

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.

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1. 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 guarantee 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 (Beebe, 2004a,b; 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.

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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., characterized 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; Linhart, unpublished data). Linhart et al. (2012b) 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 (Linhart and 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.)

2. Materials and methods

2.1. 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 km²) 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 recording. Hence, trapping could not affect behaviour of males (Linhart et al., 2012a). All birds were released in good condition immediately after banding and noting basic physical parameters (tarsus length and weight), within 5 min 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).

2.2. 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 1 min of high recording quality when the male was continuously singing (song rate: $\bar{x} \pm SD = 7.21 \pm 1.24$ 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 1500 Hz and 9000 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 $3 \times 12 = 36$ 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 recognized as conspecific, as chiffchaffs were shown to show species-specific responses even to songs with highly altered syllable interval (Schubert, 1971).

All playback experiments were conducted in the morning within 6 h 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 5 min 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 5 min during the playback. The following behavioural parameters were scored: time spent within 2 m from the speaker (s), latency of approach within 2 m (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-m 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

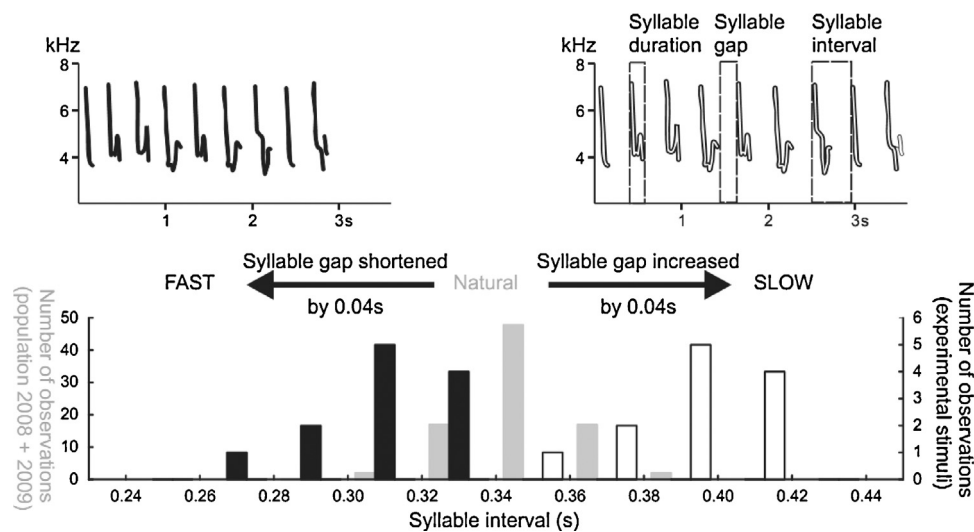


Fig. 1. Preparation of experimental stimuli and song measures taken. The upper part shows two spectrograms of the same chiffchaff song in FAST (left) and SLOW variant (right). Three measurements were taken: syllable duration, syllable gap, and syllable interval as indicated. Histogram in the lower part of the picture shows distribution of syllable interval values within our study population (natural) from two years 2008 (Linhart et al., 2012a) and 2009 (current dataset) and within experimental songs (FAST and SLOW).

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 (Linhart, pers. obs.).

2.3. 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 analyzed 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 m.

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 considered 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 (Podós,

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.

2.4. 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

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 (χ^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, χ^2	P	t, Z	P	d	CI
Time within 2 m (s)	89.7	50.3	92.8	65.3	71.3	57.4	0.48	0.62	0.84	0.41	0.34	–0.50 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	–0.70 0.98
Latency within 2 m (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
	Mean rank		Mean rank		Mean rank							
Time attacking (s)	14.3		14.8		13.4		0.48	0.79	0.14	0.89	0.24	–0.60 1.08
Attack latency (s)	20		16.2		19.3		1.13	0.57	–0.13	0.89	0.08	–0.76 0.91

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 and Cuthill (2007).

3. Results

3.1. 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, and 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 1st April; Mann–Whitney U test: $U = 122.5$, $N_{\text{attack}} = 13$, $N_{\text{non-attack}} = 23$, $P = 0.48$) 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 $P > 0.28$). The exception was the time spent within 2 m 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., 2012b).

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.

3.2. 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: $t_{33} = -2.47$,

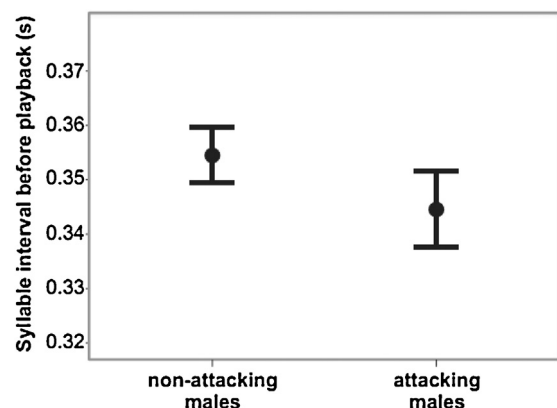


Fig. 2. Syllable interval in spontaneous songs (BEFORE playback) of attacking ($N = 13$) and non-attacking ($N = 22$) males. Means and 95% confidence intervals are displayed. The difference between the two groups is significant (see text).

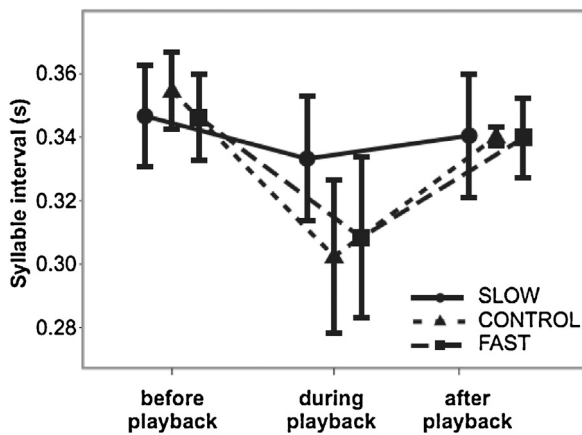


Fig. 3. Syllable intervals in the three experimental stages before, during and after playback according to the three playback types: SLOW (circle; $N=5$), CONTROL (triangle; $N=6$), and FAST (square; $N=5$). The songs recorded during playback represent those sung in aggressive context. Means and 95%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

$d = -0.86$, $CI = -1.58$ 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 sang 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: $r = -0.25$, $N = 16$, $P = 0.34$).

3.3. 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 \times DURING: $P < 0.001$, $d = -1.74$, $CI = -2.67$ to -0.81 ; BEFORE \times AFTER: $P = 0.23$, $d = -0.73$, $CI = -1.31$ to 0.15 ; DURING \times 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).

3.4. 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 , $P = 0.729$).

4. 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 and 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.

4.1. 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.

4.2. 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 analyzed – 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.

4.3. 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., 2007; 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 maximized, 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., 2012b) 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>.

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