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3-min. úsek			celkový počet zpěvů		počet zpěvů s "M-trylky"				počet zpěvů s "I-trylky"			
rok	Lokalita	kód samce	před pokus	po pokus	před pokus	po pokus	frekvence užití před pokus	frekvence užití po pokus	před pokus	po pokus	frekvence užití před pokus	frekvence užití po pokus
2012	Brdy	3	13	11	0	2	0 %	18 %	7	4	54 %	36 %
2012	Brdy	8	15	18	1	0	7 %	0 %	7	9	47 %	50 %
2012	Brdy	13	14	24	3	0	21 %	0 %	10	14	71 %	58 %
2012	Brdy	18	17	28	6	0	35 %	0 %	5	7	29 %	25 %
2012	Brdy	22	18	20	1	1	6 %	5 %	10	9	56 %	45 %
2012	Brdy	23	16	23	2	1	13 %	4 %	12	13	75 %	57 %
2012	Brdy	24	17	24	0	1	0 %	4 %	12	12	71 %	50 %
2012	Brdy	25	23	40	2	1	9 %	3 %	4	16	17 %	40 %
2012	Brdy	26	20	29	2	0	10 %	0 %	6	7	30 %	24 %
2012	Brdy	27	14	15	2	2	14 %	13 %	10	11	71 %	73 %
<b>MEDIÁN:</b>			16,5	23,5	2	1	9,3 %	3,3 %	8,5	10	54,7 %	47,5 %
<b>PRŮMĚR:</b>			16,7	23,2	1,9	0,8	11,4 %	4,8 %	8,3	10,2	52,1 %	45,9 %

**Příloha 2: Přehled celkového počtu zpěvů a zpěvů obsahujících rychlé trylky u jednotlivých samic v rámci srovnání jednonutových spontánních úseků a úseků nahraných během pokusování v Brdech v roce 2012**

1-min. úsek			celkový počet zpěvů		počet zpěvů s "M-trylky"				počet zpěvů s "I-trylky"			
rok	Lokalita	kód samce	před pokus	po pokus	před pokus	po pokus	frekvence užití před pokus	frekvence užití po pokus	před pokus	po pokus	frekvence užití před pokus	frekvence užití po pokus
2012	Brdy	3	4	4	0	2	0 %	50 %	3	3	75 %	75 %
2012	Brdy	8	4	6	1	0	25 %	0 %	2	5	50 %	83 %
2012	Brdy	13	5	6	1	0	20 %	0 %	4	5	80 %	83 %
2012	Brdy	18	6	10	1	0	17 %	0 %	3	8	50 %	80 %
2012	Brdy	22	6	7	0	0	0 %	0 %	4	4	67 %	57 %
2012	Brdy	23	6	9	0	0	0 %	0 %	3	6	50 %	67 %
2012	Brdy	24	7	7	0	1	0 %	14 %	6	5	86 %	71 %
2012	Brdy	25	7	16	1	0	14 %	0 %	2	10	29 %	63 %
2012	Brdy	26	5	10	1	0	20 %	0 %	1	6	20 %	60 %
2012	Brdy	27	6	6	0	0	0 %	0 %	1	5	17 %	83 %
<b>MEDIÁN:</b>			6	7	0,5	0	7,1 %	0 %	3	5	50,0 %	73,2 %
<b>PRŮMĚR:</b>			5,6	8,1	0,5	0,3	9,6 %	6,4 %	2,9	5,7	52,3 %	72,3 %

**Příloha 3: Přehled celkového počtu zpěvů a zpěvů obsahujících rychlé trylky u jednotlivých samic v rámci srovnání třiminutových spontánních úseků a úseků nahraných během pokusování v Brdech v roce 2013 a 2014**

3-min. úsek			celkový počet zpěvů		počet zpěvů s "M-trylky"				počet zpěvů s "I-trylky"			
rok	Lokalita	kód samce	před pokus	po pokus	před pokus	po pokus	frekvence užití před pokus	frekvence užití po pokus	před pokus	po pokus	frekvence užití před pokus	frekvence užití po pokus
2013	Brdy	28	15	8	3	0	20 %	0 %	11	8	73 %	100 %
2013	Brdy	29	9	12	3	0	33 %	0 %	5	0	56 %	0 %
2013	Brdy	30	13	23	3	2	23 %	9 %	7	8	54 %	35 %
2013	Brdy	31	10	28	2	0	20 %	0 %	4	5	40 %	18 %
2013	Brdy	32	19	24	3	2	16 %	8 %	8	12	42 %	50 %
2013	Brdy	33	14	25	1	1	7 %	4 %	8	10	57 %	40 %
2013	Brdy	34	22	23	4	2	18 %	9 %	8	9	36 %	39 %
2013	Brdy	35	17	10	1	0	6 %	0 %	6	3	35 %	30 %
2013	Brdy	36	7	14	1	0	14 %	0 %	3	4	43 %	29 %
2013	Brdy	37	17	8	1	0	6 %	0 %	4	1	24 %	13 %
2014	Brdy	38	18	28	1	0	6 %	0 %	10	10	56 %	36 %
2014	Brdy	39	15	32	1	0	7 %	0 %	4	5	27 %	16 %
2014	Brdy	40	10	19	2	0	20 %	0 %	4	1	40 %	5 %
2014	Brdy	41	13	5	0	0	0 %	0 %	1	1	8 %	20 %
2014	Brdy	42	14	19	0	0	0 %	0 %	5	6	36 %	32 %
2014	Brdy	43	18	15	1	1	6 %	7 %	6	2	33 %	13 %
<b>MEDIÁN:</b>			14,5	19	1	0	11 %	0 %	5,5	5	40 %	29 %
<b>PRŮMĚR:</b>			14,4	18,3	1,69	0,5	13 %	2 %	5,88	5,31	41 %	30 %

**Příloha 4: Přehled celkového počtu zpěvů a zpěvů obsahujících rychlé trylky u jednotlivých samic v rámci srovnání jednodominutových spontánních úseků a úseků nahraných během pokusování v Brdech v roce 2013 a 2014**

