Core Team, 2012).

Results

Song pitch and body size

Two principle components accounted for 62% of variance. PC1 was highly loaded with start F, maximum F, and peak F which could be possibly interpreted as overall song pitch. PC2 was highly loaded with average minimum F and absolute minimum F and could thus be interpreted as minimum song pitch (Table 1).

Table 1. Results of PCA on song variables. Loadings >0.70 are highlighted in bold. Means and standard deviations are given for song parameters of 30 males tested in playback experiments.

	song PC1	song PC2	mean ± SD
start F (Hz)	**0.85**	-0.22	6424 ± 708
end F (HZ)	-0.07	0.44	3233 ± 419
max F (Hz)	**0.87**	-0.15	7104 ± 489
min F (Hz)	-0.10	**0.88**	2163 ± 88
peak F (Hz)	**0.71**	0.35	3847 ± 237
min F min (Hz)	0.04	**0.82**	1952 ± 95
peak F min (Hz)	0.69	**0.25**	3278 ± 208
duration (s)			3.10 ± 0.34
Eigenvalue	2.47	1.91	
(% variability explained)	0.35	0.27	

Larger males had lower-pitched songs. Both song components correlated negatively with tarsus length (song PC1: b = -0.79, CI = -1.416 to -0.162, adj. R^2 = 0.26, $F_{1,16}$ = 7.13, P = 0.017; song PC2: b = -0.83, CI = -1.548 to -0.107, adj. R^2 = 0.22, $F_{1,16}$ = 5.93, P = 0.027; see Figure 2).

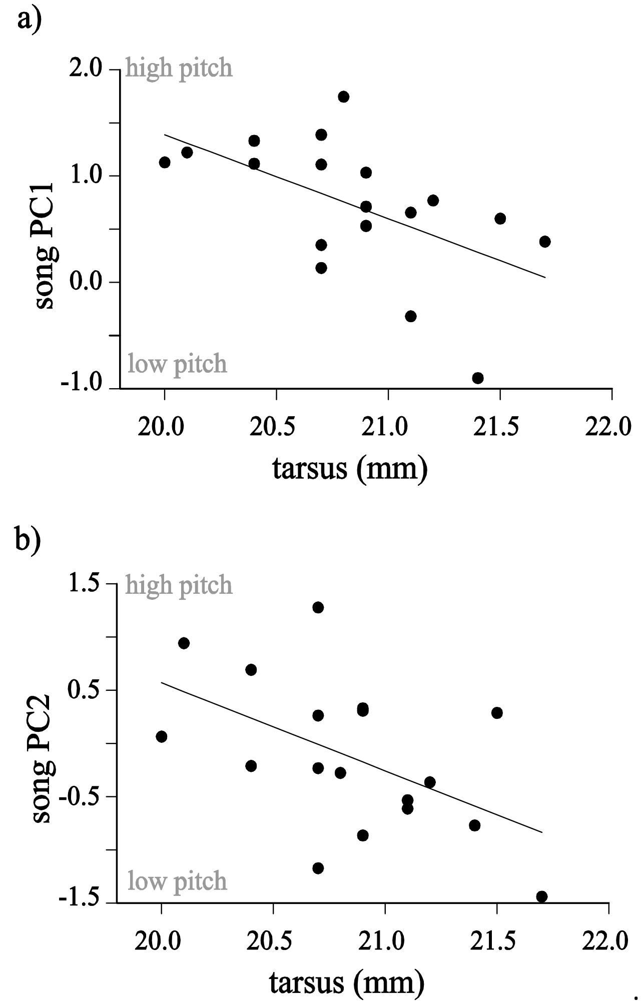

Figure 2. Relationship between body size and the two composite measures of song pitch: the overall song pitch Song PC1 (a) and the minimum song pitch song PC 2 (b). N = 18.

Playback response and song pitch

Males were clearly agitated by playback and responded with close approach, increased activity, flyovers, and wing flickering. (see Table 2 for detail). Two principle components accounted for 66% of variance. PC1 was highly loaded by approach related response variables andPC2 was highly loaded with number of flyovers and horizontal moves, hence we further call them “Approach” and “Activity” respectively (Table 2).

Table 2. Results of PCA on response strength variables in 30 tested males. Loadings >0.70 are highlighted in bold. Mean values and standard deviations of each behaviour are also shown.

	Approach (Response PC1)	Activity (Response PC2)	mean	±	SD
duration of wing flickering (s)	0.02	0.45	163.9	±	84.9
number of flyovers (n)	0.03	**0.80**	9.3	±	4.5
vertical moves (n)	-0.33	0.65	13.8	±	6.1
horizontal moves (n)	-0.33	**0.88**	21.0	±	5.3
flyover height (m)	**0.84**	-0.11	1.6	±	0.6
average height (m)	**0.90**	-0.25	2.3	±	0.6
average distance (m)	**0.87**	-0.19	4.5	±	1.1
within 2m latency (s)	0.66	-0.43	135.4	±	62.0
within 2m duration (s)	**-0.84**	0.01	56.4	±	41.6
Eigenvalue	3.62	2.33			
(% variability explained)	0.40	0.26			

Males approached closer and spent more time in a loudspeaker proximity and were more active the lower was their minimum song pitch (song PC2) in respect to stimulus minimum song pitch (Approach and song PC2: b = 0.38, CI = 0.082 to 0.673, adj. R^2 = 0.17 $F_{1,28}$ = 6.83, P = 0.014; Activity and Song PC2: b = 0.45, CI = 0.083 to 0.818, adj. R^2 = 0.15, $F_{1,28}$ = 6.29, P = 0.018; see Figure 3).

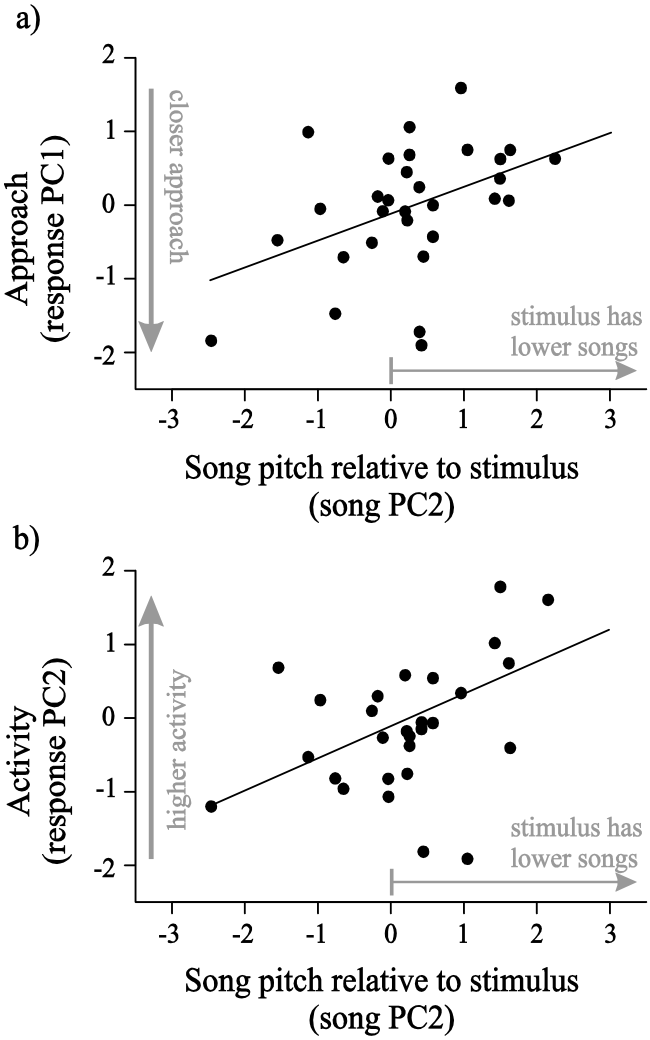

Figure 3. Relationship between male's relative song pitch (= stimulus song pitch subtracted from tested male's pitch) and the two composite playback response measures: Approach (a) and Activity (b). N = 30.

Discussion

We found song pitch to correlate negatively with size in willow warblers. Size-pitch allometry has been reported only for a few passerines (Hall et al., 2013; Linhart, Slabbekoorn, et al., 2012; Patel et al., 2010). Moreover, in willow warblers, variability in song pitch explained by tarsus length was unusually high (R^2 = 0.26 for song PC1 and R^2 = 0.22 for song PC2) compared to that found in the other bird and especially songbird species (Patel et al., 2010). In willow warblers, observed peak frequency is lower than that predicted based on body weight (average peak f = 3894 Hz; predicted 4213) and frequency range exceeded several folds the predicted range based on the males' weight (Wällschlager, 1980) (range of peak F = 3309 – 4410 Hz; predicted = 4125 – 4322) suggesting the rules working on species level may not be applicable within species. Various reasons can explain why mixed results supporting or rejecting size-pitch allometry were reported for songbirds (Patel et al., 2010). Complex nature and plasticity of many passerine songs brings methodological challenges: only some of the song spectral parameters might be related to size (Hall et al., 2013), or the allometry could be revealed in certain song types or structures (Galeotti, Saino, Sacchi, & Moller, 1997) or contexts only (Geberzahn, Goymann, Muck, & ten Cate, 2009). Also, inter-sexual selection could shape vocalizations in the opposite direction (G C Cardoso et al., 2007).

Willow warbler, belongs among the largest leaf warbler – a passerine group that exhibits inverse size-frequency relationship on an inter-species level and that probably underwent sexual selection for body size in recent evolutionary history (Mahler & Gil, 2009). Size and size signalling thus could be important for willow warblers and possibly for the whole group of leaf warblers as suggested also by the size-pitch allometry found in another species of leaf warblers - in chiffchaffs (Linhart, Slabbekoorn, et al., 2012). Further, the study on several bird species, including willow warbler, showed that smaller individuals replace males experimentally removed from their territories (Mönkkönen, 1990), suggesting that smaller males might be excluded from suitable territories in certain species. Body size has been reported to be an important factor in song bird contests (e.g. Searcy, 1979) despite generally low variation in size. Thus size signalling would be clearly beneficial to contesting males. It is not known, however, whether the song pitch is really constrained by body-size (syrinx size, etc.) in songbirds. In willow warbler, however, males and females could use songs to assess the approximate body size of the singer.

Willow warblers in our study were attentive to size-related component of acoustic variation in songs. Stimuli representing bigger opponents generally seem to elicit cautious response or retreat (e.g. Davies & Halliday, 1978; Hardouin, Reby, Bavoux, Burneleau, & Bretagnolle, 2007) which is in accordance with our results. Males got closer if their songs were relatively lower. The closer approach and lower activity in cases the resident male had relatively lower songs resembled the mixed fighting strategy found in chiffchaffs (Linhart, Slabbekoorn, et al., 2012). Only speaker occupation experiments could give an unambiguous answer whether lower songs really repel rivals.

Studying function of simple song features, like song pitch, could give us information on diversification in vocal communicative systems (Gonçalo C Cardoso, 2012). Our study provides another example that size-pitch allometry, though unexpected in small passerines, might be more common than previously thought. Our study further suggests that low song pitch could be important in male-male competition of certain species. If so, anthropogenic noise could negatively affect biology of such vulnerable species (Slabbekoorn, 2013). Despite ongoing interest in mechanisms of song production and song pitch controlling factors in songbirds (Amador & Margoliash, 2013), proximate origins of the size-pitch allometry in this taxoniomic group remain unclear. It is not know whether and how body size, syrinx or resonators' size could interact with pitch or pitch range that can be produced by an individual.

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References

Amador, A., & Margoliash, D. (2013). A Mechanism for Frequency Modulation in Songbirds Shared with Humans. *The Journal of Neuroscience*, *33*(27), 11136–11144. doi:10.1523/JNEUROSCI.5906-12.2013

Andersson, M. B. (1994). *Sexual Selection*. Chichester, United Kingdom: Princeton University Press.

Arnott, G., & Elwood, R. W. (2009). Assessment of fighting ability in animal contests. *Animal Behaviour*, *77*(5), 991–1004. doi:10.1016/j.anbehav.2009.02.010

Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of animal communication* (1. vyd.). Sunderland, MA: Sinauer Associates.

Brown, J. H., & West, G. B. (2000). *Scaling in Biology*. Oxford University Press.

Cardoso, G. C. (2012). Paradoxical calls: the opposite signaling role of sound frequency across bird species. *Behavioral Ecology*, *23*(2), 237–241. doi:10.1093/beheco/arr200

Cardoso, G. C., Mota, P. G., & Depraz, V. (2007). Female and male serins (*Serinus serinus*) respond differently to derived song traits. *Behavioral Ecology and Sociobiology*, *61*(9), 1425–1436. doi:10.1007/s00265-007-0375-5

Davies, N. B., & Halliday, T. R. (1978). Deep croaks and fighting assessment in toads *Bufo bufo*. *Nature*, *274*(5672), 683–685. doi:10.1038/274683a0

Galeotti, P., Saino, N., Sacchi, R., & Moller, A. P. (1997). Song correlates with social context, testosterone and body condition in male barn swallows. *Animal Behaviour*, *53*, 687–700. doi:10.1006/anbe.1996.0304

Geberzahn, N., Goymann, W., Muck, C., & ten Cate, C. (2009). Females alter their song when challenged in a sex-role reversed bird species. *Behavioral Ecology and Sociobiology*, *64*(2), 193–204. doi:10.1007/s00265-009-0836-0

Hall, M. L., Kingma, S. A., & Peters, A. (2013). Male Songbird Indicates Body Size with Low-Pitched Advertising Songs. *PLoS ONE*, *8*(2), e56717. doi:10.1371/journal.pone.0056717

Hardouin, L. A., Reby, D., Bavoux, C., Burneleau, G., & Bretagnolle, V. (2007). Communication of male quality in owl hoots. *The American Naturalist*, *169*(4), 552–562.

Linhart, P., Fuchs, R., Poláková, S., & Slabbekoorn, H. (2012). Once bitten twice shy: long-term behavioural changes caused by trapping experience in willow warblers *Phylloscopus trochilus*. *Journal of Avian Biology*, *43*(2), 186–192. doi:10.1111/j.1600-048X.2012.05580.x

Linhart, P., Slabbekoorn, H., & Fuchs, R. (2012). The communicative significance of song frequency and song length in territorial chiffchaffs. *Behavioral Ecology*, *23*(6), 1338–1347. doi:10.1093/beheco/ars127

Mahler, B., & Gil, D. (2009). The evolution of song in the *Phylloscopus* leaf warblers (Aves: Sylviidae): a tale of sexual selection, habitat adaptation, and morphological constraints. *Advances in the Study of Behavior*, *40*, 35–66.

Maynard-Smith, J., & Harper, D. (2004). *Animal Signals*. Oxford University Press, USA.