1-min. úsek			celkový počet zpěvů		počet zpěvů s "M-trylky"				počet zpěvů s "I-trylky"			
rok	Lokalita	kód samce	před pokus	po pokus	před pokus	po pokus	frekvence užití před pokus	frekvence užití po pokus	před pokus	po pokus	frekvence užití před pokus	frekvence užití po pokus
2013	Brdy	28	6	8	1	0	17 %	0 %	4	8	67 %	100 %
2013	Brdy	29	5	6	1	0	20 %	0 %	2	0	40 %	0 %
2013	Brdy	30	4	10	1	0	25 %	0 %	1	2	25 %	20 %
2013	Brdy	31	4	9	1	0	25 %	0 %	2	0	50 %	0 %
2013	Brdy	32	8	10	1	1	13 %	10 %	4	4	50 %	40 %
2013	Brdy	33	6	7	0	1	0 %	14 %	1	1	17 %	14 %
2013	Brdy	34	11	6	1	1	9 %	17 %	4	3	36 %	50 %
2013	Brdy	35	7	5	0	0	0 %	0 %	1	1	14 %	20 %
2013	Brdy	36	4	5	1	0	25 %	0 %	2	1	50 %	20 %
2013	Brdy	37	7	6	0	0	0 %	0 %	2	1	29 %	17 %
2014	Brdy	38	7	7	1	0	14 %	0 %	3	1	43 %	14 %
2014	Brdy	39	4	10	1	0	25 %	0 %	1	1	25 %	10 %
2014	Brdy	40	4	9	0	0	0 %	0 %	0	1	0 %	11 %
2014	Brdy	41	5	3	0	0	0 %	0 %	0	1	0 %	33 %
2014	Brdy	42	5	6	0	0	0 %	0 %	1	3	20 %	50 %
2014	Brdy	43	7	6	0	1	0 %	17 %	1	2	14 %	33 %
<b>MEDIÁN:</b>			5,5	6,5	1	0	11 %	0 %	1,5	1	27 %	20 %
<b>PRŮMĚR:</b>			5,88	7,06	0,56	0,25	11 %	4 %	1,81	1,88	30 %	27 %

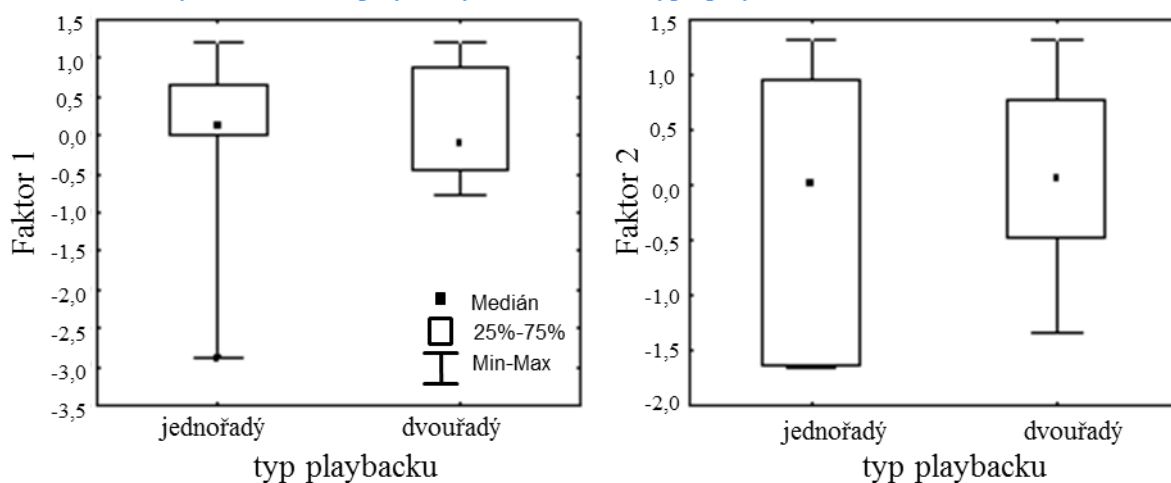
**Příloha 5: Přehled celkového počtu zpěvů a zpěvů obsahujících rychlé trylky u jednotlivých samců v rámci srovnání třiminutových spontánních úseků a úseků nahraných během pokusování v roce 2013 a 2014 na lokalitě Krkonoše a Oblík**

3-min. úsek			celkový počet zpěvů		počet zpěvů s "M-trylky"				počet zpěvů s "I-trylky"			
rok	Lokalita	kód samce	před pokus	po pokus	před pokus	po pokus	frekvence užití před pokus	frekvence užití po pokus	před pokus	po pokus	frekv. užití před pokus	frekv. užití po pokus
2013	Krkonoše	K1	20	16	0	0	0 %	0 %	8	7	40 %	44 %
2013	Krkonoše	K2	12	17	1	1	8 %	6 %	4	12	33 %	71 %
2013	Krkonoše	K3	18	10	4	1	22 %	10 %	9	4	50 %	40 %
2013	Krkonoše	K4	5	14	2	0	40 %	0 %	4	8	80 %	57 %
2013	Krkonoše	K5	21	22	1	1	5 %	5 %	5	3	24 %	14 %
2013	Krkonoše	K6	17	21	0	0	0 %	0 %	3	2	18 %	10 %
2013	Krkonoše	K7	10	10	0	1	0 %	10 %	4	3	40 %	30 %
2013	Krkonoše	K8	12	1	0	0	0 %	0 %	6	1	50 %	100 %
2013	Krkonoše	K9	9	9	2	0	22 %	0 %	4	3	44 %	33 %
2013	Krkonoše	K10	7	11	1	1	14 %	9 %	1	2	14 %	18 %
2013	Krkonoše	K11	11	25	2	3	18 %	12 %	8	13	73 %	52 %
2013	Oblík	O1	14	17	1	0	7 %	0 %	4	4	29 %	24 %
2013	Oblík	O2	19	18	0	0	0 %	0 %	2	2	11 %	11 %
2013	Oblík	O3	21	19	2	0	10 %	0 %	5	1	24 %	5 %
2014	Oblík	O4	6	27	2	0	33 %	0 %	3	1	50 %	4 %
2014	Oblík	O5	17	13	4	1	24 %	8 %	11	0	65 %	0 %
2014	Oblík	O6	16	2	1	1	6 %	50 %	9	1	56 %	50 %
2014	Oblík	O7	19	24	1	0	5 %	0 %	5	10	26 %	42 %
<b>MEDIÁN:</b>			15,0	17	1,0	0,0	8 %	0 %	4,5	3,0	40 %	32 %
<b>PRŮMĚR:</b>			14,1	15,3	1,3	0,6	12 %	6 %	5,3	4,3	40 %	34 %

**Příloha 6: Přehled celkového počtu zpěvů a zpěvů obsahujících rychlé trylky u jednotlivých samců v rámci srovnání jedninutových spontánních úseků a úseků nahraných během pokusování v roce 2013 a 2014 na lokalitě Krkonoše a Oblík**