Mönkkönen, M. (1990). Removal of Territory Holders Causes Influx of Small-Sized Intruders in Passerine Bird Communities in Northern Finland. *Oikos*, *57*(3), 281–288. doi:10.2307/3565955

Patel, R., Mulder, R. A., & Cardoso, G. C. (2010). What makes vocalisation frequency an unreliable signal of body size in birds? A study on black swans. *Ethology*, *116*(6), 554–563. doi:10.1111/j.1439-0310.2010.01769.x

R Core Team. (2012). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Dostupné z http://www.R-project.org/

Searcy, W. A. (1979). Morphological correlates of dominance in captive male red-winged blackbirds. *Condor*, *81*(4), 417–420. doi:10.2307/1366971

Slabbekoorn, H. (2013). Songs of the city: noise-dependent spectral plasticity in the acoustic phenotype of urban birds. *Animal Behaviour*, *85*(5), 1089–1099. doi:10.1016/j.anbehav.2013.01.021

Wällschlager, D. (1980). Correlation of song frequency and body weight in passerine birds. *Experientia*, *36*(4), 412–412. doi:10.1007/BF01975119

Supporting information

Table S1 - Body size and pitch relationships. Simple and multiple linear regression models showing the relationships between measures of song pitch and tarsus length. Date (number of days from 1st April), daytime (number of minutes from sunrise), and weight (g) were included as covariates.. x = independent variable, y = dependent variable; b = unstandardized slope; CI = 95% confidence interval for b unstandardized slope; B = standardized slope; significance: + $p < 0.1$, * $p < 0.05$, ** $p < 0.01$. Adjusted R^2 is given for whole models. N = 18 for all models.

y	x	b	CI	B	adj R2	t
song PC1 - simple regression						
song PC1	date	0.01	(-0.040, 0.065)	0.12	-0.04	0.51
	daytime	0.00	(-0.004, 0.002)	-0.11	-0.04	-0.5
	tarsus	-0.79	(-1.416, -0.162)	-0.56	0.26	-2.7 *
	weight	-0.16	(-0.839, 0.511)	-0.12	-0.04	-0.5
song PC1 - multiple regression						
song PC1	date	0.01	(-0.047, 0.073)	0.13		0.48
	daytime	0.00	(-0.005, 0.003)	-0.14		-0.5
	tarsus	-0.81	(-1.551, -0.062)	-0.57		-2.3 *
	weight	0.01	(-0.865, 0.882)	0.01		0.02
song PC1 - multiple regression - backward model reduction - final model						
song PC1	tarsus	-0.79	(-1.416, -0.162)	-0.56	0.26	-2.7 *
song PC2 - simple regression						
song PC2	date	0.01	(-0.049, 0.059)	0.05	-0.05	0.2
	daytime	0.00	(-0.001, 0.005)	0.34	0.07	1.59
	tarsus	-0.83	(-1.548, -0.107)	-0.52	0.22	-2.4 *
	weight	0.17	(-0.590, 0929)	0.11	-0.05	0.47
song PC2 - multiple regression						
song PC2	date	-0.01	(-0.073, 0.062)	-0.05		-0.2
	daytime	0.00	(-0.003, 0.007)	0.30		1.05
	tarsus	-0.71	(-1.551, 0.126)	-0.45		-1.8 +
	weight	-0.11	(-1.097, 0.870)	-0.08		-0.3
song PC2 - multiple regression - backward model reduction - final model						
song PC2	tarsus	-0.83	(-1.548, -0.107)	-0.52	0.22	-2.4 *
simple regression with original acoustic variables						
start F	tarsus	-345.9	(-742, 51)	-0.42	0.12	-1.9 +
max F		-9.389	(-320, 301)	-0.02	0	-0.1
peak F		-270.4	(-551, 10)	-0.45	0.16	-2 +
peak F min		-330.8	(-562, -99)	-0.60	0.32	-3 **
min F		-66.4	(-188, 55)	-0.28	0.02	-1.2
min F min		-124.1	(-241, -7.6)	-0.49	0.04	-2.3 *
end F		90.1	(-395, 575)	0.10	-0.05	0.39

Table S2 – Relative pitch and playback response intensity. Simple and multiple linear regression models showing the relationships between relative song pitch (stimulus song PC1 and song PC2 subtracted from male's song PC1 and song PC2 respectively) and playback response (Approach – response PC1; Activity = response PC2). x = independent variable, y = dependent variable; b = unstandardized slope; CI = 95% confidence interval for b; B = standardized slope; significance: * p < 0.05. Final backward selection models for multiple reggression are highlighted in bold. Adjusted R^2 is given for whole models. N = 30 for all models.

y	x	b	CI	B	adj R2	t	p	
Simple regression								
Approach	realtive song PC1	0.15	(-0.083, 0.390)	0.24	0.03	1.33	0.194	
Activity		0.11	(-0.188, 0.407)	0.14	-0.02	0.75	0.459	
Approach	relative songPC2	0.34	(0.071, 0.614)	0.44	0.16	2.59	0.015	*
Activity		0.36	(0.017, 0.707)	0.38	0.11	2.15	0.040	*
Multiple regression								
Approach	date	-0.04	(-0.073, -0.010)	-0.46		-2.72	0.011	*
	daytime	0.00	(-0.002, 0.002)	-0.07		-0.35	0.732	
	realtive song PC1	0.09	(-0.170, 0.356)	0.14		0.73	0.474	
	date (final model)	**-0.05**				**-3.02**	**0.005**	*****
Activity	date	0.01	(-0.030, 0.059)	0.13		0.68	0.506	
	daytime	0.00	(-0.004, 0.001)	-0.24		-1.04	0.310	
	realtive song PC1	0.01	(-0.358, 0.382)	0.02		0.07	0.947	
	- (final model)	**-**	**-**	**-**	**-**	**-**	**-**	
Approach	date	-0.04	(-0.069, -0.010)	-0.43		-2.76	0.010	*
	daytime	0.00	(-0.002, 0.001)	-0.07		-0.41	0.685	
	realtive song PC2	0.28	(0.030, 0.540)	0.36		2.30	0.030	*
	date (final model)	**-0.04**				**-2.94**	**0.007**	*****
	realtive song PC2 (final model)	**0.30**				**2.51**	**0.018**	*****
Activity	date	0.02	(-0.024, 0.059)	0.16		0.87	0.393	
	daytime	0.00	(-0.003, 0.001)	-0.17		-0.88	0.386	
	realtive song PC2	0.34	(-0.025, 0.704)	0.35		1.92	0.066	*
	realtive song PC2 (final model)	**0.36**						*****

Chapter 3

The communicative significance of song frequency and song length in territorial chiffchaffs

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The communicative significance of song frequency and length in territorial chiffchaffs

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Abstract

Spectral and temporal measures of vocalizations have been found to correlate with physical parameters that affect the fighting ability in various species of frogs, mammals, and birds. This correlation could play a role in communication for inter-sexual mate attraction as well as intra-sexual competition. In this study, we investigated whether the frequency and length of territorial songs reflects the body size (tarsus length, weight) of males in a small passerine species, the chiffchaff *Phylloscopus collybita*. We found a tendency for a negative correlation between song frequency and tarsus length, but no significant links with song length. Furthermore, we carried out playback experiments to see whether the variation in song frequency and length is meaningful to receiving males, and found that both parameters affected the males' responses. We found that the response strength was related to the frequency difference between the songs of the tested male and the playback stimulus, rather than only to the stimulus frequency manipulation (increased/decreased frequency). Males showed a mixed strategy of moderate attack response, combined with a low approach response, when relatively low-frequency song stimuli were played back. In contrast, manipulation of the stimulus song length (shortened/elongated) was a better predictor of response strength than the relative differences in song length. Males attacked more and approached closer in response to elongated stimuli. Our data show how multiple messages can be encoded in different signal components; song frequency most likely conveys information about some aspect of fighting ability, while song length probably signals motivation to escalate into a fight among male chiffchaffs.

Key words*:* acoustic communication, fighting ability, motivational signaling, *Phylloscopus collybita*, playback experiment

Introduction

Conflicts over resources, such as food and mates, are omnipresent in the animal kingdom, and they are often associated with aggression among males (Davies & Krebs, 1993; Dugatkin, 2008). Acoustic signals often play an important role in mate attraction, but males can also use them for moderating their aggressive interactions, and at times songs even serve both functions at the same time (Leitao & Riebel, 2003; Schmidt, Kunc, Amrhein, & Naguib, 2008). Herein, we focus on the role of song in the interactions between two competitive males. The benefits of avoiding or shortening physical fights through acoustic signaling may be mutual; saving energy, time, as well as the risk of injury or predation for both males (Bradbury & Vehrencamp, 1998; Owings & Morton, 1998).

Acoustic signaling in male-male contests has probably been most thoroughly studied in birds, who can use relatively complex vocal behaviors such as song matching, song switching, and overlapping (Searcy & Beecher, 2009; Vehrencamp, 2000). In many cases, these signals inform receivers about the short-term motivations to escalate a conflict, and can be classified as conventional signals (Vehrencamp, 2000). However, variation in species-specific vocalizations can also be used by males to assess each other's fighting abilities (e. g. Davies & Halliday, 1978); consequently, such information may affect the decision of the male to retreat or to escalate the fight (Parker, 1974). Size has been shown to be an important factor affecting fighting ability, and hence the outcome of a fight (Jonart, Hill, & Badyaev, 2007; Richner, 1989; for songbirds see: Searcy, 1979).

Spectral variation in vocalizations is a reliable indicator of body size in many taxa, including birds (Geberzahn, Goymann, Muck, & ten Cate, 2009; Hardouin, Reby, Bavoux, Burneleau, & Bretagnolle, 2007; Mager, Walcott, & Piper, 2007; Patel, Mulder, & Cardoso, 2010; see ten Cate, Slabbekoorn, & Ballintijn, 2002 for review). Songbirds have more complex syringes than non-songbirds and usually more flexible singing capacities. Nevertheless, the expected negative relationship between body size and song frequency has also been reported in comparisons among different species (Badyaev & Leaf, 1997; Bowman, 1979; Mahler & Gil, 2009; Price, Earnshaw, & Webster, 2006; Ryan & Brenowitz, 1985; Seddon, 2005; Wällschlager, 1980), as well as among individuals of the same species (Handford & Lougheed, 1991; Galeotti, Saino, Sacchi, & Møller, 1997; Forstmeier, Burger, Temnow, & Derégnaucourt, 2009; Turčoková, Osiejuk, Pavel, Glapan, & Petrusková, 2010; but see Shy, 1983; Logue et al., 2007; Gonçalo C Cardoso et al., 2008).

Despite the general occurence of body size-related variation in acoustic signals, the effect on the response behaviors of males during aggressive interactions has rarely been tested in birds, especially songbirds (see Gonçalo C Cardoso, 2012 for a review). Body size-related spectral variation among individual males has been shown to affect interactions among competing males in two non-passerine species: scops owls, *Otus scops* (Hardouin et al., 2007) and female African black coucals, *Centropus grillii* (Geberzahn et al., 2009). In a study of a passerine species, Cardoso et al. (2007) found no differences in the responses of male serins, *Serinus serinus*, to high and low-pitched songs. However, the spectral characteristics of serin songs are not correspondingly related to body size parameters (Gonçalo C Cardoso et al., 2008). Therefore, it remains an open question whether body size-dependent spectral variation can be used by males of other passerine species as an index of fighting ability.

Similarly, song length has been found to correlate with different male physical or behavioral parameters that could be associated with the fighting abilities in songbirds, including: tarsus length (a proxy for body size) in pied flycatchers, *Ficedula hypoleuca* (Lampe & Espmark, 1994); plasma testosterone levels in barn swallows, *Hirundo rustica* (Galeotti et al., 1997); and winter dominance in great tits, *Parus major* (Lambrechts & Dhondt, 1986). Congruent findings in the relationships between male characteristics, temporal song variations, and an appropriate behavioral response to this variation have been found in blue grosbeaks, *Passerina caerulea* (Lattin & Ritchison, 2009) and in the hoopoe *Upupa epops* (Martín-Vivaldi, Palomino, & Soler, 1998, 2004). Older grosbeaks had longer songs than younger grosbeaks did, and competing males responded more strongly to the playback of long songs. In the hoopoe, the length of the song was positively correlated with condition, and males with longer songs responded by a closer approach. Overall, there is a remarkable agreement among playback studies that longer songs (or longer parts of songs related to male-male interactions) elicit stronger responses, or that males with longer songs give stronger responses. However, the explanation for this consistently variable response strength differs across species and studies. This might be due to the song length functioning as an index of fighting ability (e. g. Adhikerana & Slater, 1993; Lattin & Ritchison, 2009; Martín-Vivaldi et al., 2004) or as a signal of short-term motivation (Balsby & Dabelsteen, 2001; Langemann, Tavares, Peake, & McGregor, 2000; Peter K. McGregor & Horn, 1992; Ríos-Chelén & Garcia, 2007).

The chiffchaff, *Phylloscopus collybita*, has simple songs consisting of several repeated higher- and lower-pitched syllables, which are often referred to as “chiffs” and “chaffs”. A male can have several different “chiff” and “chaff” syllable types in its repertoire. The sequence of the syllable types within a song seems random; additionally, they are likely to be functionally equal (Cramp and Brooks 1992). Both song parameters (song frequency and song length) show inter-individual variability among chiffchaff males; thus having, among other factors, the potential to signal body qualities that translate into fighting ability. McGregor (1988) tested whether there was a relationship between the length of spontaneous songs of a male chiffchaff and its subsequent response strength to playback of non-manipulated songs. However, he found no such relationship and concluded that song length in chiffchaffs is unlikely to signal fighting ability. McGregor (1988) left several aspects of male-male interactions unexplored, such as whether there is a link of the song length with any physical parameters, and the signaling function of song length from a receiver's perspective.

In this study, we investigated whether spectral and temporal variations could be used by male chiffchaffs as a signal of body size. First, we examined the relationship between physical parameters (tarsus length and body weight as proxies for body size) and song parameters (song frequency and song length). We had expected: a) for the song frequency to be negatively correlated with body size; and b) for the song length to be positively correlated with the two physical parameters (assuming that body size limits or constrains the production of vocal variation). Second, we used playback of the song stimuli, manipulated both in frequency and length in a 2 x 2 factorial design in order to find out whether variation in the spectral and temporal parameters affects the aggressive behavior of territorial males.

We had expected that song stimuli that are decreased in frequency and elongated in length would elicit a more intense response (assuming that song parameters reflect superior fighting ability, which in turn represent a greater threat to territory holders). As the level of threat from the playback stimulus could be influenced by the fighting ability of the responding male, we also tested the effect of relative song parameters, i.e. the differences in song frequency and song length, between the playback stimulus and the responding male. We expected these differences between the sender (test stimulus) and the receiver (test individual) to be a better predictor of subsequent responses than the absolute song parameters (assuming that these parameters signal fighting ability).

Materials and methods

Study Site and Animals

Our study was conducted in a wooded marshland about 1 km^2 in size, near the town of Ceske Budejovice (48°59'44"N, 14°26'23"E), South Bohemia, Czech Republic. Until 1990, the study site had been a military training area, and since then has not been used for any significant recreational or other activities. The area consists of wooded marshland with ponds and stands of willow (*Salix* spp), birch (*Betula* spp), and aspen (*Populus* spp) trees, as well as some old oak (*Quercus* spp) avenues on the edges. The first chiffchaff males come into the area in the middle of March, and after the peak arrival period (towards the end of March) there are about 60 - 70 breeding pairs. Males sing and defend their territories throughout the breeding season, until the end of June. The first eggs can be found in the second half of April, hatching occurs from the beginning of May, and the birds are present until October.

In 2008, we recorded and tested 48 previously non-banded adult males from the 14th of April until the 17th of May. The age of the males was unknown, as aging by plumage or by other external traits is unreliable, even for males in their first breeding season (Jenni & Winkler, 1994). Each bird was lured into a mist net immediately after the collection of song recordings and conducting of the playback experiments. We color-banded all tested males for further individual recognition, as well as to avoid testing the same individual twice. The two morphological measurements were taken using scales and calipers: the tarsus length with a precision of 0.1 mm, and the body weight with a precision of 0.1 g. All birds were released in good condition within 10 minutes of being caught.

Sound Recording and Analyses

We recorded the songs of each male for five minutes before playback. Typically, we collected at least 20 to 30 songs during the five minutes of recording. In the case that we had recorded only a few songs after five minutes for a particular male, we delayed the playback and prolonged the recording session until we had recorded a minimum of 15 songs. All males were recorded from a position as close as possible to the song post, which was usually within a distance of 15 meters. The songs were recorded between 06:00 - 11:00 a.m., within the first five hours after sunrise, using a Marantz PMD660 solid state recorder (sampling frequency 44,100 Hz, no compression) and a Sennheiser ME67 directional microphone, equipped with a Rycote Softie windshield.

We only used high-quality song recordings, and performed acoustic analyses on 17.81 ± 2.40 (mean ± SD) songs per individual. We used Avisoft SASLab software for the acoustic processing and analyses (Raimund Specht, Berlin). Before taking any measurements, all recordings were down-sampled to a sampling rate of 22,050 Hz, band-pass filtered between 1,500 Hz and 9,000 Hz (well outside the frequency use of chiffchaff song: ca. 2,500 - 8,000 Hz), and were standardized to 90% of the maximum amplitude. We used the automatic measurements tool in Avisoft SASLab to assess the spectral and temporal characteristics of each syllable (see Figure 1), taking -15 dB from the maximum amplitude in the song as the lower cut-off point. Spectrogram settings were: FFT-length: 1,024, frame: 100%, window: Hamming, overlap: 87.5%; resulting in a temporal resolution of 5.8 ms, and a spectral resolution of 22 Hz.

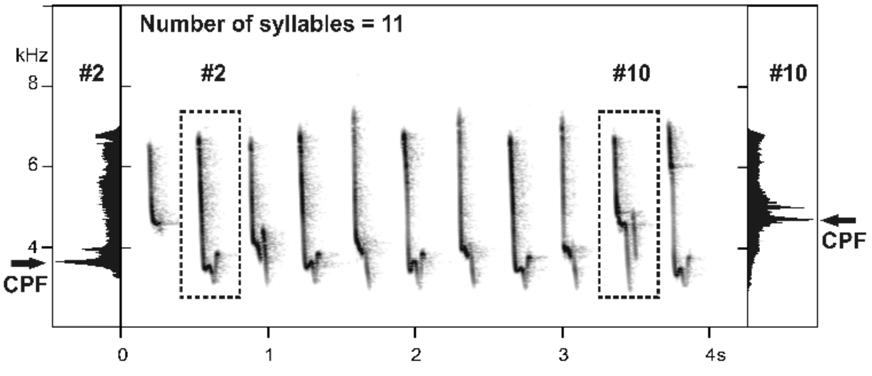

Figure 1. Spectrogram of a typical chiffchaff male song showing spectral and temporal measurements. The cumulative peak frequency (CPF) measure was the highest value in a cumulative power spectrum of a syllable (black arrows). We have indicated the cumulative spectra of a typical “chaff” (syllable #2) and a “chiff“ (syllable #10) on both sides of spectrogram. Song length was expressed as number of syllables within the song.

We measured the highest peak in the accumulated energy distribution over a whole syllable, which we refer to as the cumulative peak frequency (CPF). We obtained the mean CPF for each bird by averaging the syllable CPFs within, and then across the songs. The CPF reflects well which frequencies are emphasized in the songs (Galeotti et al. 1997; Cardoso et al. 2008), and it is less affected by recording quality and relative recording levels than are the minimum and maximum frequencies. To assess the relationship between CPF and the other commonly-assessed peak frequency (PF) (defined as the frequency with the maximum amplitude within a sound unit, at a single sampling point in time), we measured PFs and CPFs of all syllables of five songs (40 - 66 syllables) in five individuals. The CPF of syllables is highly correlated to the PF (Pearson correlation: all $r > 0.93$).