1-min. úsek			celkový počet zpěvů		počet zpěvů s "M-trylky"				počet zpěvů s "I-trylky"			
rok	Lokalita	kód samce	před pokus	po pokus	před pokus	po pokus	frekvence užití před pokus	frekvence užití po pokus	před pokus	po pokus	frekv. užití před pokus	frekv. užití po pokus
2013	Krkonoše	K1	7	6	0	0	0 %	0 %	3	4	43 %	67 %
2013	Krkonoše	K2	4	7	1	0	25 %	0 %	3	6	75 %	86 %
2013	Krkonoše	K3	8	8	2	1	25 %	13 %	3	4	38 %	50 %
2013	Krkonoše	K4	1	5	0	0	0 %	0 %	1	3	100 %	60 %
2013	Krkonoše	K5	7	10	0	0	0 %	0 %	0	1	0 %	10 %
2013	Krkonoše	K6	7	6	0	0	0 %	0 %	0	1	0 %	17 %
2013	Krkonoše	K7	4	7	0	1	0 %	14 %	0	3	0 %	43 %
2013	Krkonoše	K8	5	1	0	0	0 %	0 %	2	1	40 %	100 %
2013	Krkonoše	K9	4	3	1	0	25 %	0 %	2	1	50 %	33 %
2013	Krkonoše	K10	3	2	1	0	33 %	0 %	1	0	33 %	0 %
2013	Krkonoše	K11	3	9	1	0	33 %	0 %	3	5	100 %	56 %
2013	Oblík	O1	6	5	1	0	17 %	0 %	1	1	17 %	20 %
2013	Oblík	O2	7	3	0	0	0 %	0 %	1	0	14 %	0 %
2013	Oblík	O3	7	4	0	0	0 %	0 %	3	0	43 %	0 %
2014	Oblík	O4	2	9	1	0	50 %	0 %	2	0	100 %	0 %
2014	Oblík	O5	7	7	4	1	57 %	14 %	7	0	100 %	0 %
2014	Oblík	O6	7	2	0	1	0 %	50 %	2	1	29 %	50 %
2014	Oblík	O7	7	7	1	0	14 %	0 %	5	2	71 %	29 %
<b>MEDIÁN:</b>			6,5	6	0,5	0,0	7 %	0 %	2,0	1,0	41 %	31 %
<b>PRŮMĚR:</b>			5,3	5,61	0,7	0,2	16 %	5 %	2,2	1,8	47 %	34 %

**Příloha 7: Rozdíly v reakcích na playbacky v závislosti na typu playbacku v Brdech v letech 2013-2014**





## Variation in Trill Characteristics in Tree Pipit Songs: Different Trills for Different Use?

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### Abstract

Transmitting information about singer's quality is an important function of song in many bird species, and this information should be useful in territorial interactions. Fast trills, being physically demanding song structures, are particularly suitable candidates for signalling of quality or aggressive motivation. We have evaluated trill characteristics in songs within a population of the Tree Pipit, a common European songbird with no sexual dimorphism, in which song apparently plays a key role in territory defence as well as mate choice. Two types of relatively fast trills (each of them in multiple variants differing in complexity) were commonly observed in repertoires of Tree Pipit males. Trill rates significantly differed among individuals, suggesting that these song structures may carry information about male quality in this species. We tested by playback experiments whether both trill types are used in territorial encounters. Only one of the trill types was sung by males in response to playback, regardless on the trill type played to them. In an immediate response to playback, they increased the frequency of use of this trill, and also significantly increased the trill rate in comparison with spontaneous songs. This confirmed field observations, suggesting that this trill is important in male–male interactions. On the contrary, the use of the fastest, apparently more demanding, trill type actually decreased after the simulated territorial intrusion. We hypothesize that the latter one is more directed towards females, and that while performance of both trill types may reflect male quality, they are primarily used in different contexts.

### Introduction

Birdsong has been intensively studied for many decades, especially in the context of its two main functions, mate attraction and territorial defence (e.g. Krebs et al. 1978; Catchpole & Slater 2008). Despite this long tradition, scientific interest in animal vocalizations does not cease. Recent fast technical development of recording devices as well as bioacoustic software has allowed researchers to study birdsong in subtle details (Catchpole & Slater 2008). Many studies focused on contexts of singing behaviour. Use of distinct song types in specific situations has been shown in many bird species (e.g. Byers 1996; Trillo & Vehrencamp 2005; Benedict et al. 2012), in some cases

involving song types of different vocal performance level (e.g. Beebee 2004b; Molles 2006; Cardoso et al. 2009). Specific singing behaviour has been documented in the context of aggressive encounters, for example the use of soft songs (e.g. Dabelsteen et al. 1998; Searcy & Nowicki 2006; Ballentine et al. 2008). In other species, song overlapping has been shown to be a threatening signal (Naguib & Kipper 2006; Vehrencamp et al. 2007; Fitzsimmons et al. 2008), although it has become a matter of recent debate whether it can be generally considered a signal of aggressiveness (Searcy & Beecher 2009, 2011; Naguib & Mennill 2010).

Other song characteristics that seem associated with aggressive signalling are less ambiguous. Increasing

evidence shows that physically demanding song types or structures are often used during territorial encounters (e.g. Beebe 2004a,b; Cardoso et al. 2009; DuBois et al. 2009). Variation in the song vocal performance seems meaningful to receivers: various experimental studies found different responses of females (Ballentine et al. 2004; Weiss et al. 2012) as well as males (Illes et al. 2006; Cramer & Price 2007; de Kort et al. 2009; DuBois et al. 2011; but see also Cramer 2013), and correlations with male reproductive or pairing success (Byers 2007; Schmidt et al. 2008) seem to support the hypothesis that the vocal performance signals singer's quality.

Fast trills, being physically demanding song structures, are particularly suitable candidates for signalling of quality or aggressive motivation. Vocal performance of trills is often expressed by trill rate and element bandwidth. Experimental evidence suggesting physiological constraints for these trill parameters was brought by Podos (1997). Such constraints would predetermine trills to be a reliable index signal of quality. Occurrence of fast trills in aggressive encounters has been documented for several species. For example, Cramer and Price (2007) showed that Red-winged Blackbirds (*Agelaius phoeniceus*) discriminated between trills of low and high performance. Similarly, Banded Wrens (*Thryothorus pleurostictus*) responded differently to trills closer to the performance limit (Illes et al. 2006). Schmidt et al. (2008) found that rapid broadband trills are a signal of aggression in Common Nightingales (*Luscinia megarhynchos*), and DuBois et al. (2009) documented increase of trill rate as well as bandwidth during aggressive encounters in Swamp Sparrows (*Melospiza georgiana*). Reliable measurement of bandwidth from field recordings nevertheless poses methodological challenges due to varying recording quality and amplitude (Zollinger et al. 2012); measuring trill or syllable rate itself seems thus more robust to bias. Cardoso et al. (2007a) suggested that syllable rate alone might be an important signal in some species, as documented, for example, by Drăgănoiu et al. (2002) or Cardoso et al. (2007a,b). Interestingly, syllable rate seems to play a role also in territorial defence in Chiffchaff (*Phylloscopus collybita*), a songbird species with a slow song (Linhart et al. 2013).