We described song length as the number of syllables within a song, following the approach of McGregor (1988); with the mean song length determined for every individual this way. The number of syllables is a crucial trait, which also directly determines the length of songs in terms of time duration (Pearson correlation: all $r > 0.993$; data on 5 males, 10 songs per male). Furthermore, there is low variability within and between individuals in syllable lengths and intervals (P K McGregor, 1988; pers. obs. Schubert, 1971); therefore, the syllable number is a reliable measure of song length between males.

Playback Stimuli and Experimental Design

We used the songs of 12 chiffchaff males recorded at our study site either in 2006 or in 2007. Recorded males were color-banded, and none of these males were present at the study area in 2008. We selected one minute of high-quality recording during which the male sang intensively (mean ± SD = 7.21 ± 1.24 songs). A band-pass filter was used to remove noise under 1,500 Hz and above 9,000 Hz. If there were any sounds of other birds in the recording, we removed them as well, if possible without affecting the target signal. The high-quality song recordings were subsequently normalized to 90 percent of the maximum volume. There were no significant differences between the recorded songs of the whole study population and the selection of experimental songs before manipulation for song length (Mann-Whitney U test: *U* = 281, *N population* = 48, *N stimuli* = 12, *P* = 0.91) or in the CPF measure of song frequency (Mann-Whitney U test: *U* = 245, *N population* = 48, *N stimuli* = 12, *P* = 0.43).

The standardized set of songs was used to generate four categories of manipulated songs, leading to 4 x 12 = 48 different playback stimuli. Song frequency was manipulated by moving the whole song up 250 Hz (‘increased’) or down 250 Hz (‘decreased’), using the ‘Frequency domain transformation’ tool in Avisoft SASLab. Song length was manipulated by omitting (‘shortened’) or repeating (‘elongated’) three syllables (the 3rd to 5th syllable in a sequence), while retaining the original interval lengths between the syllables. The spectral and temporal manipulations led to the following categories: ‘increased-shortened’, ‘increased-elongated’, ‘decreased-shortened’, and ‘decreased-elongated’. The manipulation of length did not affect the CPF of the songs (Matched-pair t-test: *t* = -0.59, *N* = 12, *P* = 0.567). Song CPF and the lengths in the songs of the males in our study population did not correlate; we therefore believe it is valid to manipulate both parameters independently. The scale of the manipulations corresponds approximately to the standard deviations for the two parameters within the study population (see Table 1). The final stimuli were within the natural range of variation for both of the parameters. Thus, all four treatments represented biologically relevant song variants.

Table 1. Overview of the non-manipulated experimental songs parameters, physical, and song parameters of tested males.

	N	mean	±	SD	Min	Max
Morphological measures:						
Weight (g)	45	7.92	±	0.37	7.2	8.8
Tarsus length (mm)	45	20.48	±	0.5	19.2	21.7
Song parameters:						
Song CPF (Hz)	48	4 351	±	225	974	787
Song length (n)	48	11.7	±	3	6.6	21.9
Stimuli before manipulation:						
Stimuli CPF (Hz)	12	4 328	±	395	786	146
Stimuli length (n)	12	11.4	±	3.6	5.7	18.5

All playback experiments were performed between 6:00 - 12:00 a.m. in the morning. The 48 playback stimuli (12 different song variants in 4 treatments) were played back to 48 territorial chiffchaff males (each male was tested with only one stimulus) from inside their territories (within the area demarcated by their song perchest). Neighboring males were never tested in two successive experiments. The four experimental treatment categories were applied in four successive playback experiments, in a random order. Additionally, the treatment variants from the set of 12 songs were randomly selected for the experiments. Each song variant was used four times, once for each treatment. PL, who carried out the experiments, was blind to the actual treatment being broadcast. The songs were played back for five minutes from a JBL CONTROL 1X loudspeaker, at a peak volume of 80 dB (A) measured 1 m from the speaker. We started the playback from a shelter 10 - 15 m away from the speaker, while the focal male was in sight and singing. The response behavior was observed for five minutes during the playback. The following behavioral parameters were scored: number of fly-overs (the focal male flying over the loud-speaker); number of attacks (focal male in physical contact with the loudspeaker: hitting it when flying-over, or sitting on top and pecking it);

duration of wing flicking(s); latency of interest(s) (focal male stopped singing and moved towards the loud-speaker); latency of fly-overs(s); latency of attack(s); time spent within 2.0 meters from the speaker(s); and the latency of coming within 2.0 meters. The distance to the speaker was estimated with the help of two-meter markers on the ground around the speaker. We did not evaluate the vocal response, as the chiffchaff males typically remained rather silent during the playback.

Statistical Analyses

We used multiple linear regression when examining the possible relationships between song, body size, and two environmental variables: date (number of days since the first experimental day; mean = 20.4, SD = 11.6) and time of the day (number of minutes after sunrise; mean = 196.0, SD = 96.2). We included these environmental parameters into the analysis to control for their possible influence on both the spectral and temporal song parameters. The variance inflation factor (VIF) for all of the predicting variables were between 1.11 and 1.36 (respective tolerance values = amount of variance that was not explained by the other predictors: 0.74 - 0.90), suggesting that multicolinearity was not a problem for our data. We also checked the residuals from regression models to confirm that they were normally distributed, were homoscedastic, and did not systematically vary across the range of predicted values.

We used the Bonferroni correction to correct α-values for multiple comparisons. However, using the Bonferroni correction substantially increases the probability of making a Type II error, especially in the behavioral sciences, where the statistical power in most cases is already low; as highlighted in some recent discussions (Jennions & Møller, 2003; Nakagawa, 2004; D. R. Smith, Hardy, & Gammell, 2011). We reported effect sizes along with 95% confidence intervals (CI), which allows for a better interpretation of significance levels (Colegrave & Ruxton, 2003; Jennions & Møller, 2003; Nakagawa, 2004). We computed the effect sizes and Cohen's *d* exact CIs using R scripts as outlined by Nakagawa and Cuthill (2007).

As the first step of playback response analysis, we applied a Principal Component Analysis (PCA) to get a reduced number of uncorrelated intensity response measures (P K McGregor, 1992), based on the eight behavioral variables scored during the playback experiments. Subsequently, we used 2 x 2 factorial ANOVAs to analyze the differences in each of the derived response measures between treatments (manipulation of CPF, manipulation of song length, plus the interaction between the two).

Because the receiver's response could be influenced by the sender's qualities as well as by his own qualities, we further compared models that only included a stimulus manipulation and/or a relative male-stimulus difference in the CPF and song length. We used general linear models and Akaike's information criterion (AIC) model selection to find out whether the behavioral response was affected by the frequency manipulation (F), and/or the length manipulation (L) of the stimulus songs (factors: increased/decreased, and shortened/elongated); or by the receiver-stimulus frequency difference (FD), and/or the receiver-stimulus length difference (LD) (continuous predictors defined as: tested male's song CPF - stimulus CPF; tested male's song length - stimulus song length. The following steps were taken: 1) We computed the statistics for all possible models that included single predictors only (F, L, FD, LD), their additive combinations (F+L, F+LD, FD+L, FD+LD), interactions (F:L, F:LD, FD:L, FD:LD), and the full models (F*L, F*LD, FD*L, FD*LD). Note that F*L stands for the original 2 x 2 factorial design that we referred to above. 2) We employed AIC model selection, which allows the comparing of alternative hypotheses, based on their relative likelihoods (Burnham, Anderson, & Huyvaert, 2011; Burnham & Anderson, 2002; Garamszegi et al., 2009). As the ratio between sample size (n) and number of estimated parameters (K) was < 40 we used a bias-adjusted AICc (Burnham & Anderson, 2002). We selected the plausible models, based on ∆AICc, by the rule of thumb for each response measure (∆AICc < 2 suggests that there is substantial evidence for the model) (op. cit.). 3) We checked for the consistency of the plausible models; as those supported across response measures should be preferred as the most parsimonious.

Some studies have suggested that the receiver's response could be unimodal, with the most intense responses occurring when the difference between the sender's and receiver's fighting ability / motivation is minimal (Collins, 2004; de Kort, Eldermire, Cramer, & Vehrencamp, 2009; Searcy & Nowicki, 2000). Therefore, we checked the scatterplots of the response measures plotted against the frequency and length differences, to see whether the use of linear models was adequate. All scatterplots suggested linear, rather than unimodal, response patterns. All statistical analyses were done in R (Ihaka & Gentleman, 1996).

Results

Song and Physical Parameters

When considering the effect of body size and environmental parameters on CPF in a simple regression, CPF was negatively related to the tarsus length (Table 2). None of the other parameters affected CPF significantly. Again, tarsus length was the only significant predictor of CPF in a multiple regression. After the stepwise removal of the insignificant variables from the model, only the tarsus length was retained in the model (adjusted R^2 = 0.09, $F_{1, 43}$ = 5.44, unstandardized slope b = -141, 95% CI: -263 – -19, P = 0.024) (Figure 2). The significant relationship turned into a trend after application of the Bonferroni correction for multiple comparisons (corrected α = 0.013).

Table 2. Results of simple and multiple regression of cumulative peak frequency (CPF) and song length on body size and environmental parameters. Significant predictors are highlighted in bold. Unstandardized slopes (b) along with 95% confidence intervals (95% CI) and values of adjusted R2 are presented.

	Simple regression								**Multiple regression**					
	CPF (Hz)				Song length (syllables)				CPF (Hz)			Song length (syllables)		
	b (95% CI)	*t*	R^2	*P*	*b* (95% CI)	*t*	R^2	*P*	*b* (95% CI)	*t*	*P*	*b* (95% CI)	*t*	*P*
date	-1 (-7, 4)	-0.48	-0.02	0.63	0.1 (-0.0, 0.2)	1.86	0.05	0.07	-2 (-8, 4)	-0.75	0.46	0.1 (-0.0, 0.2)	1.95	0.06
daytime	0 (-1, 0)	-0.79	0	0.43	0 (-0.0, 0.0)	1.51	0.03	0.14	0 (-1, 0)	-1.02	0.32	0 (-0.0, 0.0)	1.58	0.12
weight	-69 (-245, 106)	-0.8	0	0.43	2.3 (-0.2, 4.7)	1.87	0.05	0.07	44 (-154, 243)	0.45	0.65	1 (-1.8, 3.8)	0.75	0.46
tarsus	-141 (-263, -19)	-2.33	0.09	0.02	0.8 (-1.1, 2.6)	0.84	-0.01	0.4	-152 (-287, -17)	-2.27	0.03	0.5 (-1.4, 2.4)	0.51	0.61

Song length tended to increase with both date and weight in a simple regression (Table 2). However, when controlled for the other variables in a multiple regression, there was no longer any indication for the effect of weight on song length. Song length seemed to be more affected by environmental parameters than by body size parameters. In a full model, there was a trend for songs getting longer with the recording date (Table 2). When insignificant variables were removed from the model, there was a significant positive effect of the date, and a trend for a positive effect of time of day on song length (adjusted R^2 = 0.11, $F_{2,\ 43}$ = 5.44, P = 0.031; date: b = 0.09, 95% CI: 0.01 - 0.16, P = 0.030; daytime: b = 0.01, 95% CI: 0.00 - 0.02, P = 0.056). None of the relationships were significant after the Bonferroni correction for multiple comparisons (corrected α = 0.013).

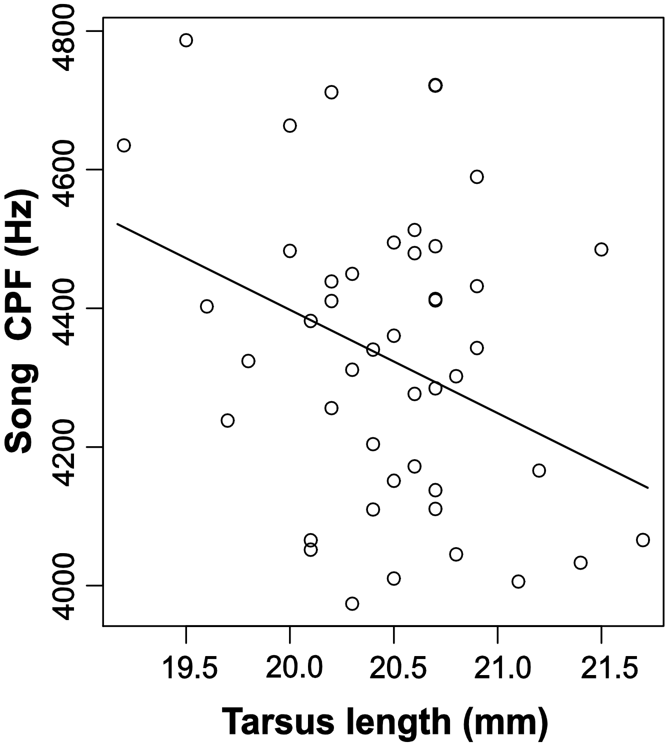

Figure 2. Relationship between the cumulative peak frequency (CPF) of chiffchaff males' songs and their tarsus lengths. See text for details.

Playback Response

The first two principal components of our analysis explained 66.33% of the total variation in the playback response data, and were the only ones with eigenvalues > 1.0. The first component (PC1) explained 45.27% and had particularly high loadings related to the latency of approach within 2.0 meters (-0.85) and the time spent within 2.0 meters (0.86), and will be referred to as the 'Approach Response'. The second component (PC2) explained 21.06% and had particularly high loadings for latency to attack (-0.70) and number of attacks (0.82), and will be referred to as 'Attack Response'. Factor loadings for all original behavioral measures are reported in Table 3.

Table 3. Results of principal component analysis on separate behavioral response measures. The table shows loadings of original behavioral elements scored during experiment and variability explained by the two principal components with eigenvalues >1. Loadings equal to 0.70 and higher (or -0.70 and lower) are highlighted in bold and were used to interpret the PCs.

	PC1 Approach Response	PC2 Attack Response
latency of interest	-0.59	0.31
time spent within 2m	**0.86**	0.15
within 2m latency	**-0.85**	0.15
number of flyovers	0.64	-0.51
latency of flyovers	**-0.81**	0.33
number of attacks	0.48	**0.82**
latency of attacks	-0.61	**-0.7**
duration of wing flickering	0.37	-0.14
Eigenvalue	3.62	1.68
(% variability explained)	45.27	-21.06%

On average, males approached closer (high PC1 values) to elongated stimuli (ANOVA: $F_{1,44}$ = 6.19, P = 0.017, d = 0.73, 95% CI: 0.44 – 4.49). There was no significant effect of CPF on Approach Response although frequency-increased stimuli elicited a stronger Approach Response (ANOVA: $F_{1,44}$ = 1.33, P = 0.254, d = 0.34, 95% CI: -0.83 – 3.11); however, the interaction of the two effects was significant (ANOVA: $F_{1,44} = 4.19$, $P = 0.047$). Post-hoc comparison of all level combinations revealed that males approached significantly closer in response to 'increased-elongated' songs (Fisher LSD test: 'increased-elongated' vs. 'increased-shortened' $P = 0.003$; 'increased-elongated' vs. 'decreased-elongated' $P = 0.029$; 'increased-elongated' vs. 'decreased-shortened' $P = 0.013$). There were no other significant differences between the four treatment groups (Fisher LSD test: all $P > 0.350$).

Males also attacked more (higher PC2 values) in response to long stimuli (ANOVA: $F_{1,44} = 4.50$, $P = 0.040$, $d = 0.63$, 95% CI: 0.10 – 4.11). Neither the stimulus frequency manipulation (ANOVA: $F_{1,44} = 0.00$, $P = 0.955$, $d_{\text{increased}} = -0.10$, 95% CI: -2.01 – 1.91) nor the interaction between stimulus frequency and length manipulation affected the attack response. (ANOVA: $F_{1,44} = 0.75$, $P = 0.390$). There was only one significant difference in post-hoc comparisons of factor level combinations. Males attacked more in response to 'increased-elongated' than to 'increased-shortened' stimuli (Fisher LSD test, $P = 0.040$). There were no significant differences between the other level combinations (Fisher LSD test, all $P > 0.131$).

The above results indicate that males pay attention to the variation in song length, and to a lesser extent to the variation in CPF, as well (for elongated songs). However, it might be that these results are influenced by the fact that they only account for the variation in the sender's CPF and song length; however, they do not account for the variation in these parameters on the receiver's side of the interaction. Therefore, we further compared AIC values for models consisting of stimulus manipulation effects (frequency increased / decreased, elongated / shortened) and the effects of relative differences in song parameters between the tested male and the stimulus. We also considered models combining stimulus manipulation of one parameter and male-stimulus differences for the other parameter.

There were six candidate models for the Approach Response (PC1) and three candidate models for the Attack Response (PC2) that passed the threshold level of ΔAICc < 2 (see Table 4). The candidate model consisting of additive main effects of receiver-stimulus difference and stimulus length manipulation (FD+L) was the best candidate model for both the Approach Response as well as Attack Response. Furthermore, it was the only candidate model that passed the threshold for both response measures. These results indicate that chiffchaffs responded to stimulus frequency based on a relative criterion (receiver-stimulus frequency difference), while they responded to stimulus length based on an absolute criterion (stimulus length manipulation).