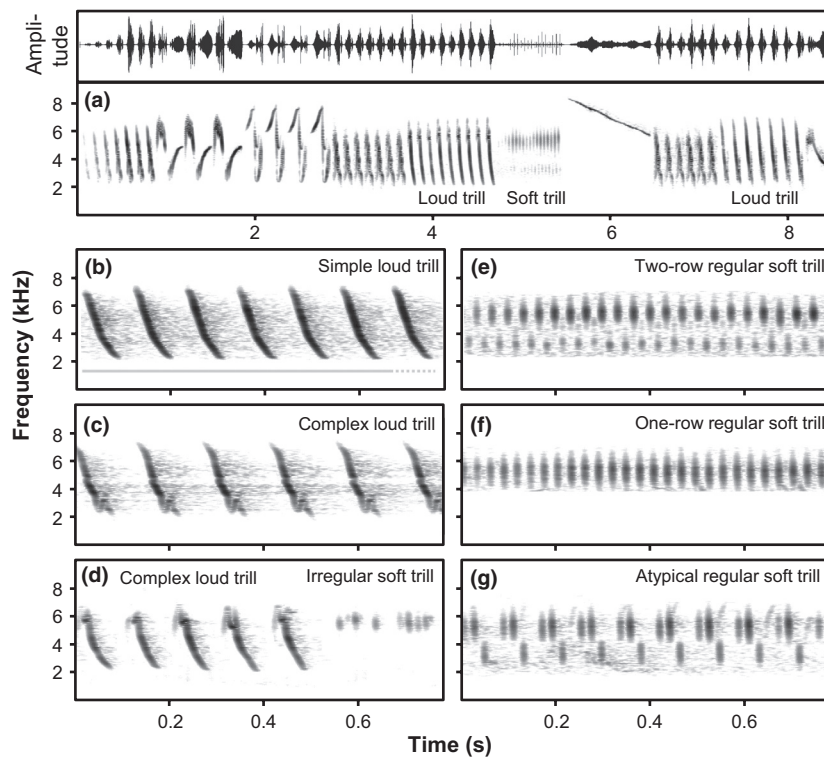
In this study, we focused on the possible role of trill phrases and their syllable rate in songs of the Tree Pipit (*Anthus trivialis*; Passeriformes: Motacillidae). This is a territorial socially monogamous passerine with moderate song complexity, with average syllable repertoire sizes of approximately 11–12 syllable types per male (Petrusková et al. 2008). In our previous

study, we documented for this species a high geographic variation in songs, as evaluated from composition of male syllable repertoires (Petrusková et al. 2010). A substantial proportion of syllable types was restricted to one or only a few of the studied populations. In contrast with this finding, we revealed that very fast trill phrases of relatively low amplitude (further labelled 'soft trills', examples shown in Fig. 1e, f) occurred regularly in all studied populations as well as in other parts of the species' range. Furthermore, these trills (labelled as 'M syllables' or 'M phrase' in Petrusková et al. 2008, 2010) strongly varied in length among 90 examined males from five studied Czech localities (Petrusková et al. 2008). We therefore suggested that such conservative structures in the song might serve as a male quality indicator.

Recently, in a more detailed study of Pipit song behaviour, another type of relatively fast trills attracted our attention (examples in Fig. 1b, c, d). Syllables forming those trills (additional ones labelled  $H_x$  and I are apparent in spectrograms in Petrusková et al. 2008, 2010) are characterized by downsweeps with predominantly constant frequency modulation and occur in either simple (Fig. 1b) or more complex variants (as in Fig. 1c, d). Due to perceived prominence of such trills, we further labelled them 'loud trills'. They were widespread and frequently used by all Tree Pipit males examined by us so far. Loud trills occurred much more often than soft trills, and we observed that they were regularly used in perched songs during natural male–male singing interactions at the studied locality (T. Petrusková, unpubl. data).

We examined the role of both types of fast trills in the Tree Pipit song behaviour and focused on the following research questions and hypotheses. First, we tested the hypothesis that males differ in vocal performance (reflected in trill rate) of both trill types, suggesting that both might be quality indicators. This was tested directly by evaluating the variation of trill rates among males. Second, we focused on question whether males performing faster, that is presumably higher-quality, trills are more successful in male–male interactions. As a proxy for success in intrasexual interactions, we focused on the males' ability to maintain stable territories. Thus, we tested the hypothesis that males occupying territories during the whole season sing faster trills than those without a stable territory. Third, assuming that different trill types carry information about different levels of aggressive motivation (e.g. soft trills possibly expressing stronger threat than loud trills), we expected that males will respond to the playback containing one particular trill type with increased use of the same one. We thus





**Fig. 1:** Example of a flight song of a Tree Pipit male including both soft and loud trills (a), and details of various trill types used by Tree Pipit males at the studied locality: loud trills with simple (b) and more complex elements (c, d left), two-row and single-row soft trills (e and f, respectively), atypical but regular soft trill (g), and irregular soft trill sung by an apparently poor-quality male (d right). Amplitude envelope curve is provided for the song in a. Note the differences in amplitude between trills within this song, and in rates of the three trill phrases (first loud trill: 9.1 elements per s, soft trill: 47.7, second loud trill: 7.1). Duration measures taken to calculate the trill rate are marked by grey lines in b (dotted line indicates duration of the last element, which is excluded from the calculation; see Methods for the respective formula).

evaluated the frequency and characteristics of trills used in spontaneous songs sung before and after playback stimuli containing one or the other trill type.

## Methods

### Study Species and Locality

The Tree Pipit is a widespread Palearctic songbird, relatively common in suitable habitats within the Czech Republic (Šiastný et al. 2006). It is a small, cryptically coloured, ground-nesting passerine with no sexual dimorphism. However, singing behaviour of its males defending the territories is very conspicuous and thus the species is easy to recognize. During the continuous singing bouts, lasting up to several minutes, individual songs are well separated by gaps of silence. Two types of songs are typical for territory defence – shorter perched songs sung from high posts, typically tree tops (with average duration ca 2.4 s), and characteristic for the species, usually longer, flight songs (on average 6.5 s; Petrusková et al. 2008).

Our study was conducted at the edge of the hilly area Brdská vrchovina in Central Bohemia, Czech Republic (49°84'N, 14°10'E; elevation 370–440 m a.s.l., study area dimensions approx. 2300 × 800 m), where Tree Pipit males establish territories at the for-

est edge bordering gently sloping managed meadows, along bands of sparsely growing trees and bushes intersecting those meadows, and at forest clearings. Data were collected during two consecutive breeding seasons (2011–2012) from mid-April to early July during a day time. First Tree Pipit males arrive to the area during the second part of April with a peak at the beginning of May, but newcomers appear sporadically even during late June. The presence and distribution of males at the locality was checked at least once a week, in May and the first half of June more frequently (2–3 times a week).

Songs of Tree Pipit males were recorded using Marantz PMD 660 and 661 recorders equipped with Sennheiser ME-67 shotgun microphones (44.1 kHz sampling rate and a 16 bit depth, no data compression). Most of those detected for the first time were subsequently captured with a mist net (Ecotone, Gdynia) by luring them by playback of conspecific songs recorded at the same locality in 2010. The mist-netted males were colour-ringed to ease future identification, weighted, and their wing and tarsus lengths were measured. All birds were released in a good condition immediately after this procedure, within a few minutes. As there are no reliable features allowing age assignment in the Tree Pipit, we adopted the method used by Węgrzyn and Leniowski (2010), where birds

recorded for the first time are considered at least 1 yr old (labelled 1+), and those recorded also in a consecutive year are labelled 2+.

Altogether, we observed 45 males. We detected 29 males at the studied locality in 2011 (21 of which were colour-ringed during the season), and 25 males in 2012 (21 colour-ringed). Nine of the birds detected in 2012 were already recorded in 2011. To avoid bias in behaviour due to negative experience associated with playback and mist-netting (Linhart et al. 2012, pers. obs.), none of the 10 males tested in playback experiments (performed in 2012 only) was captured earlier during that season.

### Song Recording and Trill Analyses

Singing bouts (consisting of perched as well as flight songs) of all individual males present in the study area were repeatedly recorded throughout the whole season (with frequency according to their presence at the locality and singing activity; only males recorded at least five times within at least 1 mo period, altogether 21 individuals, were included in subsequent analyses). Position and identity of each singing male were noted for further assessment of territory maintenance; furthermore, we also recorded activities indicating possible pairing or breeding success. Males were unambiguously identified either by their unique colour ring combination observed during the recording session, or during analyses based on individual characteristics of recorded songs (syllable repertoires and syntax) that consistently differed among males (T. Petrusková & I. Pišvejcová, unpubl. data). Based on positions where individual birds were recorded throughout the season, we distinguished males that were able to maintain the territory during the whole breeding season ('territory holders') and those that changed their positions several times (at least twice but up to five times) during the season ('non-holders'). Most territory holders established territories at sites apparently preferred by Tree Pipits within a study area, as these sites were always occupied first during the season.

Of all recorded males, we selected for further evaluation of trill characteristics 21 individuals, for which we obtained a sufficient number of recordings covering a substantial part of the breeding period (on average, 10 recordings per male, range 5–23; see Table 1). For individuals present at the locality in both studied years, only one season (better covered by recordings) was used. For all selected males, very fast soft trill and slower loud trill types (as shown in Fig. 1) were located in recordings, and their characteristics were

measured in the Avisoft SASLab Pro 5.x software (Specht 2007) with the following settings (as in Petrusková et al. 2008): FFT-length 512, Frame 100%, Hamming window, temporal overlap 87.5%. All recordings were band-pass filtered between 1.5 and 10 kHz (well outside the frequency range of Tree Pipit song) to remove background noise. To reveal possible differences in trill production among males, we calculated the rate for all trills containing at least seven elements. To take into account the duration of the gap between repeated elements in the trill, we calculated the trill rate using the following formula (as in Illes et al. 2006):  $(\text{number of elements} - 1) / (\text{trill duration} - \text{last element duration})$ . Note that the denominator in the formula is equivalent to measuring the trill duration from the beginning of the first element to the beginning of the last element (see Fig. 1b). We did not take the frequency measures to calculate trill bandwidth, as this might be substantially affected by the different quality of recordings (Zollinger et al. 2012), which cannot be easily standardized in fieldwork.

The initial evaluation of loud trill characteristics revealed that males often had more than one loud trill variant in their repertoires (see examples in Fig. 1b–d) that were used apparently interchangeably (in the same contexts and parts of songs). Furthermore, the rates of trills sung by the same bird frequently followed bi- or multimodal distributions (which was not the case for soft trills). Often, some loud trills sung by the same bird were consistently faster than others, as demonstrated by a clear gap separating such fast loud trills in histograms evaluating trill rate (see Fig. S1, and also apparent difference in the two loud trills in Fig. 1A). In such birds, slower as well as faster loud trills were observed in most recordings throughout the season (with trill rates in these different rate categories very similar over time). Assuming that the fastest trills are most relevant in male–male interactions and/or for evaluation of singer quality, we decided to compare only their characteristics among birds, rather than pooling loud trills of all different rate categories for the analysis. Thus, we constructed a trill rate histogram for each male (examples are shown in Fig. S1), and in case of bi- or multimodal distribution (observed in 15 of 21 evaluated birds), only the fast category was further selected. This fast category could have involved any of the loud trill variants observed in the studied population (i.e. those shown in Fig. 1b–d) or their mix, depending on individual males (Table 1).

During the fieldwork, we noticed that while the loud trills were usually very conspicuous, the soft trills were always noticeably quieter than the rest of the

**Table 1:** Basic characteristics of studied Tree Pipit males and their trills

Male no.	Year	Territory holder	Weight (g)	Wing length (mm)	Tarsus length (mm)	No. recordings	First date	Last date	Loud trills (total)	Fast category of loud trills	Loud trill rate median (range)	Soft trills analysed	Soft trill rate median (range)
1	2011	No	22	87	21.0	5	22.4.2011	6.6.2011	47 (CD)	45 (C)	8.2 (7.7–10.3)	<5 recorded	
2	2012	No	26	87	21.0	18	2.5.2012	29.6.2012	119 (BD)	119 (B'D)	8.2 (7.4–11.3)	31	41.9 (29.9–58.6)
3	2012	No	Not captured			10	17.4.2012	17.6.2012	123 (CD)	86 (CD')	8.3 (7.6–8.6)	7	50.0 (41.0–64.8)
4	2011	No	21	85	20.2	13	1.5.2011	15.6.2011	126 (BCD)	70 (BC')	9.2 (8.5–10.2)	19	53.2 (34.8–69.6)
5	2011	No	22.5	89	20.7	16	22.4.2011	6.6.2011	220 (BCD)	97 (BC')	9.5 (8.0–11.0)	69	58.7 (33.7–68.6)
6	2012	Yes?	23	88	20.7	9	25.4.2012	8.6.2012	64 (CD)	28 (D)	8.5 (8.1–10.0)	27	65.6 (45.4–67.5)
7	2012	Yes?	21	93	20.8	15	17.4.2012	20.6.2012	33 (B)	33 (B)	8.7 (8.0–10.1)	6	35.6 (32.5–45.1)
8	2011	Yes	22	86	21.2	6	5.5.2011	15.6.2011	40 (BD)	34 (B'D)	8.3 (7.8–10.1)	11	49.6 (31.1–62.4)
9	2012	Yes	21	88	21.4	23	25.4.2012	25.6.2012	204 (BD)	202 (B)	9.5 (7.8–10.4)	42	56.8 (29.5–62.6)
10	2011	Yes	Not captured			13	22.4.2011	13.6.2011	237 (BCD)	201 (B'C'D)	10.0 (9.2–11.5)	12	47.3 (28.9–64.1)
11	2011	Yes	Not captured			7	5.5.2011	5.6.2011	35 (B)	35 (B)	10.3 (8.9–10.5)	11	46.8 (29.4–66.7)
12	2012	Yes	22.5	88	20.8	10	1.5.2012	6.6.2012	132 (B)	37 (B)	10.7 (9.9–11.0)	25	41.1 (28.4–63.4)
13	2012	Yes	22.5	89	20.8	12	1.5.2012	29.6.2012	193 (BD)	99 (B)	10.7 (9.6–11.1)	28	54.0 (30.4–62.9)
14	2011	Yes	21	88	20.3	6	21.4.2011	6.6.2011	52 (BCD)	27 (B'CD)	10.9 (9.4–12.1)	19	44.9 (30.4–64.5)
15	2011	Yes	23.5	90	21.8	7	21.4.2011	6.6.2011	33 (B)	22 (B)	11.0 (10.6–11.6)	9	61.1 (53.6–66.8)
16	2012	Yes	Not captured			7	17.4.2012	11.6.2012	67 (BD)	37 (B'D)	11.0 (10.8–11.3)	16	64.5 (59.7–69.2)
17	2011	Yes	22	89	21.1	10	28.4.2011	22.6.2011	34 (B)	34 (B)	11.3 (11.1–11.9)	None recorded	
18	2012	Yes	21.75	89	20.7	9	28.4.2012	21.6.2012	36 (B)	36 (B)	11.4 (10.3–11.8)	23	44.3 (28.7–54.7)
19	2011	Yes	23	90	20.4	7	21.4.2011	15.6.2011	90 (B)	58 (B)	11.8 (9.7–13.1)	<5 recorded	
20	2011	Yes	22	90	19.9	8	22.4.2011	27.6.2011	46 (BCD)	34 (B'C)	11.8 (10.8–12.3)	8	50.1 (26.6–56.8)
21	2011	Yes	22.5	85	21.5	5	12.5.2011	13.6.2011	56 (B)	45 (B)	12.2 (11.5–12.7)	28	58.9 (29.8–67.0)