Table 4. Akaike's information criterion comparison of all possible models of response composite measures on stimulus modification or relative receiver-stimulus differnece.

PC1 (Approach Response)						**PC2 (Attack Response)**					
model	AICc	K	?AICc	ω	ER	model	AICc	K	?AICc	ω	ER
FD+L	**133.6**	**4**		**0.19**		**FD+L**	**136.2**	**4**		**0.3**	
F:LD	**133.9**	**4**	**0.3**	**0.17**	**1.13**	**L**	**137.2**	**3**	**1**	**0.18**	**1.62**
F*LD	**134.3**	**5**	**0.7**	**0.14**	**1.4**	**FD**	**138.2**	**3**	**2**	**0.11**	**2.73**
FD*L	**135.3**	**5**	**1.7**	**0.08**	**2.35**	FD*L	138.7	5	2.5	0.09	3.47
F*L	**135.3**	**5**	**1.7**	**0.08**	**2.38**	FD+LD	138.8	4	2.6	0.08	3.78
F:L	**135.3**	**5**	**1.7**	**0.08**	**2.38**	F+L	139.5	4	3.3	0.06	5.36
L	136.1	3	2.5	0.05	3.52	FD:L	140.6	4	4.4	0.03	8.94
FD*LD	136.6	5	3	0.04	4.42	LD	141.1	3	4.9	0.03	11.35
FD:LD	137.1	3	3.5	0.03	5.76	FD*LD	141.2	5	5	0.02	12.29
F+L	137.2	4	3.6	0.03	5.94	F*L	141.2	5	5	0.02	12.29
FD+LD	137.5	4	3.9	0.03	7.04	F:L	141.2	5	5	0.02	12.29
LD	137.6	3	4	0.03	7.31	FD:LD	141.2	3	5	0.02	12.29
FD	138.1	3	4.5	0.02	9.5	F	141.8	3	5.6	0.02	16.39
F+LD	138.5	4	4.9	0.02	11.88	F:LD	142.6	4	6.4	0.01	26.82
FD:L	140.1	4	6.5	0.01	27.14	F+LD	143.4	4	7.2	0.01	36.88
F	140.6	3	7	0.01	31.67	F*LD	145.1	5	8.9	0	98.33

Single predictors alone (F – stimulus frequency manipulation, L – stimulus length manipulation, FD – receiver-stimulus frequency difference, LD – receiver-stimulus length difference), additive combinations of predictors (F+L, F+LD, FD+L, FD+LD), interactions between predictors (F:L, F:LD, FD:L, FD:LD), and full models (F*L, F*LD, FD*L, FD*LD). Models are sorted in ascending order by the bias adjusted Akaike's information criterion (AICc) and the models below the threshold level ΔAICc < 2 are highlighted in bold. We report the number of estimated parameters (K), the difference from the minimum AICc model (ΔAICc), the Akaike's weight (ω) and the evidence ratio (ER).

The model indicated that elongated stimuli elicited a significantly stronger response for both response measures (Approach response: *d* = 0.78, 95% CI: 0.59 – 4.66; Attack response: *d* = 0.61, 95% CI: 0.05 – 4.06). However, the effect of relative CPF difference between the male and the stimulus is more complex. Males approached more when their song CPF was lower, relative to the stimulus CPF (Approach response: standardized slope β = -0.30, 95% CI: -0.56 – -0.03). However, in contrast, they seem to attack more if their song CPF was higher, relative to the CPF of the stimulus song (Attack response: β = -0.25, 95% CI: -0.03 – 0.52) (Figure 3).

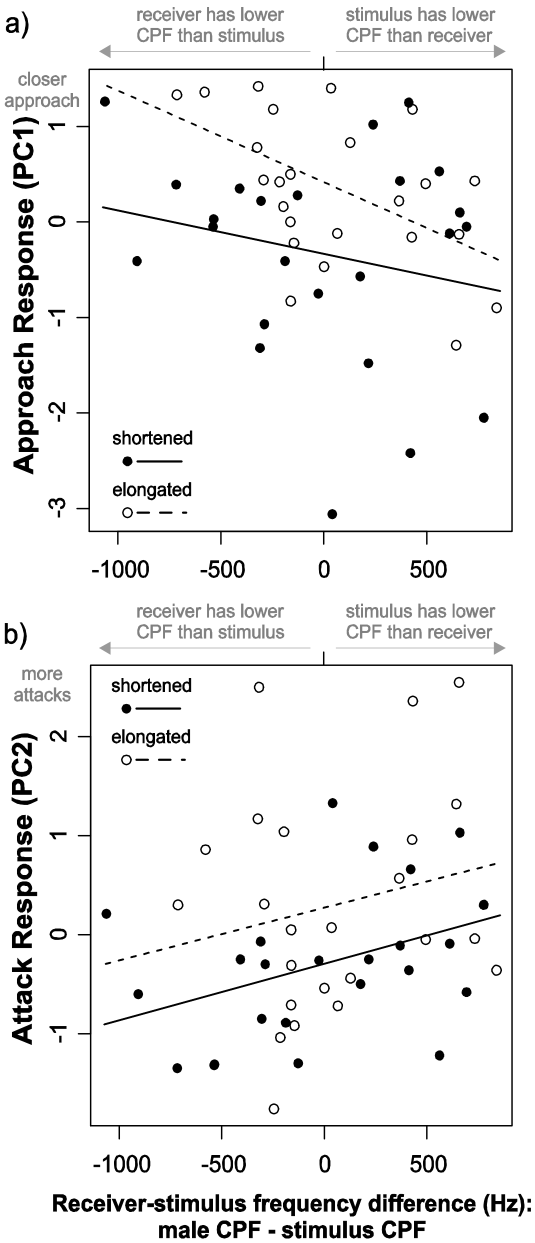

Figure 3. Effect of receiver-stimulus frequency difference (x-axis) and song length manipulation (shortening: filled circles, full line; elongation: empty circles, dashed line) on Approach (a) and Attack Response (b). Note that positive values of response strength measures represent more intense response (closer approach - PC1; and more attacks - PC2). Negative values of receiver-stimulus frequency differences represent males singing lower than the playback stimulus.

Discussion

Our study on acoustic communication in chiffchaffs revealed a tendency for a correlation between the sender and signal characteristics, as well as experimental evidence of signal variation having behavioral relevance to the receivers. Song frequency showed a trend for a negative correlation with tarsus length of males, while there was no correlation between body characteristics and song length. Nevertheless, both song frequency and song length influenced the behavior of males during playback. Males discriminated between frequency-increased and frequency-decreased stimuli in our Approach Response measure, but only with the elongated stimulus variants, while they consistently discriminated between the shortened and elongated stimulus variants in both response measures. Further comparisons of hypothetical response models suggested that, in the case of frequency, males responded to differences between their own songs and those of the stimulus, while this receiver-sender difference did not appear as important for males in the context of song length. It seemed that spectral information, indicating relatively strong competitors, caused more moderate response levels, and more cautious response strategies, than did signals of relatively weak competitors; while temporal information on highly motivated signal senders were matched with high response levels from the signal receivers, independent of their own pre-playback motivation, as reflected in song length.

Sender characteristics reflected in song parameters

Signal frequency variation can play a role in size assessment among males if acoustic and morphometric parameters covary within a population. A negative relationship between the body size or body mass parameters and the vocalization frequency within a population has been found in different taxa including insects, fish, birds, and mammals (Gonçalo C Cardoso, 2012 for review; see ten Cate et al., 2002). Correlative studies in songbirds have led to mixed results (correlation present: Galeotti et al., 1997; Forstmeier et al., 2009; Turčoková et al., 2010; correlation absent: Logue et al., 2007; Gonçalo C Cardoso et al., 2008). In our study, we found no strong pattern, but there was a tendency for spectral characteristics of song to negatively correlate with the tarsus length of male chiffchaffs.

However, it is apparent that this relationship is not as obvious as in several non-passerine species (Hardouin et al., 2007; Mager et al., 2007; c. f. Miyazaki & Waas, 2003). The explanation for this may lie in the fact that passerines possess a relatively complex syrinx, which allows them to produce highly variable sounds of a facultative wide bandwidth (Gaunt, 1983). Further, spectral parameters in the songs of passerines show some degree of plasticity depending on e.g. environmental conditions. For instance, it has been shown that chiffchaffs can actively increase song frequency in response to natural and experimental variations in environmental noise conditions (Verzijden, Ripmeester, Ohms, Snelderwaard, & Slabbekoorn, 2010). Also, variability in body size is much smaller in chiffchaffs than in most of the other species that have been investigated so far, which is particularly relevant to the reliability of signals related to body size (Patel et al., 2010). These facts can all blur the relationship between song frequency and body size. On the other hand, the effect of size for the tarsus length - CPF relationship in chiffchaffs is relatively large compared to other songbirds (Patel et al., 2010).

A correlation between the characteristics of the song and the body of the singer may also depend on the social context of singing. The chiffchaff songs in the current data set were recorded when males were singing spontaneously and not obviously interacting with other males. It is possible that the males do not push their vocal abilities to their limits in this context, and that size- or strength-related correlations would become stronger during the vocal interaction. For example, Montezuma oropendolas, *Psarocolius montezuma*, decrease the frequency of their songs during vocal contests (Price et al., 2006), as also occurs in females of the African black coucal, a species with reversed size dimorphism, where the relationship between body size and song frequency is only apparent in songs recorded during agonistic interaction (Geberzahn et al., 2009). A similar analysis for chiffchaffs has to wait for future studies, as we only recorded songs before playback. We aimed primarily to get strong behavioral responses by using playback from well within the territory boundaries, which seemed to inhibit a vocal response (pre-playback: 4.5 songs / min; playback: 1.4 songs / min, with many males not singing at all). Therefore, future experiments presenting the stimuli at or just outside the territory boundaries might better capture the potential dynamics in vocal response variations.

Although in our study on chiffchaffs, song length was not correlated with physical features; other studies in other species do report such correlations. Song length has been found to be correlated with several male attributes that could be associated with superior fighting abilities, such as: tarsus length (Lampe & Espmark, 1994), age (Lattin & Ritchison, 2009), winter dominance at feeders and survival (Lambrechts & Dhondt, 1986), condition (Martín-Vivaldi et al., 1998), or blood androgen levels (Galeotti et al., 1997). We cannot exclude the possibility that song length in the chiffchaff is correlated with some other parameter of male quality (e.g. age) that was not measured in our study.

Signal variation affecting receiver responses

Studies in many animal species have reported variations in response levels to the playback of high- and low-frequency variants of acoustic signals, typically representing relatively small and large males respectively (Arak, 1983; e. g. toads: Davies & Halliday, 1978; owls: Hardouin et al., 2007; loons: Mager et al., 2007; deer: Reby et al., 2005). However, in songbirds there is an apparent lack of experimental data on the signaling function of frequency in male-male contests (Gonçalo C Cardoso, 2012). We are aware of only one published study on this specific topic. Serin males (*Serinus serinus*) did not show different responses to frequency-increased and frequency-decreased variants of the stimulus songs (G C Cardoso et al., 2007). This is in contrast with our findings in chiffchaffs, who did respond to variations in spectral parameters and specifically to the difference in receiver-stimulus song frequency. Although Cardoso et al. (2007) did not consider such relative measures, we do not think that this is the only factor that explains the discrepancy in the findings of the two studies, as the stimulus manipulation in a study by Cardoso et al. was much greater (+/-

1000 Hz) than ours; therefore, the overlap between the stimulus song versions and receivers' songs was much lower.

Instead, spectral song parameters could have different signaling functions in different species. Serins possess unusually high-frequency songs relative for their body size, and do not exhibit a correlation between body size and spectral song parameters (Gonçalo C Cardoso et al., 2008). High songs also seem to be attractive to females in this species (G C Cardoso et al., 2007). Therefore, it is possible that intersexual selection has shaped the spectral parameters of songs in serins, while intrasexual selection has driven signal evolution in the case of chiffchaffs. In chiffchaffs, it has been suggested that females choose the place for the nest after arrival to the breeding area, and males follow them and monopolize females by defending the neighboring area (Rodrigues, 1996a; Wesolowski, 1987). This favors the use of songs primarily in male-male competition and corresponds to their unusually high song rates throughout the season (Rodrigues, 1996b). Furthermore, a relationship between song frequency and body size has not been found in several other *Serinus* species (GonçAlo C. Cardoso & Mota, 2007), while there is such a relationship in *Phylloscopus* warblers (Mahler & Gil, 2009). This is congruent with the hypothesis that song frequency has been under different selection pressures in serins and chiffchaffs. We therefore predict that female chiffchaffs prefer low-pitched songs, in contrast with the female preferences for high-pitched songs as has thus far been found in most of the songbirds studied (Gonçalo C Cardoso, 2012).

In many songbird species, territorial males respond more strongly to those stimuli that represent a greater threat (see Searcy & Beecher, 2009 for review); however, response behavior may be more complex, and response intensity curves may vary depending on species or signals under study (de Kort et al., 2009). We had expected to get a more intense response to the relatively lower (or frequency-decreased) song stimuli. Yet, we only found a tendency in this direction for the Attack Response measure, and not for the Approach Response measure (which was stronger to song stimuli, with frequencies higher than those of the receiver). This suggests that chiffchaff males confronted with a high-risk intruder (assuming a lower song means a bigger body, which in turn means a higher threat) have a mixed strategy consisting of a slightly higher attack response combined with a lower approach response. Hence, males might compensate for the higher risk associated with attacks by reducing the time spent close to an intruder. A very similar divergent response pattern was found for banded wrens approaching songs with faster trills first; yet spending less time in close proximity to these songs, which presumably represented stronger birds and a greater threat (Illes, Hall, & Vehrencamp, 2006). Other studies have also suggested that such mixed response strategies may be widespread across taxa, including toads and birds (Arak, 1983; e. g. Davies & Halliday, 1978; Geberzahn, Goymann, & ten Cate, 2010; Hardouin et al., 2007); this may reflect the fact that spending more time exposed in close proximity to the stronger competitor may be risky.

Our playback response data were congruent with the literature in showing stronger reactions to longer songs (Adhikerana & Slater, 1993; Balsby & Dabelsteen, 2001; Lattin & Ritchison, 2009). However, the interpretation of why longer songs are perceived as requiring a stronger response varied among studies. Basically, two main interpretations prevail: 1) Song length serves as an indicator of fighting abilities, such as male vigor, strength, or endurance (Adhikerana & Slater, 1993; Lambrechts & Dhondt, 1986); and 2) Song length serves as a signal of short-term variation in the motivation to escalate a conflict (Balsby & Dabelsteen, 2001; Langemann et al., 2000; Lattin & Ritchison, 2009; Peter K. McGregor & Horn, 1992). The second explanation seems the most likely for chiffchaffs, as we did not find any strong relationship between song length and body size, nor to body condition. There is also some evidence that chiffchaff males can alter song length extensively in both directions in different contexts (i.e. spontaneous / challenged singing); hence, would be suitable for motivation signaling (P K McGregor, 1988, pers. obs.).

Multiple messages in different signal components

We believe that our data suggest that chiffchaffs are able to communicate multiple messages in different signal components. The playback data shows that response variations of chiffchaff males are related to relative stimulus frequency (receiver-stimulus frequency difference). This suggests that song frequency is involved in signaling fighting ability in this species, as the outcome of a contest is also dependent on the relative differences between the fighting abilities of both contestants (Hammerstein, 1981; Parker, 1974; J. M. Smith & Parker, 1976). The tendency for a negative correlation between body size (tarsus) and song frequency (CPF) of the males further supports the hypothesis that song frequency is involved in signaling fighting ability, just as large body size is likely to be connected with superior fighting abilities (e. g. Searcy, 1979).

On the other hand, manipulation of the stimulus length was a better predictor of response strength than the relative song parameter of the length difference between stimulus song and the responder’s own song. We think this suggests that song length is more likely to convey the information about the motivational state of the signaler. The fighting motivation was always high for tested males, due to the resource value of the territory; therefore, it is the level of fighting motivation displayed by the intruder (stimulus) alone that should drive the male’s response strength (Hammerstein, 1981; Parker, 1974; J. M. Smith & Parker, 1976). Accordingly, we did not find a correlation between song length and physical features, which may be a reflection that the costs of singing shorter or longer songs do not vary very much (Oberweger & Goller, 2001). This would make song length suitable for conventional motivation signaling (Vehrencamp, 2000).

Conclusions

Our study provides more insight into the role of song frequency in territorial signaling about male qualities. Although we only found a tendency for the spectral variation to be correlated to tarsus length, the potential for the communicative importance of this relationship was emphasized by the playback response data. Song frequency differences between males are detected and meaningful to the birds. A better understanding of the relationship between spectral song characteristics and body measures in passerines may come from further studies on songs produced in the context of increased arousal. Our data also contributes an additional example to the literature which show stronger responses to longer songs, which is a consistent pattern across species. However, in searching for interpretations, both a message signaling fighting ability and a message signaling the motivation to escalate a fight remain plausible avenues for future explorations.