Males are listed in the same order as in Fig. 2. Basic body measurements and weight are provided for each captured individual. For loud trills, total number in the recordings and the number of fast-category loud trills selected for rate measurements (see Methods and Fig. S1) are provided separately. Capital letters in parentheses indicate loud trill variants (as in Fig. 1) used by the particular male; if multiple variants have been included in the fast category, the dominant one is marked by an apostrophe. Two males defending particularly small territories are marked by a question mark in the 'territory holder' column.

song (see also the amplitude envelope curve in Fig. 1a). We thus decided to quantify these consistently perceived amplitude differences between loud and soft trills. Due to unavoidable presence of background noise, including overlapping songs of other bird species present at the locality, this could only be measured on high-quality recordings. For this comparison, we selected suitable recordings containing both soft and loud trills from 22 of the males recorded during the study (per each male, originating from 1 yr only). Due to variation of recording conditions even within a single recording, which might strongly affect overall amplitude (Zollinger et al. 2012), we did not compare the amplitudes of the trills directly but rather focused on the ratios between amplitudes of trills and adjacent phrases within a song. For representative high-quality recordings of each male, we evaluated root-mean-square (RMS) amplitude (using the Avisoft function 'Copy RMS of marked section') for (1) three randomly selected loud and soft trills, and (2) sections of the song immediately preceding the respective trills. The RMS amplitude was always measured from an equally long part of a trill and of a preceding phrase (duration based on whichever of the two was shorter). To characterize the amplitude ratios, the measured amplitude values of the two phrases (expressed in dB, i.e. in logarithmic scale) were subtracted from each other (Bradbury & Vehrencamp 2011). Such within-song comparison should not be strongly affected by variation of recording conditions, as all syllables should be affected similarly in adjacent parts of a single song.

### Playback Experiments

We used songs of 10 males recorded at the locality during the breeding season 2011 and not present in the following season to prepare playback stimuli. These were used in the 2012 season in experiments simulating a territory intrusion by a singing male. We selected 10 adjacent songs from a good-quality recording of a particular male (typically from a section approx. 90 s long), making sure that those songs were sung solo, that is not in response to a challenge of another conspecific male. As the playbacks were conducted from a stationary loudspeaker, we included only perched songs in the playback stimuli. The songs were processed in Avisoft SASLab Pro with the same settings as for song analyses (see above). Natural sequences of songs were kept but the song rate was slightly adjusted, if necessary, to six songs per minute (an average value for recordings of spontaneous singing, i.e. without territorial intrusion; T. Petrusková &

A. Kinštová, unpubl. data). All trills in those songs were replaced with either loud or soft trills originating from other recordings of the same bird (to manipulate all songs in an equal fashion). Each playback stimulus obtained thus contained the same number of randomly distributed songs with either loud or soft trills. The altered song sequence was doubled to create the final 3-min playback recording, which was used only once during the experiments.

Between end of April and beginning of June 2012, 10 Tree Pipit males were tested by playback of one of the stimulus types (loud or soft), that is, five males were tested with each type. First, we recorded spontaneous song of the tested male (using the equipment described above) for 3 min from a distance of approx. 25 m. After, the playback stimulus was played for 3 min from a portable digital player using a wireless transmission to a loudspeaker Mipro MA-202 placed into the male's territory. Focal male's singing activity was recorded during the playback as well as for 3 min after the end of the playback (to evaluate the post-playback singing behaviour). Neighbouring males were never tested during the same day.

### Data Analyses

Due to data distributions not conforming to normal distributions or homogeneity of variance, and/or relatively low number of males tested in playback experiments, we mostly analysed the data by non-parametric tests calculated in Statistica 6.1 (StatSoft, Inc., Tulsa, OK, USA). The differences in trill rates of both soft and loud trills among individual males were evaluated by Kruskal–Wallis test (only males for which at least five trills of that particular type could be analysed were included in the test). The relationships between soft and loud trill rates of the same individual, and between trill rates and body measures (body weight, wing length and tarsus length) were evaluated by calculating the respective correlation coefficients. Furthermore, we compared the performance of fast loud trills between territory holders and non-holders, by evaluating the median values of trill rates by Mann–Whitney *U*-test (with exact probability associated with the respective *U*-statistics); body measures between these two groups of males were compared the same way. Wilcoxon signed-rank test was used to compare the amplitude ratios of trills and immediately preceding phrases, between soft and loud trills sung by the same males (median value of three measurements was used for both types of trills).

To evaluate singing response to territorial intrusion simulated by playback experiments, we first compared

by Wilcoxon signed-rank test the number of songs sung in spontaneous songs recorded from the ten tested males, and in songs sung by them as a response to playback, that is, after the bird has started to respond to playback by singing. This comparison was performed for 1- and 3-min periods before the experiments and in response to playback. To test for a possible change in frequency of trill use in these periods, we evaluated the counts of songs with presence/absence of either loud or soft trills by generalized estimating equation (GEE) with the binomial distribution of errors and exchangeable correlation structure, using the package 'geeproc' (Højsgaard et al. 2006) implemented in R version 3.0 (R Core Team 2013). Finally, we used Wilcoxon signed-rank test to compare the median trill rate and duration in these songs for loud trills (which, unlike soft trills, were used sufficiently frequently by males; see Results).