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References

Adhikerana, A. S., & Slater, P. J. B. (1993). Singing interactions in coal tits, *Parus ater*: an experimental approach. *Animal Behaviour*, *46*(6), 1205–1211. doi:10.1006/anbe.1993.1311

Arak, A. (1983). Sexual selection by male–male competition in natterjack toad choruses. *Nature*, *306*(5940), 261–262. doi:10.1038/306261a0

Badyaev, A. V., & Leaf, E. S. (1997). Habitat associations of song characteristics in *Phylloscopus* and *Hippolais* warblers. *Auk*, *114*(1), 40–46.

Balsby, T. J. S., & Dabelsteen, T. (2001). The meaning of song repertoire size and song length to male whitethroats *Sylvia communis*. *Behavioural Processes*, *56*(2), 75–84.

Bowman, R. I. (1979). Adaptive morphology of song dialects in Darwin's finches. *Journal of Ornithology*, *120*(4), 353–389. doi:10.1007/BF01642911

Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of animal communication* (1. vyd.). Sunderland, MA: Sinauer Associates.

Burnham, K. P., & Anderson, D. (2002). *Model selection and multi-model inference* (2nd vyd.). New York: Springer.

Burnham, K. P., Anderson, D. R., & Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, *65*(1), 23–35. doi:10.1007/s00265-010-1029-6

Cardoso, G. C. (2012). Paradoxical calls: the opposite signaling role of sound frequency across bird species. *Behavioral Ecology*, *23*(2), 237–241. doi:10.1093/beheco/arr200

Cardoso, G. C., Mamede, A. T., Atwell, J. W., Mota, P. G., Ketterson, E. D., & Price, T. D. (2008). Song frequency does not reflect differences in body size among males in two oscine species. *Ethology*, *114*(11), 1084–1093. doi:10.1111/j.1439-0310.2008.01552.x

Cardoso, G. C., & Mota, P. G. (2007). Song diversification and complexity in canaries and seedeaters (*Serinus* spp.). *Biological Journal of the Linnean Society*, *92*(1), 183–194. doi:10.1111/j.1095-8312.2007.00837.x

Cardoso, G. C., Mota, P. G., & Depraz, V. (2007). Female and male serins (*Serinus serinus*) respond differently to derived song traits. *Behavioral Ecology and Sociobiology*, *61*(9), 1425–1436. doi:10.1007/s00265-007-0375-5

Colegrave, N., & Ruxton, G. D. (2003). Confidence intervals are a more useful complement to nonsignificant tests than are power calculations. *Behavioral Ecology*, *14*(3), 446–447. doi:10.1093/beheco/14.3.446

Collins, S. A. (2004). Vocal fighting and flirting: the functions of birdsong. In P. R. Marler & H. Slabbekoorn (Ed.), *Nature's music: the science of birdsong* (s. 39–79). San Diego, CA: Elsevier Academic Press.

Davies, N. B., & Halliday, T. R. (1978). Deep croaks and fighting assessment in toads *Bufo bufo*. *Nature*, *274*(5672), 683–685. doi:10.1038/274683a0

Davies, N. B., & Krebs, J. R. (1993). *An introduction to behavioural ecology* (3rd Edition.). Oxford: Wiley-Blackwell.

De Kort, S. R., Eldermire, E. R. B., Cramer, E. R. A., & Vehrencamp, S. L. (2009). The deterrent effect of bird song in territory defense. *Behavioral Ecology*, *20*(1), 200–206. doi:10.1093/beheco/arn135

Dugatkin, L. A. (2008). *Principles of animal behavior* (2nd Edition.). New York: W. W. Norton & Company.

Forstmeier, W., Burger, C., Temnow, K., & Derégnaucourt, S. (2009). The genetic basis of zebra finch vocalizations. *Evolution*, *63*(8), 2114–2130. doi:10.1111/j.1558-5646.2009.00688.x

Galeotti, P., Saino, N., Sacchi, R., & Møller, A. P. (1997). Song correlates with social context, testosterone and body condition in male barn swallows. *Animal Behaviour*, *53*(4), 687–700. doi:10.1006/anbe.1996.0304

Garamszegi, L. Z., Calhim, S., Dochtermann, N., Hegyi, G., Hurd, P. L., Jorgensen, C., … Nakagawa, S. (2009). Changing philosophies and tools for statistical inferences in behavioral ecology. *Behavioral Ecology*, *20*(6), 1363–1375. doi:10.1093/beheco/arp137

Gaunt, A. S. (1983). An hypothesis concerning the relationship of syringeal structure to vocal abilities. *Auk*, *100*(4), 853–862.

Geberzahn, N., Goymann, W., Muck, C., & ten Cate, C. (2009). Females alter their song when challenged in a sex-role reversed bird species. *Behavioral Ecology and Sociobiology*, *64*(2), 193–204. doi:10.1007/s00265-009-0836-0

Geberzahn, N., Goymann, W., & ten Cate, C. (2010). Threat signaling in female song-evidence from playbacks in a sex-role reversed bird species. *Behavioral Ecology*, *21*(6), 1147–1155. doi:10.1093/beheco/arq122

Hammerstein, P. (1981). The role of asymmetries in animal contests. *Animal Behaviour*, *29*(1), 193–205. doi:10.1016/S0003-3472(81)80166-2

Handford, P., & Lougheed, S. C. (1991). Variation in duration and frequency characters in the song of the rufous-collared sparrow, *Zonotrichia capensis*, with respect to habitat, trill dialects, and bodysize. *Condor*, *93*(3), 644–658. doi:10.2307/1368196

Hardouin, L. A., Reby, D., Bavoux, C., Burneleau, G., & Bretagnolle, V. (2007). Communication of male quality in owl hoots. *The American Naturalist*, *169*(4), 552–562.

Ihaka, R., & Gentleman, R. (1996). R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, *5*(3), 299–314.

Illes, A. E., Hall, M. L., & Vehrencamp, S. L. (2006). Vocal performance influences male receiver response in the banded wren. *Proceedings of the Royal Society of London B Biological Sciences*, *273*(1596), 1907–1912. doi:10.1098/rspb.2006.3535

Jenni, L., & Winkler, R. (1994). *Moult and Ageing of European Passerines* (1. vyd.). London: Academic Press Limited.

Jennions, M. D., & Møller, A. P. (2003). A survey of the statistical power of research in behavioral ecology and animal behavior. *Behavioral Ecology*, *14*(3), 438 –445. doi:10.1093/beheco/14.3.438

Jonart, L. M., Hill, G. E., & Badyaev, A. V. (2007). Fighting ability and motivation: determinants of dominance and contest strategies in females of a passerine bird. *Animal Behaviour*, *74*(6), 1675–1681. doi:10.1016/j.anbehav.2007.03.012

Lambrechts, M., & Dhondt, A. A. (1986). Male quality, reproduction, and survival in the Great tit (*Parus major*). *Behavioral Ecology and Sociobiology*, *19*(1), 57–63.

Lampe, H. M., & Espmark, Y. O. (1994). Song structure reflects male quality in pied flycatchers, *Ficedula hypoleuca*. *Animal Behaviour*, *47*(4), 869–876. doi:10.1006/anbe.1994.1118

Langemann, U., Tavares, J. P., Peake, T. M., & McGregor, P. K. (2000). Response of great tits to escalating patterns of playback. *Behaviour*, *137*, 451–471. doi:10.1163/156853900502178

Lattin, C., & Ritchison, G. (2009). Intra- and intersexual functions of singing by male blue grosbeaks: the role of within-song variation. *The Wilson Journal of Ornithology*, *121*(4), 714–721. doi:10.1676/09-026.1

Leitao, A., & Riebel, K. (2003). Are good ornaments bad armaments? Male chaffinch perception of songs with varying flourish length. *Animal Behaviour*, *66*, 161–167. doi:10.1006/anbe.2003.2167

Logue, D. M., Droessler, E. E., Roscoe, D. W., Vokey, J. R., Rendall, D., & Kunimoto, R. M. (2007). Sexually antithetical song structure in a duet singing wren. *Behaviour*, *144*(3), 331–350. doi:10.1163/156853907780425749

Mager, J. N., Walcott, C., & Piper, W. H. (2007). Male common loons, *Gavia immer*, communicate body mass and condition through dominant frequencies of territorial yodels. *Animal Behaviour*, *73*(4), 683–690. doi:10.1016/j.anbehav.2006.10.009

Mahler, B., & Gil, D. (2009). The evolution of song in the *Phylloscopus* leaf warblers (Aves: Sylviidae): a tale of sexual selection, habitat adaptation, and morphological constraints. *Advances in the Study of Behavior*, *40*, 35–66.

Martín-Vivaldi, M., Palomino, J. J., & Soler, M. (1998). Song structure in the Hoopoe (*Upupa epops*) - Strophe length reflects male condition. *Journal Fur Ornithologie*, *139*(3), 287–296. doi:10.1007/BF01653339

Martín-Vivaldi, M., Palomino, J. J., & Soler, M. (2004). Strophe length in spontaneous songs predicts male response to playback in the hoopoe *Upupa epops*. *Ethology*, *110*(5), 351–362.

McGregor, P. K. (1988). Song length and „male quality" in the chiffchaff. *Animal Behaviour*, *36*(2), 606–608.

McGregor, P. K. (1992). Quantifying responses to playback: one, many, or composite multivariate measures? In P. K. McGregor (Ed.), *Playback and studies of animal communication* (s. 79–96). New York: Plenum Press.

McGregor, P. K., & Horn, A. G. (1992). Strophe length and response to playback in great tits. *Animal Behaviour*, *43*(4), 667–676. doi:10.1016/S0003-3472(05)81025-5

Miyazaki, M., & Waas, J. R. (2003). Acoustic properties of male advertisement and their impact on female responsiveness in little penguins *Eudyptula minor*. *Journal of Avian Biology*, *34*(3), 229–232. doi:10.1034/j.1600-048X.2003.03099.x

Nakagawa, S. (2004). A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behavioral Ecology*, *15*(6), 1044 –1045. doi:10.1093/beheco/arh107

Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews*, *82*(4), 591–605. doi:10.1111/j.1469-185X.2007.00027.x

Oberweger, K., & Goller, F. (2001). The metabolic cost of birdsong production. *Journal of Experimental Biology*, *204*(19), 3379 –3388.

Owings, D. H., & Morton, E. S. (1998). *Animal vocal communication: a new approach* (1. vyd.). Cambridge: Cambridge University Press.

Parker, G. A. (1974). Assessment strategy and the evolution of fighting behaviour. *Journal of Theoretical Biology*, *47*(1), 223–243.

Patel, R., Mulder, R. A., & Cardoso, G. C. (2010). What makes vocalisation frequency an unreliable signal of body size in birds? A study on black swans. *Ethology*, *116*(6), 554–563. doi:10.1111/j.1439-0310.2010.01769.x

Price, J. J., Earnshaw, S. M., & Webster, M. S. (2006). Montezuma oropendolas modify a component of song constrained by body size during vocal contests. *Animal Behaviour*, *71*, 799–807. doi:10.1016/j.anbehav.2005.05.025

Reby, D., McComb, K., Cargnelutti, B., Darwin, C., Fitch, W. T., & Clutton-Brock, T. (2005). Red deer stags use formants as assessment cues during intrasexual agonistic interactions. *Proceedings of the Royal Society London Series B: Biological Sciences*, *272*(1566), 941–947. doi:10.1098/rspb.2004.2954

Richner, H. (1989). Phenotypic correlates of dominance in carrion crows and their effects on access to food. *Animal Behaviour*, *38*(4), 606–612. doi:10.1016/S0003-3472(89)80005-3

Ríos-Chelén, A. A., & Garcia, C. M. (2007). Responses of a sub-oscine bird during playback: effects of different song variants and breeding period. *Behavioural Processes*, *74*(3), 319–325. doi:10.1016/j.beproc.2006.11.007

Rodrigues, M. (1996a). Parental care and polygyny in the chiffchaff *Phylloscopus collybita*. *Behaviour*, *133*, 1077–1094. doi:10.1163/156853996X00602

Rodrigues, M. (1996b). Song activity in the chiffchaff: territorial defence or mate guarding? *Animal Behaviour*, *51*(3), 709–716. doi:10.1006/anbe.1996.0074

Ryan, M. J., & Brenowitz, E. A. (1985). The role of body size, phylogeny, and ambient noise in the evolution of bird song. *American Naturalist*, *126*(1), 87–100.

Searcy, W. A. (1979). Morphological correlates of dominance in captive male red-winged blackbirds. *Condor*, *81*(4), 417–420. doi:10.2307/1366971

Searcy, W. A., & Beecher, M. D. (2009). Song as an aggressive signal in songbirds. *Animal Behaviour*, *78*(6), 1281–1292. doi:10.1016/j.anbehav.2009.08.011

Searcy, W. A., & Nowicki, S. (2000). Male-male competition and female choice in the evolution of vocal signaling. In Y. Espmark, T. Amundsen, & G. Rosenqvist (Ed.), *Animal signals: signalling and signal design in animal communication* (1. vyd., s. 277–300). Trondheim: Tapir Academic Press.

Seddon, N. (2005). Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. *Evolution*, *59*(1), 200–215.

Shy, E. (1983). The relation of geographica variation in song to habitat characteristics and body size in north-american tanagers (Thraupinae, *Piranga*). *Behavioral Ecology and Sociobiology*, *12*(1), 71–76. doi:10.1007/BF00296935

Schmidt, R., Kunc, H. P., Amrhein, V., & Naguib, M. (2008). Aggressive responses to broadband trills are related to subsequent pairing success in nightingales. *Behavioral Ecology*, *19*(3), 635 –641. doi:10.1093/beheco/arn021

Schubert, G. (1971). Experimentelle Untersuchungen über die Artkennzeichnenden Parameter im Gesang des Zilpzalps, *Phylloscopus c. collybita* (Vieillot). *Behaviour*, *38*, 289–314.

Smith, D. R., Hardy, I. C. W., & Gammell, M. P. (2011). Power rangers: no improvement in the statistical power of analyses published in Animal Behaviour. *Animal Behaviour*, *81*(1), 347–352. doi:10.1016/j.anbehav.2010.09.026

Smith, J. M., & Parker, G. A. (1976). The logic of asymmetric contests. *Animal Behaviour*, *24*(1), 159–175. doi:10.1016/S0003-3472(76)80110-8

Ten Cate, C., Slabbekoorn, H., & Ballintijn, M. R. (2002). Birdsong and male-male competition: Causes and consequences of vocal variability in the collared dove (*Streptopelia decaocto*). *Advances in the Study of Behavior*, *31*, 31–75.

Turčoková, L., Osiejuk, T. S., Pavel, V., Glapan, J., & Petrusková, T. (2010). Song divergence of two Bluethroat subspecies (*Luscinia s. svecica* and *L. s. cyanecula*). *Ornis Fennica*, *87*(4), 168–179.

Vehrencamp, S. L. (2000). Handicap, index, and conventional signal elements of bird song. In Y. Espmark, T. Amundsen, & G. Rosenqvist (Ed.), *Animal signals: signalling and signal design in animal communication* (1. vyd., s. 277–300). Trondheim: Tapir Academic Press.

Verzijden, M. N., Ripmeester, E. A. P., Ohms, V. R., Snelderwaard, P., & Slabbekoorn, H. (2010). Immediate spectral flexibility in singing chiffchaffs during experimental exposure to highway noise. *Journal of Experimental Biology*, *213*(15), 2575–2581. doi:10.1242/jeb.038299

Wällschlager, D. (1980). Correlation of song frequency and body weight in passerine birds. *Experientia*, *36*(4), 412–412. doi:10.1007/BF01975119

Wesolowski, T. (1987). Polygyny in three temperate forest passerines (with a critical reevaluation of hypotheses for the evolution of polygyny). *Acta Ornithologica*, *23*, 273–302.

Chapter 4

Being angry, singing fast? Signalling of aggressive motivation by syllable rate in a songbird with slow song

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Being angry, singing fast? Signalling of aggressive motivation by syllable rate in a songbird with slow song

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Abstract

Syllable rate has been shown to play a role in male-male aggressive interactions and has been proposed to serve as a male quality indicator in several bird species. In those with fast syllable rates, males often increase rates when singing in aggressive context, and respond differently to test stimuli of varying rates. We asked whether the syllable rate fulfils a similar signalling function in the chiffchaff (*Phylloscopus collybita*), a songbird species with a slow syllable rate. We confronted 36 chiffchaff males with one of three playback types differing in syllable rate: control (non-manipulated rate), fast, or slow (artificially increased and decreased syllable rate, respectively). We recorded tested males' songs and behaviour before and during the experiment. Our results indicate that syllable rate might be an aggressive signal in chiffchaff. Males that physically attacked the loudspeaker during experiments sang faster songs spontaneously, and those that continued singing during the playback responded to fast and non-manipulated stimuli with substantial increase of syllable rate. Indirect evidence further suggests that syllable rate in chiffchaff is unlikely constrained by respiratory demands; thus, we propose

that syllable rate in this species functions as a conventional signal of male aggressiveness rather than an index of quality.

Keywords: bird song, syllable rate, vocal performance, male-male competition, male quality, chiffchaff, *Phylloscopus collybita*, playback experiment, conventional signalling, personality

Introduction

Bird song has an important function both in mate attraction and territorial defence (Catchpole and Slater 2008; Collins 2004). It is well documented that both males and females are able to assess qualities of singers based on their song performance (Collins 2004; Searcy and Nowicki 2005; ten Cate et al. 2002). This is only possible due to different costs (e.g., production costs, time costs, receiver retaliation) or constraints associated with the production of different song variants, which guarantees their signalling reliability (Gil and Gahr 2002; ten Cate et al. 2002; Vehrencamp 2000).

Certain temporal characteristics of songs, especially of repeated elements (expressed as syllable or trill rate), are particularly suitable candidates to reliably reflect intrinsic male qualities (Vallet et al. 1998). Good performance of such song structures apparently depends on fine co-ordination of syringeal, respiratory, and vocal tract muscles, and has been documented to be constrained by respiratory demands (Podos and Nowicki 2004; Suthers et al. 1999). To allow continuous song renditions, birds insert minibreaths between syllables (Calder 1970; Hartley and Suthers 1989). In species examined so far, longer syllables are accompanied by longer minibreaths (Cardoso et al. 2007b; Hartley and Suthers 1989) or deeper minibreaths (Wild et al. 1998), probably compensating for greater air loss during production of longer syllables (Franz and Goller 2003; Hartley and Suthers 1989). Physiological constraints on the rate of syllable delivery, experimentally documented by Podos (1996), predetermine the syllable rate as a candidate for an index signal of male quality.

The signalling function of the syllable rate has mainly been studied in combination with the song bandwidth, as a compound song performance trait (e.g. Ballentine et al. 2004; Cramer and Price 2007; DuBois et al. 2011; Illes et al. 2006). Several studies found an increase in subjects' song vocal performances from a non-aggressive to aggressive context and speculated that this effect is due to singer emphasizing its own qualities (Beebee 2004b, 2004a; Cardoso et al. 2009; DuBois et al. 2009). Studies testing whether variation in the relevant parameters of song vocal performance is meaningful to receivers found different responses of females (Ballentine et al. 2004) as well as males (Cramer and Price 2007; de Kort et al. 2009; DuBois et al. 2011; Illes et al. 2006), generally supporting the hypothesis that the vocal performance may signal male quality.

Both parameters constituting performance (bandwidth and syllable rate) may be also treated independently, as slower trill types (i.e., longer syllables) may accumulate more frequency modulation intrinsically (Cardoso et al. 2007b). Therefore, syllable rate alone could be a parameter important for the signalling process in some species (Cardoso et al. 2007b). Within *Serinus* spp., characterised by very fast songs, syllable rate is apparently used for assessing singer's qualities by both females (Cardoso et al. 2007a; Drăgănoiu et al. 2002; Vallet and Kreutzer 1995) and males (Cardoso et al. 2007a). To our knowledge, experiments testing if the changes in syllable rate are meaningful to rivals in bird species with much slower rates are lacking.

The chiffchaff (*Phylloscopus collybita*) is suitable for such a test, as it is a common species with a simple song of rather slow syllable rate (mostly ranging between 2.6 and 3.4 Hz; P. Linhart, unpublished data). Linhart et al. (2012) recently documented for this species that peak frequency of songs conveys information about the fighting ability, while song length probably signals motivation to escalate the conflict. However, males obviously modify also the song tempo during male-male interactions (P. Linhart and P. Jaška, pers. obs.). Therefore, we hypothesized that syllable rate might be another component of signalling in the context of male-male competition in chiffchaff. Given the relatively slow syllable rate of this species, it was nevertheless unclear whether the apparent increase is sufficiently demanding to signal male quality.

Based on these hypotheses, we made the following predictions to be experimentally evaluated. First, if syllable rate functions as a signal of aggression (Searcy and Beecher 2009), it should increase during the playback stimulation, and it should correlate positively with the intensity of singer's response. Receiving males should also discriminate between stimulus types with different syllable rates. Second, if the syllable rate reflects male quality through song performance constrained by respiratory demands, longer syllables should be followed by longer syllable gaps. (However, other potential physiological constraints, such as those imposed by syrinx and oropharyngeal musculature or mechanics, would not be detectable by this approach.)

Materials and methods

Study site and population

Our study was conducted at the edge of České Budějovice, South Bohemia, Czech Republic (48° 59.5'N, 14° 26.5'E). The study area (ca. 1 km^2) consists of a wooded marshland with ponds and stands of willow (*Salix*), birch (*Betula*), and aspen (*Populus*) trees, and some old oak (*Quercus*) avenues at the edges. The first chiffchaff males arrive to the area in the middle of March; after the peak of arrival by the end of March, there are about 80 breeding pairs. Males sing and defend their territories throughout the breeding season until the end of June. The first eggs are laid in the second half of April, hatching occurs from early May.

We conducted playback experiments on 36 males from April 22 to May 3, 2010. This period corresponds to fertile and post-fertile period of chiffchaff females, when the singing activity of males reaches its peak (Rodrigues 1996). To avoid testing the same male twice, each bird was lured into a mist net (Ecotone, Gdynia) and colour-banded immediately after conducting playback experiments and subsequent song recordings. All birds were released in good condition immediately after banding and noting basic physical parameters (tarsus length and weight), within 5 minutes after capture. Banding and field playback experiments were approved by the Czech Bird Ringing Centre (licence no. 1067), and the Czech Animal Welfare Commission (permission no. 7956/2008-30).

Preparation of playback stimuli and experimental design

For preparation of playback stimuli, we used songs of 12 chiffchaff males recorded at our study site in 2006 or 2007, using a Marantz PMD660 solid state recorder (sampling frequency 44,100 Hz) and a Sennheiser ME67 directional microphone, equipped with a Rycote Softie windshield. For each male, we selected one minute of high recording quality when the male was continuously singing (song rate: $\bar{x} \pm$ SD = 7.21 ± 1.24 songs. min^{-1}). These songs were processed in Avisoft SASLab Pro software (Raimund Specht, Berlin) to prepare the playback stimuli. First, all recordings were down-sampled to a sampling rate of 22,050 Hz, band-pass filtered between 1,500 Hz and 9,000 Hz (well outside the frequency range of chiffchaff song: ca 2500 – 8000 Hz) and standardized to 90% of maximum amplitude. This standardized set of songs was used to generate three experimental categories of manipulated songs: CONTROL (songs without manipulation), SLOW, and FAST, leading to 3 x 12 = 36 different playback stimuli. FAST stimuli were prepared by shortening of syllable gap by 0.04 s (i.e., 0.04 s long section between syllables was cut out of the recording). To create SLOW stimuli, we inserted additional section of silence between the syllables, thus increasing the syllable gap by 0.04 s. We chose the manipulation interval of 0.04 s because it roughly corresponds to half of the range between minimal and maximal mean values of syllable interval in spontaneous songs in the population studied (Fig. 1). Thus, we obtained experimental stimuli at and slightly beyond both extremes of natural variation in spontaneous syllable intervals (Fig. 1). Such songs were likely to be still recognised as conspecific, as chiffchaffs were shown to show species-specific responses even to songs with highly altered syllable interval (Schubert 1971).

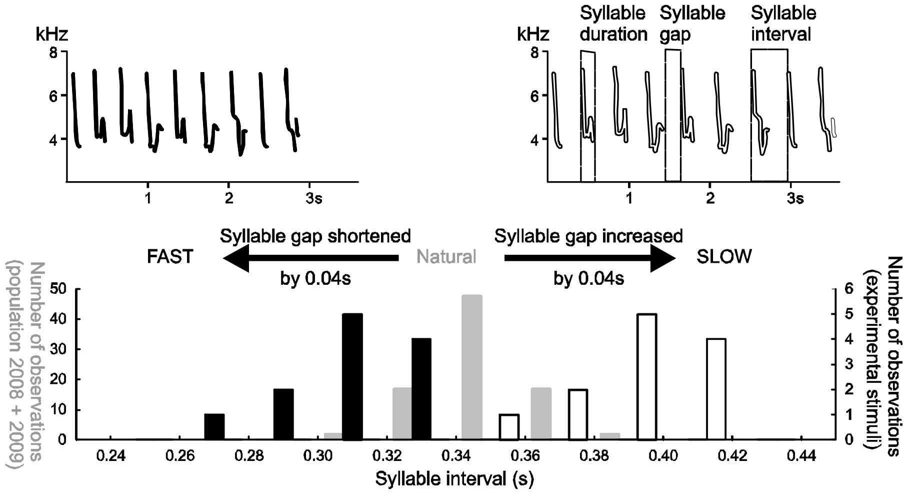

Figure 1. Preparation of experimental stimuli and song measures taken. The upper part shows two spectrograms of the same chiffchaff song in FAST (left) and SLOW variant (right). Three measurements were taken: syllable duration, syllable gap, and syllable interval as indicated. Histogram in the lower part of the picture shows distribution of syllable interval values within our study population (Natural) from two years 2008 (Linhart et al., unpublished data) and 2009 (current dataset) and within experimental songs (FAST and SLOW).

All playback experiments were conducted in the morning within six hours after sunrise, i.e., between 6 and 12 AM. Each of the 36 experimental songs was played back only once, to a single chiffchaff male. Neighbouring males were never tested in two successive experiments. Song stimuli were played back in a loop for five minutes from a JBL Control 1x loudspeaker at a peak volume of 80 dB(A) SPL, measured at 1 m distance (using a Voltcraft SL-200 sound level meter, with Fast response setting). The loudspeaker was placed inside the tested male's territory. We started the playback from a shelter 10 to 15 m away from the loudspeaker while the focal male was in sight and singing.

The response behaviour of tested males was observed for five minutes during the playback. The following behavioural parameters were scored: time spent within 2 meters from the speaker (s), latency of approach within 2 meters (s), number of fly-overs (the focal male flying over the loudspeaker), latency of fly-overs (s), time spent attacking (focal male being in physical contact with loudspeaker), and latency of attack (s). Distance of the male from the speaker was estimated with the help of 2-meter markers on the ground around the speaker. We particularly focused on occurrence of physical attacks, as these are the most intense and clearly aggressive type of response, while other responses that may be considered a general “agonistic” response do not have to be necessarily aggressive (Searcy and Beecher 2009).

Some recent studies based on playback experiments used an artificial dummy or a taxidermic mount together with acoustic stimuli (Ballentine et al. 2008; Petrusková et al. 2007; Searcy et al. 2006; Turčoková et al. 2011), and even recommended using such visual stimulus to allow the tested bird to focus aggressive response (Akçay et al. 2011; Petrusková et al. 2008). We did not use mount in the experiments, however, our observations suggest that chiffchaff males respond similarly during experiments with and without a taxidermic mount. The loudspeaker attacks are very similar to mount attacks; the latter are nevertheless more severe (up to the intensity that may quickly result in a destruction of the mount). In both cases, similar set of behavioural reactions is exhibited by males: there may be air strikes, i.e., male flying close over the loudspeaker and kicking it, or longer landings on the loudspeaker occasionally accompanied by pecking (P. Linhart, pers. obs.).

Song analyses

We recorded songs of each tested male for 5 min in three experimental stages: before (spontaneous songs), during (aggressive context), and after playback. We typically obtained at least 20 - 30 songs during the 5 min of recording. For each individual, we analysed last 15 songs of sufficient recording quality (excluding those overlapped by singing birds or other sounds) from the recordings before the playback, up to first 15 songs during the playback, and first 15 songs after the playback (i.e., on average within 148 s after the end of the playback; minimum = 75 s, maximum = 257 s). All males were recorded from a position as close as possible to the song post, which was usually within a distance of 15 meters.

Before analyses of song parameters, the recordings were processed in Avisoft SASLab Pro the same way as the songs used for playbacks (down-sampled, band-pass filtered, and normalized; see above). We used the Avisoft SASLab Pro Automatic measurements tool to assess temporal characteristics of each syllable, taking -15 dB from the maximum amplitude in the song as the lower cut-off point for syllable detection. Results of the tool were checked visually and syllables that were not detected correctly were omitted from further analyses. Spectrogram parameters were set as follows: FFT-length: 512, frame: 100%, window: Hamming, overlap: 87.5%; this resulted in a temporal resolution of 1.5 ms and a spectral resolution of 86 Hz.

As the main measure of repetition rate in chiffchaff songs, we measured syllable interval (SI, from the start of the syllable to the start of the following syllable; see Fig. 1). Additionally, we also measured syllable duration (SD, time from the start to the end of the syllable) and syllable gap (SG, time from the end of the syllable to the beginning of the following syllable), which constitute together the syllable interval (i.e., SI = SL + SG; Fig. 1).

Most previous studies consider the syllable rate jointly with the frequency bandwidth, using a compound song performance or vocal deviance parameter, because it has been shown that there is a trade-off between syllable rate and song bandwidth (Podos 1997). In chiffchaffs, there is also a negative correlation between bandwidth and syllable rate; however, it is not as prominent as in other species, and songs of almost all bandwidths can be produced at almost every syllable rate (see Supplementary material 1). Furthermore, the bandwidth is much more liable to bias due to different amplitudes of recorded songs (Zollinger et al. 2012) than the syllable rate based on syllable interval (measured as given above, and thus well comparable among different songs). Therefore, we considered appropriate to examine syllable rate alone.

Statistical analyses

The statistical analyses were done in R (R Core Team 2012). All statistical tests were two-tailed. Characteristics of attacking and non-attacking males were compared by a two sample *t*-test (or Mann-Whitney *U* test as a non-parametric alternative). A Fisher exact test was used to evaluate whether the occurrence of physical attacks was associated with singing during the playback.

We used linear mixed effect model (LME) to compare average syllable intervals (SI) in songs of chiffchaff males before, during, and after playback stimulation. The stimulus type and experimental stage were used as fixed factors, and male identity as a random factor in the analysis; Tukey's test was used for post-hoc comparisons performed by the 'ghlt' function from 'multcomp' R package (Hothorn et al. 2008). Residuals for the model approximated a normal distribution, were homoscedastic, and did not increase, decrease, or vary nonlinearly across the range of predicted values.

To investigate whether syllable rate is constrained by respiratory demands, we used a paired t-test to compare if syllable gap (SG), syllable duration (SD), or both of these parameters change between songs sung spontaneously and in aggressive context (i.e., before and during the playbacks, respectively). To test the relationship between syllable duration and subsequent syllable gap, we first computed linear regressions of syllable gap on syllable duration for each male separately. Further, we used one sample *t*-test to evaluate whether the obtained Pearson correlation coefficients are different from zero, and a paired *t*-test was used to test whether correlation coefficients changed between songs sung before and during the playback.

To compare the response strength of tested males towards the three stimulus types (SLOW/CONTROL/FAST), we used one-way ANOVA when the response variable values were normally distributed (time spent within 2 m, latency within 2 m, number of flyovers, square root transformed latency of flyovers) and Kruskal-Wallis test in case they were not (time spent attacking, latency of attacks). Subsequently, we also used *t*-test and Mann-Whitney U test to compare responses to FAST and SLOW stimuli and computed effect sizes from this comparison to provide information comparable to other studies that mostly test for differences between the variants of stimuli. Reported effect sizes (Cohen's *d*) were computed according to suggestions and scripts provided by Nakagawa & Cuthill (2007).

Results

Responses of males to experimental stimuli

There were no significant differences in the syllable rate between spontaneously sung songs of the 36 tested males (recorded before playback) and the selection of experimental songs before manipulation for the syllable rate (*t*-test: t_{46} = −0.31, *P* = 0.755). Males in the three treatment groups (SLOW, CONTROL, FAST) differed neither in physical parameters (tarsus length: ANOVA: $F_{2,33}$ = 0.36, *P* = 0.70, weight: ANOVA: $F_{2,33}$ = 0.84, *P* = 0.44) nor in the syllable rates in their spontaneous songs (syllable interval: ANOVA: $F_{2,32}$ = 0.14, *P* = 0.87). All tested males showed non-vocal responses of various intensities to playbacks of all three playback types; typical responses included close approach, flyovers and occasionally even attacks on the loudspeaker (see Table 1).

Physical attack was the most intensive response observed. Altogether, 13 out of 36 tested males physically attacked the loudspeaker; these attacking males were equally distributed among all three tested groups (SLOW: 4 /12 males attacked; CONTROL: 5 /12; FAST: 4 /12). There were no significant differences between attacking and non-attacking males either in date when tested (expressed as number of days from 1th April; Mann-Whitney *U* test: *U* = 122.5, N_{attack} = 13, $N_{\text{non-attack}}$ = 23, *P* = 0.48) or in time of the day when tested (expressed as number of minutes since sunrise; *t*-test: t_{34} = −1.49, *P* = 0.15). The two groups also did not differ significantly in body size measures (tarsus length: t_{34} = −1.14, *P* = 0.26; weight: t_{34} = −0.80, *P* = 0.43), and there were no differences in most other non-vocal response measures (t-test, Mann-Whitney *U* tests: all *P* > 0.28).

Table 1. Effect of playback type on behaviour response strength measures. Sample size was 12 males for each of the three treatments. One-way ANOVA (F) and t-test (t) were used when data were normally distributed. Latency of flyovers was square-root transformed to obtain normal distribution. Kruskal-Wallis test ($\chi 2$) and Mann-Whitney U test (Z) were used for non-normally distributed data. Effect sizes for the FAST vs. SLOW comparisons are also shown (d – Cohen's d, CI – upper and lower 95% confidence intervals for Cohen's d). Cohen's d for non-normally distributed data was computed on ranked data.