## Results

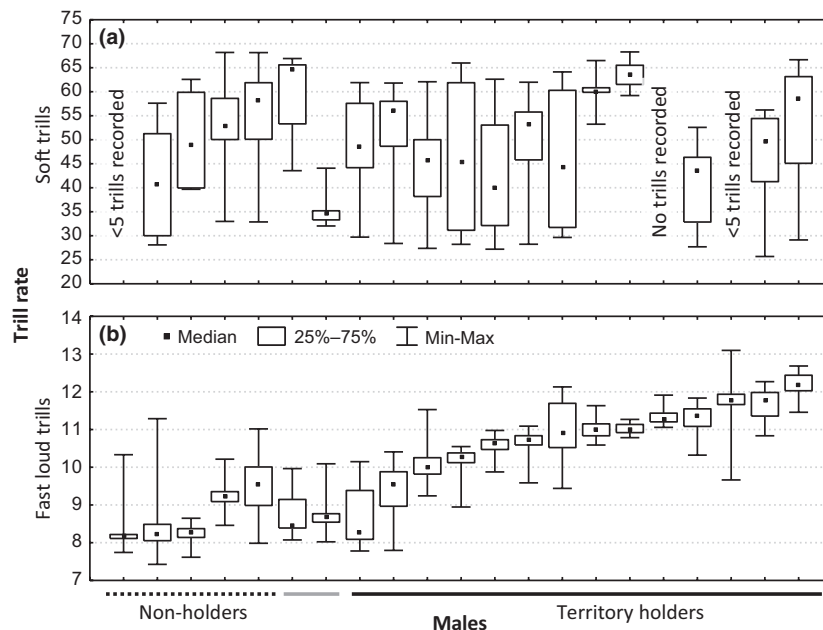
### Trill Characteristics and Rate Variation

We measured trill characteristics of 21 Tree Pipit males from the studied population (17 recorded in 2011, and four in 2012), for which a sufficient number of recordings from the respective season were available (see Methods). Altogether, 2022 loud trills and 391 soft trills were measured. All males frequently sang songs containing loud trills. Most of them also used soft trills at least occasionally but no

such trills were recorded for one male, and two others used them apparently only rarely, so <5 soft trills were found in recordings of their songs (Table 1, Fig. 2).

After evaluating the distribution of loud trill rates within each male's songs, we selected 1379 fast loud trills for further analyses. In the studied population, males sang three distinct variants of loud trills differing in element shape (as shown in Fig. 1b–d) but apparently used in the same contexts. Some males used only one of those variants (usually the one with a simple element), but others alternated two or even all three loud trill variants in their songs, and used them interchangeably (Table 1). In some cases, loud trill variants sung by the same male did differ in trill rate (Fig. S1), and only one of the variants was preferentially sung with high trill rate. The males singing particularly fast loud trills tended to use predominantly the simple element (shown in Fig. 1b). However, the trill rates within a bird's repertoire did not depend consistently on the trill variant used. The dominant variant in the fast category of loud trills comprised simple syllables (Fig. 1b) in some individuals, but more complex syllables (Fig. 1c, d left) in others (Table 1). Overall, males significantly differed in the trill rate of loud trills independently of the type they used (Fig. 2b).

We also observed variation in delivery of soft trills. Some males usually sang very fast trills in which high- and low-frequency elements were regularly alternating (as in Fig. 1e); a few of them used only trills containing the high-frequency elements



**Fig. 2:** Differences in trill rates of both studied categories of trills among Tree Pipit males. Males significantly differed in trill rate of both soft trills (a; Kruskal–Wallis test,  $df = 17$ ,  $n = 391$ ,  $H = 136$ ,  $p < 0.001$ ), and fast loud trills (b;  $df = 20$ ,  $n = 1379$ ,  $H = 1134$ ,  $p < 0.001$ ). Black line at the bottom indicates males which were able to maintain the territory during the whole breeding season (territory holders); grey line indicates two males, which were present throughout the season at the same place but their defended territories were substantially smaller than of other territory holders. Remaining males (indicated by a dotted line) changed their position during the season and are considered non-holders. Within each of these groups, males are ordered according to median values of their loud trill rate.

(Fig. 1f), and yet others attempted soft trills with both rows that were often irregular, missing some of the low-frequency elements. One presumably young male (according to its overall poor song performance; I. Pišvejcová, unpubl. data) sang particularly irregular single-row trills (Fig. 1d, right) in the first study season (2011), but in the subsequent season it substantially improved in trill production (as in Fig. 1e) as well as in delivery of other syllable types within songs. Another notable exception was a male singing very consistent soft trills, in which two high-frequency elements regularly alternated with one low-frequency element (Fig. 1g).

The above-mentioned variation in delivery of soft trills was reflected in their rate, with two-row soft trills having substantially higher rate than those with one row only. Further rate variation was observed within these groups and thus the males significantly differed in soft trill rates (Fig. 2a).

There were no significant correlations between median trill rates of soft and loud trills of the same male ( $n = 18$ ,  $r = 0.16$ ,  $p = 0.53$ ). Furthermore, trill rates were not significantly correlated with any of the body measures taken for the captured males (soft trills,  $n = 14$ : rate vs. weight,  $r = 0.08$ , wing length,  $r = -0.31$ , tarsus length,  $r = 0.30$ , all  $p > 0.29$ ; loud trills,  $n = 17$ : rate vs. weight,  $r = -0.11$ , wing length,  $r = 0.17$ , tarsus length,  $r = -0.10$ , all  $p > 0.51$ ).

Males that were able to maintain the territory at preferred locations (occupied each year as first) during the whole breeding season ( $n = 14$ ; indicated by black line in Fig. 2) were able to sing significantly faster loud trills (i.e. with higher median trill rate) than those without a stable territory ( $n = 5$ , dotted line in Fig. 2; Mann–Whitney  $U$ -test,  $U = 3$ , exact  $p = 0.0012$ ). These groups of males did not differ in their body measures (e.g. tarsus length:  $N_{\text{holders}} = 11$ ,  $N_{\text{non-holders}} = 4$ ,  $U = 16.5$ , exact  $p = 0.49$ ). The differences in loud trill rate between the two male groups were significant regardless of possible inclusion (to either territory holders or non-holders) of two ‘intermediate’ males (indicated by grey line in Fig. 2) that defended unusually small territories for this species (in comparison with other males at this as well as other Czech localities; see Kumstátová et al. 2004).

#### Amplitudes of Trills Relative to Adjacent Song Parts

Loud trills were always sung at substantially higher amplitudes than soft trills sung by the same birds. When compared with immediately preceding song phrases, loud trills were usually produced at similar and often higher amplitude (ratios of RMS amplitudes

of the two sections ranging from  $-9.2$  to  $5.6$  dB; overall median  $-1.2$  dB; negative values indicate that the trill amplitude was higher than that of the preceding phrase). In contrast, the soft trills that were perceived as notably quieter were indeed always of lower amplitude than preceding phrases (ratios in RMS amplitudes between  $7.8$  and  $24.1$  dB, overall median  $14.7$  dB). As such, a paired test comparing the amplitude ratios of loud and soft trills and preceding song phrases (taken as median values of the measurements for each bird) revealed highly significant differences (Wilcoxon signed-rank test,  $n = 22$ ,  $W = 0$ ,  $Z = 4.1$ ,  $p < 0.0001$ ).