	SLOW		**CONTROL**		**FAST**				**SLOW vs FAST comparison**				
	mean	SD	mean	SD	mean	SD	*F*, c^2	*P*	*t*, *Z*	*P*	*d*	*CI*	
Time within 2m (s)	89.7	50.3	92.8	65.3	71.3	57.4	0.48	0.62	0.84	0.41	0.34	-0.5	1.18
Number of flyovers (n)	12.8	7.1	12.3	7.2	11.8	7.4	0.06	0.94	0.34	0.74	0.14	-0.7	0.98
Latency within 2m (s)	97.6	41.4	109.1	50.8	124.9	63.3	0.82	0.45	-1.25	0.224	-0.51	-1.36	0.34
Latency of flyovers (s)	10.4	3.2	8.8	3.1	11	3.5	1.35	0.27	-0.37	0.71	-0.15	-0.99	0.69
	rank		rank		rank								
Time attacking (s)	14.3		14.8		13.4		0.48	0.79	0.14	0.89	0.24	-0.6	1.08
Attack latency (s)	20		16.2		19.3		1.13	0.57	-0.13	0.89	0.08	-0.76	0.91

The exception was the time spent within two meters from loudspeaker, where the attacking males spent significantly more time than non-attacking ones (average time spent within 2 m: attacking males = 126 s, non-attacking males = 60 s; *t*-test: $t_{33} = 3.54$, $d = 1.35$, $CI = 0.59$ to 2.11, $P = 0.002$). Finally, neither did attacking and non-attacking males differ in their average spontaneous song length (*t*-test: $t_{34} = 0.76$, $P = 0.46$), nor did experimental songs they received differ in average length (*t*-test: $t_{34} = -0.45$, $P = 0.66$), confirming that the song length did not confound our results (see Linhart et al. 2012).

While males sang frequently before and after playback, nine males out of 36 did not sing at all during playback; such non-singing birds were more frequent among attacking than non-attacking males (singing / non-singing males: attacking 7 / 6, non-attacking 20 / 3; Fisher exact test: $P = 0.046$). Total song output for singing males (number of songs) tended to be negatively related to time spent within 2 m (Spearman rank correlation: $r = -0.34$, $N = 27$, $P = 0.087$). Only 16 males sang five or more songs during playback (SLOW: 5 males; CONTROL: 6 males; FAST: 5 males); these were included into analyses of context-dependent syllable interval changes. Males with fewer songs were excluded, as the estimate of syllable interval in their songs would not be reliable.

All but one response variable (attack latency) indicated slightly more intense response towards SLOW playback stimulus (Table 1). However, the type of playback stimulus did not significantly affect the response intensity of males in any evaluated response strength measures, either when all three treatments were compared or in the SLOW / FAST comparison.

Relationship between syllable interval and attack occurrence

We compared SI before playback of attacking ($N = 13$) and non-attacking males ($N = 22$) to evaluate whether syllable rate is related to male aggressive behaviour (one male, which did not sing enough songs before playback, was excluded from this analysis). Attacking males sang significantly faster before playback than non-attacking males (Fig. 2;

average SI before playback: attacking males = 0.344 s, non-attacking males = 0.354 s; *t*-test: $t_{33} = -2.47$, $d = -0.86$, $CI = -1.58$ to -0.15, $P = 0.019$). We could not compare SI in songs recorded during playbacks for attacking and non-attacking males because attacking males sung little or not at all during playback (there were only three attacking males that sang five or more songs). For 16 males (SLOW: 5 males; CONTROL: 6 males; FAST: 5 males) that sung more than 5 songs, males singing faster during playback had a tendency to spend more time within 2 m from the loudspeaker, though this relationship was not significant (Spearman rank correlation: $r = -0.25$, N = 16, p = 0.34).

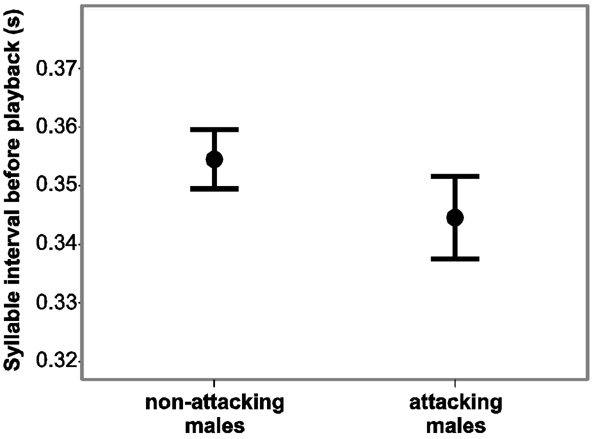

Figure 2. Syllable interval in spontaneous songs (BEFORE playback) of attacking (N = 13) and non-attacking (N = 22) males. Means and 95% confidence intervals are displayed. The difference between the two groups is significant (see text).

Syllable interval change in aggressive context

Males dramatically changed their song syllable rate between the three experimental stages: before, during, and after playback (LME: $F_{8,26} = 25.54$, $P < 0.001$, see Fig. 3). They significantly shortened SI during playback but subsequently slowed down the song tempo by elongating SI after playback, so it was again comparable to SI before playback (average SI: BEFORE = 0.350 s, DURING = 0.314 s, AFTER = 0.340 s; post-hoc tests: BEFORE x DURING: $P < 0.001$, $d = -1.74$, $CI = -2.67$ to -0.81; BEFORE x AFTER: $P =$

0.23, *d* = −0.73, *CI* = −1.31 to 0.15; DURING x AFTER: *P* < 0.001, *d* = 1.31, *CI* = 0.43 to 2.20).

Overall, the syllable rates of males that received SLOW, CONTROL or FAST stimuli were comparable; SI in songs of males from the three playback treatment groups was not significantly different (LME: $F_{8,26}$= 0.74, *P* = 0.50). However, stimulus type affected how much the males increased their syllable rates during playback; there was a significant interaction between playback treatment and context (LME: $F_{8,26}$= 2.78, *P* = 0.048). The males shortened SI significantly only in response to CONTROL and FAST stimuli (post-hoc tests: CONTROL-BEFORE vs. CONTROL-DURING: *P* < 0.001; FAST-BEFORE vs. FAST-DURING: *P* < 0.001; SLOW-BEFORE vs. SLOW-DURING: *P* = 0.87, see Fig. 3).

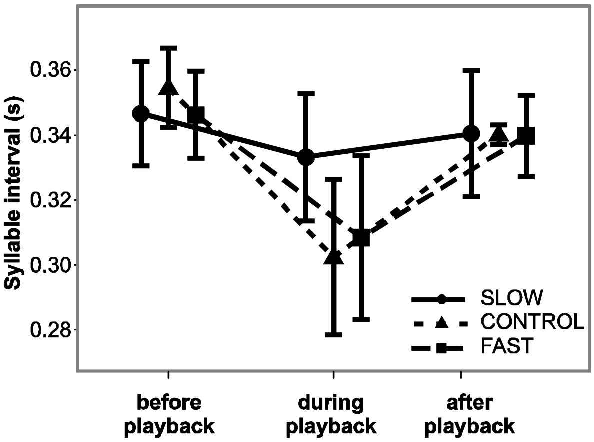

Figure 3. Syllable intervals in the three experimental stages before, during and after playback according to the three playback types: SLOW (circle; N = 5), CONTROL (triangle; N = 6), and FAST (square; N = 5). The songs recorded during playback represent those sung in aggressive context. Means and 95% confidence intervals are displayed.

Syllable rate and respiratory demands

Several findings indicated that the syllable rate in songs of the tested males was not constrained markedly by respiratory demands. Males were able to shorten syllable gap significantly (BEFORE vs. DURING: t_{15} = 5.06, *P* < 0.001) while the syllable duration remained the same in both contexts (BEFORE vs. DURING: t_{15} = 0.79, P = 0.44) suggesting that there is no trade-off between duration of syllables and gaps.

Further, longer syllables were followed by shorter, not longer, gaps in songs before as well as during the playback. In songs before playback, syllable gap (SG) was significantly negatively correlated with syllable duration (SD) in all but one male (for that particular male the negative correlation was non-significant). The mean correlation coefficient between SG and SL for 16 males was −0.58 (range = −0.81 to −0.18) and was significantly smaller than zero (one-sample *t*-test: t_{15} = −11.75, *CI* = −0.68 to −0.47, $P < 0.001$).

In songs during playback, syllable gap (SG) was also significantly negatively correlated with syllable duration (SD) in all but four males (for those, three correlation coefficients were negative but non-significant, and one correlation coefficient was positive but non-significant). The mean correlation coefficient between SG and SL for 16 males was −0.55 (range = −0.84 to 0.27) and was significantly smaller than zero (one-sample *t*-test: t_{15} = −7.97, *CI* = −0.69 to −0.40, $P < 0.001$). Correlation coefficients did not increase or decrease systematically between songs recorded before and playbacks (paired *t*-test: t_{15} = −0.35, *CI* = −0.22 to 0.16, P = 0.729).

Discussion

Our results suggest that syllable rate might play a role of aggressive signal during territorial encounters in chiffchaffs. The three criteria suggested by Searcy & Beecher (2009) for aggressive signal (context, predictive, and response criterion) seem to be met at least partially. Tested males that continued to sing during simulated playback intrusion responded with substantial increase of syllable rate. The males also apparently considered differences between playbacks of different syllable rates, as they significantly increased the syllable rates only in response to non-manipulated and fast syllable rates but not to artificially slowed-down songs. We could not answer the question whether syllable rate during the playback predicts attack. However, males that attacked the loudspeaker sang faster spontaneous songs, which points to a potential link between faster songs and male aggressive behaviour. We did not find any evidence for syllable rate being constrained by respiratory demands.

Syllable rate and respiratory demands in chiffchaffs

Negative relationship between duration of syllables and subsequent silent periods in slow chiffchaff songs (2.6 – 3.4 Hz) contrasts markedly with the positive relationship found in much faster singing canaries (3 – 35 Hz; Hartley and Suthers 1989) and dark-eyed juncos (6 – 22 Hz; Cardoso et al. 2007a). Apparently, chiffchaff males can comfortably compensate for the air loss associated with phonation during syllable gaps regardless of the singing rate.

Further, individual chiffchaffs were able to substantially modulate their syllable rates during simulated rival challenge; in comparison with spontaneous singing, they decreased their syllable interval on average by 71% (average decrease of syllable interval = 0.036 s) of between-male variation in this parameter (average syllable intervals before playback in 16 males with known syllable interval change = 0.324 – 0.375 s). Although quality index signals can be modulated to some extent (see Searcy and Nowicki 2005 for review), the change of syllable rate in chiffchaffs is very high in contrast with birds for which vocal performance has been shown to be an index signal of quality (Ballentine et al. 2004; DuBois et al. 2011, 2009).

Due to the absence of apparent respiratory constraints for syllable rate, and to the extent to which chiffchaffs are able to modify this song characteristic, we suggest that syllable rate might rather serve as a conventional signal in this species. Indeed, song rhythm and its changes may be an efficient way of conventional signalling in species with simple vocalizations (Ręk and Osiejuk 2010). However, other possible factors limiting the syllable rate in chiffchaff, such as constraints imposed by syrinx and oropharyngeal musculature or mechanics, cannot be ruled out unless controlled laboratory studies are carried out.

Syllable rate as a predictor of attack

Different vocal as well as non-vocal behaviours were found to predict conflict escalation in songbirds (see Searcy and Beecher 2009 for review). A physical attack during territorial encounters is considered as the highest level of aggressive behaviour (e.g., Dunn et al. 2004; Petrusková et al. 2007; Searcy et al. 2006), and conventional signalling allows avoiding such risky behaviour (Hurd and Enquist 1998; Ręk and Osiejuk 2010). Recently, particular attention has been paid to soft vocalizations that predict attack in various species (Ballentine et al. 2008; Ręk and Osiejuk 2011; Searcy et al. 2006). We suggest that high syllable rate in spontaneous songs might be a relevant predictor of attack behaviour too and chiffchaffs might use syllable rate when assessing their rivals' temper. Although the average difference between syllable intervals in attacking and non-attacking males seems to be small (10 ms), it represents about 10% of the overall variation within the studied population (see Fig. 1), and thus it is likely that chiffchaffs may be able to perceive such differences if relevant in the context of territorial contests. Bobwhite quails (*Colinus virginianus*) can discriminate artificial tones in range from 400 ms to 600 ms differing in duration by only 3% (Brown and Bailey 1990), which corresponds to 10 ms difference in syllable intervals between attacking and non-attacking chiffchaff males. Some other bird species, however, showed less pronounced abilities in experiments testing temporal discrimination of artificial tones or manipulated songs (Dooling and Haskell 1978; Maier and Klump 1990).

We were not able to show that increased syllable rate during playback predicts attack, as observed for other aggressive signals for example by Searcy et al. (2006) and Ballentine et al. (2008). Singing response was inhibited during playback especially in attacking chiffchaffs. Apparently, chiffchaffs stop signalling at certain level of fight escalation; this is also indicated by a negative correlation of total song output and duration spent within 2 m from the loudspeaker. The response to territorial intrusion is often graded and uses multiple signalling levels. For example, in black-capped chickadees, increase in song rate was the first step in escalating aggression, but then the physical attack was advertised by specific “gargle” calls (Baker et al. 2012). We presume that a graded response may be used by chiffchaff males as well. Attacking males, which had shorter syllable intervals in spontaneous songs, usually stopped singing during the playback and attacked, while those singing during the experiment reacted by shortening SI (especially when exposed to CONTROL or FAST playback stimuli). This was also true for two out of three attacking males whose songs were analysed – firstly they increased their already high syllable rates and subsequently attacked the speaker (the last bird, which did not react by increasing syllable rate, was exposed to SLOW stimulus and its spontaneous syllable rate was already fast). Thus, syllable rate in the aggressive context might primarily reflect response intensity during territorial conflicts of lower escalation level. This seems to be the case, e.g., for banded wrens (*Thryophilus pleurostictus*), for which syllable rate increase during song playback positively correlated with aggressive response expressed as time spent within 15 m from the loudspeaker (Vehrencamp et al. 2013). Apparently, these birds were much less likely to escalate the conflict to close approaches and physical attacks than chiffchaffs, at least in experiments performed by Vehrencamp et al. (2013).

Higher spontaneous syllable rate in attacking than non-attacking males cannot be explained by aggressive context. However, it may reflect overall rather than immediate willingness to escalate conflict, and could be explained by differences in personalities of tested males (e.g., Groothuis and Carere 2005; Sih and Bell 2008). Although data relating bird personalities and singing behaviour are relatively scarce, Amy et al. (2010) showed in playback experiments with great tits (*Parus major*) clear differences not only in song production but also in loudspeaker approach corresponding with male’s personalities. Males with higher exploratory scores sang fewer but longer songs with higher element rates, and approached loudspeaker for longer time and closer than did males with lower scores; this matches differences in behaviour of attacking and non-attacking males in our experiments. The strength of non-vocal response of chiffchaffs could then reflect males’ personalities – shy individuals, unwilling to attack physically, may have mostly increased syllable rate, while the bolder ones rather reduced or stopped singing, and approached or directly attacked the loudspeaker.

Significance of syllable rate for males

Chiffchaff males apparently differentiated between playback treatments, as they increased their syllable rate in response to FAST and CONTROL songs, but not in response to SLOW songs. In contrast with other playback-based studies focusing on response to song variants with different syllable rates (Cardoso et al. 2007a; Illes et al. 2006), we did not find differences in other behavioural measures of response between the playback treatments. Overall, chiffchaffs showed very strong responses, including physical attacks, in more than one third of the cases. It is therefore possible that, regardless the type of song played back, the non-vocal responses to the simulated intrusion in the territory centre of most tested males were maximised, depending on tested male quality or personality. Such “ceiling effect” seems common for some types of signals (Stoddard et al. 1991). In those cases, playback from the territory boundary might better reveal possible effects of playback type on males’ responses. However, previous playback experiments on chiffchaff with altered song length and song pitch (Linhart et al. 2012) elicited different behavioural responses in the centre of territory. This might indicate that length and pitch are more important features of song for contesting chiffchaffs.

To conclude, we present several lines of evidence that syllable rate might be another component of aggressive signalling in chiffchaff. Studies on song performance focused their attention primarily on species with songs probably reaching their performance limits. We show that similar phenomena occur in a species with slow songs without an apparent production constraint, thus providing interesting comparative data. We suggest that syllable rate is a simple song trait that can be modified substantially (at least in some species) and instantly (from song to song or possibly even within a song) and as such it has a substantial potential to signal immediate changes in motivation in species with simple as well as complex songs.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.beproc. 2013.06.012.
(note; I also added Supplementary data after References for the purposes of PhD thesis)

References

Akçay, Ç., Tom, M. E., Holmes, D., Campbell, S. E., Beecher, M. D., 2011. Sing softly and carry a big stick: signals of aggressive intent in the song sparrow. Anim. Behav., 82, 377–382.

Amy, M., Sprau, P., Goede, P. de, Naguib, M., 2010. Effects of personality on territory defence in communication networks: a playback experiment with radio-tagged great tits. Proc. R. Soc. B., 277, 3685–3692.

Baker, T. M., Wilson, D. R., Mennill, D. J., 2012. Vocal signals predict attack during aggressive interactions in black-capped chickadees. Anim. Behav., 84, 965–974.

Ballentine, B., Hyman, J., Nowicki, S., 2004. Vocal performance influences female response to male bird song: an experimental test. Behav. Ecol., 15, 163–168.

Ballentine, B., Searcy, W. A., Nowicki, S., 2008. Reliable aggressive signalling in swamp sparrows. Anim. Behav., 75, 693–703.

Beebee, M. D., 2004a. The functions of multiple singing modes: experimental tests in yellow warblers, *Dendroica petechia*. Anim. Behav., 67, 1089–1097.

Beebee, M. D., 2004b. Variation in vocal performance in the songs of a wood-warbler: evidence for the function of distinct singing modes. Ethology, 110, 531–542.

Brown, L. J., Bailey, E. D., 1990. Duration discrimination in Northern bobwhite quail. Bioacoustics, 2, 317–326.

Calder, W. A., 1970. Respiration during song in canary (*Serinus canaria*). Comp. Biochem. Physiol., 32, 251–&.

Cardoso, G. C., Mota, P. G., Depraz, V., 2007a. Female and male serins (*Serinus serinus*) respond differently to derived song traits. Behav. Ecol. Sociobiol., 61, 1425–1436.

Cardoso, G. C., Atwell, J. W., Ketterson, E. D., Price, T. D., 2007b. Inferring performance in the songs of dark-eyed juncos (*Junco hyemalis*). Behav. Ecol., 18, 1051–1057.

Cardoso, G. C., Atwell, J. W., Ketterson, E. D., Price, T. D., 2009. Song types, song performance, and the use of repertoires in dark-eyed juncos (*Junco hyemalis*). Behav. Ecol., 20, 901–907.

Catchpole, C. K., Slater, P. J. B., 2008. Bird Song: Biological Themes and Variations, 2nd ed. Cambridge University Press, New York

Collins, S. A., 2004. Vocal fighting and flirting: the functions of birdsong, in: Marler, P. R., Slabbekoorn, H. (Eds.), Nature's music: the science of birdsong, Elsevier Academic Press, San Diego, CA, pp. 39–79.

Cramer, E. R. A., Price, J. J., 2007. Red-winged blackbirds *Ageliaus phoeniceus* respond differently to song types with different performance levels. J. Avian. Biol., 38, 122–127.

De Kort, S. R., Eldermire, E. R. B., Cramer, E. R. A., Vehrencamp, S. L., 2009. The deterrent effect of bird song in territory defense. Behav. Ecol., 20, 200–206.

Dooling, R. J., Haskell, R. J., 1978. Auditory duration discrimination in the parakeet (*Melopsittacus undulatus*). J. Acoust. Soc. Am., 63, 1640–1643.

Drăgănoiu, T. I., Nagle, L., Kreutzer, M., 2002. Directional female preference for an exaggerated male trait in canary (*Serinus canaria*) song. Proc. R. Soc. B., 269, 2525–2531.

DuBois, A. L., Nowicki, S., Searcy, W. A., 2009. Swamp sparrows modulate vocal performance in an aggressive context. Biol. Lett., 5, 163–165.

DuBois, A. L., Nowicki, S., Searcy, W. A., 2011. Discrimination of vocal performance by male swamp sparrows. Behav. Ecol. Sociobiol., 65, 717–726.

Dunn, M., Copelston, M., Workman, L., 2004. Trade-offs and seasonal variation in territorial defence and predator evasion in the European Robin *Erithacus rubecula*. Ibis, 146, 77–84.

Franz, M., Goller, F., 2003. Respiratory patterns and oxygen consumption in singing zebra finches. J. Exp. Biol., 206, 967–978.

Gil, D., Gahr, M., 2002. The honesty of bird song: multiple constraints for multiple traits. Trends Ecol. Evol., 17, 133–141.

Groothuis, T. G. G., Carere, C., 2005. Avian personalities: characterization and epigenesis. Neurosci. Biobehav. Rev., 29, 137–150.

Hartley, R. S., Suthers, R. A., 1989. Airflow and pressure during canary song: direct evidence for mini-breaths. J. Comp. Physiol. A, 165, 15–26.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical J., 50, 346–363.

Hurd, P. L., Enquist, M., 1998. Conventional signalling in aggressive interactions: the importance of temporal structure. J. Theor. Biol., 192, 197–211.

Illes, A. E., Hall, M. L., Vehrencamp, S. L., 2006. Vocal performance influences male receiver response in the banded wren. Proc. R. Soc. B., 273, 1907–1912.

Linhart, P., Slabbekoorn, H., Fuchs, R., 2012. The communicative significance of song frequency and song length in territorial chiffchaffs. Behav. Ecol., 23, 1338–1347.

Maier, E. H., Klump, G. M., 1990. Auditory duration discrimination in the European starling (*Sturnus vulgaris*). J. Acoust. Soc. Am., 88, 616–621.

Nakagawa, S., Cuthill, I. C., 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol. Rev., 82, 591–605.

Petrusková, T., Petrusek, A., Pavel, V., Fuchs, R., 2007. Territorial meadow pipit males (*Anthus pratensis*; Passeriformes) become more aggressive in female presence. Naturwissenschaften, 94, 643–650.

Petrusková, T., Petrusek, A., Pavel, V., Fuchs, R., 2008. When an alien sings at a rival's post: a passerine excited by conspecific stimulus may show aggressive behaviour towards heterospecific individuals. Folia Zool,, 57, 201–211.

Podos, J., 1996. Motor constraints on vocal development in a songbird. Anim. Behav., 51, 1061–1070.

Podos, J., 1997. A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes:Emberizidae). Evolution, 51, 537–551.

Podos, J., Nowicki, S., 2004. Performance limits on birdsong, in: Marler, P. R., Slabbekoorn, H. (Eds.), Nature's Music: The Science of Birdsong, Elsevier Academic Press, San Diego, CA, pp. 318–342.

R Core Team, 2012. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria

Ręk, P., Osiejuk, T. S., 2010. Sophistication and simplicity: conventional communication in a rudimentary system. Behav. Ecol., 21, 1203–1210.

Ręk, P., Osiejuk, T. S., 2011. Nonpasserine bird produces soft calls and pays retaliation cost. Behav. Ecol., 22, 657 –662.

Rodrigues, M., 1996. Song activity in the chiffchaff: territorial defence or mate guarding? Anim. Behav., 51, 709–716.

Searcy, W. A., Beecher, M. D., 2009. Song as an aggressive signal in songbirds. Anim. Behav., 78, 1281–1292.

Searcy, W. A., Nowicki, S., 2005. The Evolution of Animal Communication: Reliability and Deception in Signaling Systems, Princeton University Press, Princeton, New Jersey

Searcy, W. A., Anderson, R. C., Nowicki, S., 2006. Bird song as a signal of aggressive intent. Behav. Ecol. Sociobiol., 60, 234–241.

Schubert, G., 1971. Experimentelle Untersuchungen über die Artkennzeichnenden Parameter im Gesang des Zilpzalps, *Phylloscopus c. collybita* (Vieillot). Behaviour, 38, 289–314.

Sih, A., Bell, A. M., 2008. Insights for behavioral ecology from behavioral syndromes. Adv. Study. Behav., 38, 227–281.

Stoddard, P. K., Beecher, M. D., Horning, C. L., Campbell, S. E., 1991. Recognition of individual neighbors by song in the song sparrow, a species with song repertoires. Behav. Ecol. Sociobiol., 29, 211–215.

Suthers, R. A., Goller, F., Pytte, C., 1999. The neuromuscular control of birdsong. Philos. Trans. R. Soc. B, 354, 927–939.

Ten Cate, C., Slabbekoorn, H., Ballintijn, M. R., 2002. Birdsong and male-male competition: Causes and consequences of vocal variability in the collared dove (*Streptopelia decaocto*). Adv. Study. Behav., 31, 31–75.

Turčoková, L., Pavel, V., Chutný, B., Petrusek, A., Petrusková, T., 2011. Differential response of males of a subarctic population of Bluethroat *Luscinina svecica svecica* to playbacks of their own and foreign subspecies. J. Ornithol., 152, 975–982.

Vallet, E., Kreutzer, M., 1995. Female canaries are sexually responsive to special song phrases. Anim. Behav., 49, 1603–1610.

Vallet, E., Beme, I., Kreutzer, M., 1998. Two-note syllables in canary songs elicit high levels of sexual display. Anim. Behav., 55, 291–297.

Vehrencamp, S. L., 2000. Handicap, index, and conventional signal elements of bird song, in: Espmark, Y., Amundsen, T., Rosenqvist, G. (Eds.), Animal signals: signalling and signal design in animal communication, 1st ed. Tapir Academic Press, Trondheim, pp. 277–300.

Vehrencamp, S. L., Yantachka, J., Hall, M. L., Kort, S. R. de, 2013. Trill performance components vary with age, season, and motivation in the banded wren. Behav. Ecol. Sociobiol., 67, 409–419.

Wild, J. M., Goller, F., Suthers, R. A., 1998. Inspiratory muscle activity during bird song. J. Neurobiol., 36, 441–453.

Zollinger, S. A., Podos, J., Nemeth, E., Goller, F., Brumm, H., 2012. On the relationship between, and measurement of, amplitude and frequency in birdsong. Anim. Behav., 84, e1–e9.

Appendix A. Supplementary data

Supplementary material 1

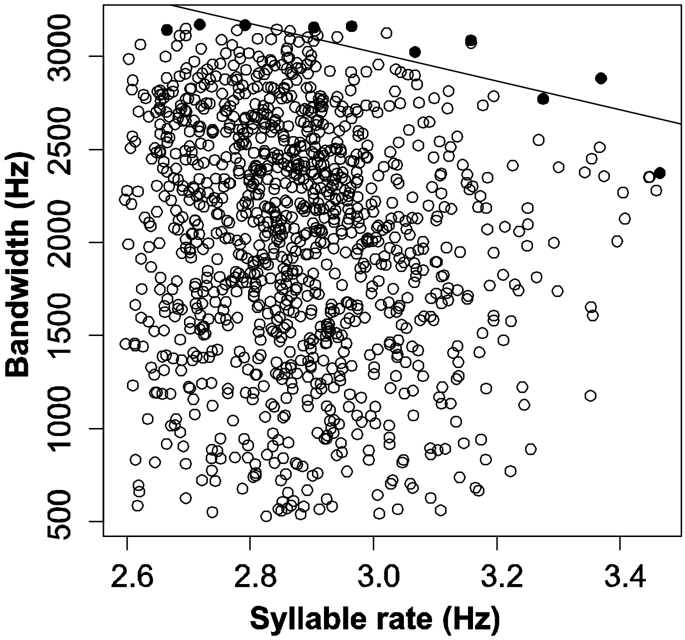

Figure. Trade-off between syllable rate and bandwidth in 1129 spontaneous songs collected from 84 males during breeding seasons 2008 and 2009. Data points used for computing upper-bound regression are shown as black filled circles and upper-bound regression line is also shown (intercept = 5336.5, ß = -771.2, F = 17.72, p = 0.003).Negative correlation between syllable rate and bandwidth was also significant (Pearson r = -0.11; p < 0.001). Despite the existence of trade-off between bandwidth and syllable rate, it is apparent that most of the bandwidths can be sung at any syllable rate.

Methods:

To assess the trade-off between song syllable rate and song bandwidth we followed methods described by Podos (1997). We averaged individual syllable bandwidths within a song to compute song bandwidth; minimum and maximum frequency within each syllable were assessed at threshold -15 dB from the maximum amplitude level. Song bandwidths ranged from 527 Hz to 3171 Hz.

We got song syllable rate by inversing average syllable interval within a song (= time from syllable onset to the onset of following syllable); this measure is equivalent to syllable rate got by the equation: (number of syllables – 1) / (song length – syllable length) used by Illes et al. (2006) and it describes how many syllables can be produced per second. Syllable rate ranged from 2.60 to 3.47 Hz.

To find the data points to be used for upper-bound regression, we split data by their syllable rate into 10 bins (each bin covered 1/10 of syllable rate range = 0.087 Hz). We then selected the point with the maximum bandwidth in each of the bins and computed the regression.

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PUBLICATIONS:

Linhart, P. & Fuchs, R. (submitted Animal Behaviour). Song frequency indicates body size and influences males' response to playback in a songbird.

Maruščáková, I. L., **Linhart, P.**, Ratcliffe, V. F., Reby, D., & Špinka, M. (submitted to Journal of Comparative Psychology). Cross-species judgement of emotional content in vocalizations: effects of call features and listeners' psychology.

Ptáček, L., Machlica, L., **Linhart, P.**, Jaška, P. & Müller, L. (submitted to Naturwissenschaften). Automatic identification of bird individuals by raw recording using GMM-UBM method.

Linhart, P., Jaška, P., Petrusková, T., Petrusek, A. & Fuchs, R. 2013. Being angry, singing fast? Signalling of aggressive motivation by syllable rate in a songbird with slow song. *Behavioural Processes*, 100, 139-145.

Tallet, C., **Linhart, P.**, Policht, R., Hammerschmidt, K., Šimeček, P., Kratinová, P. & Špinka, M. 2013. Encoding of situations in the vocal repertoire of piglets (*Sus scrofa*): a comparison of discrete and graded classifications. *PLoS ONE*, 8, e71841.

Linhart, P., Fuchs, R., Poláková, S. & Slabbekoorn, H. 2012. Once bitten twice shy: long-term behavioural changes caused by trapping experience in willow warblers *Phylloscopus trochilus*. *Journal of Avian Biology*, 43, 186–192.

Linhart, P., Slabbekoorn, H. & Fuchs, R. 2012. The communicative significance of song frequency and song length in territorial chiffchaffs. *Behavioral Ecology*, 23, 1338–1347.

Petruskova, T., Osiejuk, T., **Linhart, P.** & Petrusek, A. 2008. Structure and complexity of perched and flight songs of the tree pipit (*Anthus trivialis*). *Anales Zoologici Fennici*, 45, 135–148.

INTERNATIONAL PRESENTATIONS (oral):

Linhart, P., Reby, D., Ratcliffe, V. & Špinka, M. 2014. Acoustic coding of arousal in piglets: call types matter. The European Conference on Behavioural Biology (ECBB), Prague, Czech Republic

Linhart, P., Ptáček, L., Machlica, L., Muller, L., Jaška, P., Průchová, A., Chrenková M., Kipson, M., Šálek, M., 2014. Acoustic monitoring of individual in birds: lessons from owls and songbirds. Ecology and Acoustics, Paris, France.

Linhart, P & Špinka, M. 2013. Coding of emotions in piglet calls. CogBio Talks, Department of Cognitive Biology, University of Vienna, Austria.

Linhart, P & Špinka, M. 2013. Acoustic features of piglet 'Scream' and 'Grunt' calls reflect level of situation urgency. Joint Eastern and Western Europe Regional ISAE Meeting, October 2013, Scopje, Macedonia.

Linhart, P., Poláková, S., Fuchs, R. & Slabbekoorn, H. 2010. The long-term memory in willow warbler: Never fly into same mist net twice. Annual Meeting of the Netherlands Society for Behavioural Biology. November 2010, Soesterberg, Netherlands.

Linhart, P., Fuchs, R. & Slabbekoorn, H. 2009. Rival assessment in chiffchaff: Effect of frequency and length of the song. Annual Meeting of the Netherlands Society for Behavioural Biology. November 2009, Dalfsen, Netherlands.

INTERNSHIPS:

Sussex University, UK, December 2012, School of Psychology, Sussex University, Dr. David Reby

Leiden University, Netherlands, October – December 2010, Institute of Biology, Leiden University, Dr. Hans Slabbekoorn

Leiden University, Netherlands, November 2009, Institute of Biology, Leiden University, Dr. Hans Slabbekoorn.

AWARDS:

GAJU, Student Award of University of South Bohemia, 2009, 4000€

SGA, Student Award of Faculty of Science, University of South Bohemia, 2007, 1000€

TEACHING:

I organize and teach a substantial part of **Introduction to Bioacoustics** course at University of South Bohemia and Charles University, Prague (together with Dr. Tereza Petruskova, Charles University, Prague).

I participated in teaching of following courses at University of South Bohemia: **Ethology practicals**, **Summer school of ethology**, **Ornithology**, **Methods in behavioural research**, **Biology field course**.

Student supervision: I am / was a consultant of several undergraduate theses.

OTHER PROFESSIONAL ACTIVITIES:

Reviewing: Journal of Avian Biology, Wilson Journal of Ornithology, Sylvia (journal of Czech Ornithological Society)

I also reviewed undergraduate thesis from University of South Bohemia and Charles University and student research projects from Charles University Grant Agency (GAUK).

Memberships: Czech and Slovak Ethological Society, Czech Ornithological Society, Association of Bird Ringing Centre Collaborators

NGO Ho Avy (http://hoavy.org/en/): in 2009 – 2011 I helped to run the Czech Republic based department of Ho Avy NGO focused on restoration of spiny bush forest and sustainable community development at southwestern Madagascar; web administration; fundrising; series of public talks

POPULAR SCIENCE

I consider popularization of science an important part of the scientific work. I give popular talks on birdsong and domestic animals for high school students (within larger science popularization project organized by Faculty of Science). I contributed scientific news articles to magazines Abicko and Vesmir and talked about bird song research on a radio program.

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