#### Playback Experiments

In contrast to our hypothesis, we did not observe substantial differences in responses to playback stimuli containing either loud or soft trills. After both types of stimuli, the tested birds frequently included loud trills in their songs but the use of soft trills remained rare, even in birds exposed to playback containing those trills. Two of five males exposed to soft trill stimulus actually did not use such trills at all within 3 min after the playback started, and none of them sung more than two songs containing a soft trill. Similarly, two of five males exposed to loud trill stimulus did not use any soft trill after the playback; the three remaining ones used this type of trill once. In contrast, each male sung in the same period at least seven (up to 16) songs containing loud trills (in males exposed to soft trill stimuli, 24–73% songs sung after the playback contained a loud trill, in males exposed to loud trill stimuli, this proportion ranged from 25% to 58%). As the use by the two trill types by males did not seem strongly dependent on the playback type, we pooled the males’ responses for further analyses.

As a response to the playback, males increased the singing activity: the number of songs in the 3-min recordings was larger in post-playback than in spontaneous periods for all but one male (on average by over two songs per minute, from  $5.6$  to  $7.1$ ; median increase  $2.3$  songs/min); a similar, although weaker, pattern was observed for 1-min periods (three males sung the same number of songs, others increased the song output by 1–9 songs, median 3). Differences in both evaluated time periods were significant (Wilcoxon signed-rank test,  $n = 10$ ,  $W = 2.5$ ,  $Z = 2.55$ ,  $p = 0.011$  for 3 min, and  $n = 10$ ,  $W = 0$ ,  $Z = 2.37$ ,  $p = 0.018$  for 1 min, respectively).

As an immediate response, most males usually sang short songs from perches relatively close to the loudspeaker and increased the proportion of songs

containing loud trills. This was particularly pronounced in the period just after the onset of singing activity (that usually occurred during the playback itself): the proportion of songs containing loud trills increased significantly in the first minute of singing response in comparison with the spontaneous singing before the experiment (on average 52% before playback, range 17–86%; average after playback 72%, range 52–83%; GEE, binomial family,  $p = 0.009$ ). Afterwards, however, the males tended to resume singing activity to include other syllable types, and actually decreased the use of loud trills towards the post-playback silent period; the difference in the proportion of songs containing loud trills in 3-min stretches of the recording was thus no longer significant (averages 52% vs. 46%; ranges 17–75% vs. 24–73%; GEE,  $p = 0.23$ ).

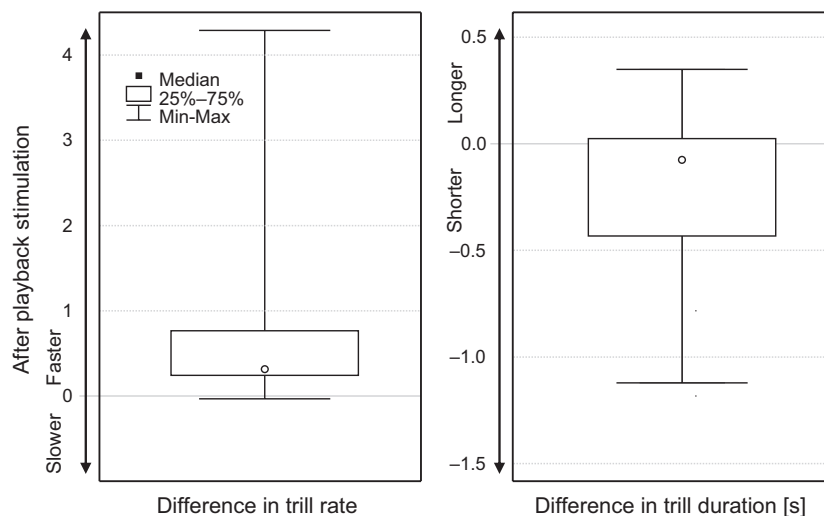
In contrast to loud trills, the proportion of songs containing soft trills, which are generally rarer, further decreased during the playback: eight of ten tested birds used these trills less frequently within the 3-min period, and actually did not sing any soft trill within 1 min. In a 3-min period of spontaneous recording before the playback, two birds did not use any soft trill, the others sang up to six such trills (median 2); during the playback, the numbers of soft trills sung further decreased (median 1, four birds did not use any, two sang two such trills). The difference in frequency of songs containing soft trills in 3-min periods before (average 11.4%) and during the playback stimulus (average 4.7%) was thus significant (GEE,  $p = 0.019$ ).

Finally, we evaluated the changes in trill rate of loud trills in spontaneous songs and those sang in response to playback. This comparison revealed that males significantly increased the trill rate in response

to the stimulus (Fig. 3; Wilcoxon signed-rank test,  $n = 10$ ,  $W = 1$ ,  $Z = 2.70$ ,  $p = 0.007$ ). No such pattern was observed for trill duration; on the contrary, the trills during the playback had a tendency to be shorter but the difference was not significant (Fig. 3;  $n = 10$ ,  $W = 14$ ,  $Z = 1.38$ ,  $p = 0.17$ ).

## Discussion

As we expected, males significantly differed in the production of both trill types (Figs 1 and 2). Faster soft trills showed more inconsistencies in element delivery among males – from those singing most often poor irregular soft trills (see example in Fig. 1d, right) to those commonly using two-row regular ones (example in Fig. 1e). These differences were consequently reflected in the trill rate (Fig. 2a). Trill rate of fastest loud trills substantially varied among males as well (Fig. 2b). Interestingly, the ability to produce faster loud trills correlated positively with the territory maintenance, and these trills were frequently used in male–male interactions (T. Petrusková, pers. obs.). After the playback stimulus, the males increased the overall song production, and all but one male speeded up loud trills in comparison with spontaneous singing recorded before the experiment (Fig. 3). Most of them increased the proportion of perched songs containing loud trills for a short period. Contrary to our expectation, no tested male used a substantial number of soft trills in response to playback containing only soft trill songs; on the contrary, the proportion of songs containing these songs actually decreased after the playback. This evidence suggests that unlike loud trills that are used in male–male short-distance interactions and apparently may play an important role in territory



**Fig. 3:** Differences in loud trill rate (left) and duration (right) in 3-min recording after the onset of singing response to the playback stimulus and in spontaneous singing. For each bird, median values are subtracted; the differences are highly significant for trill rate (Wilcoxon signed-rank test,  $n = 10$ ,  $W = 1$ ,  $Z = 2.70$ ,  $p = 0.007$ ) but not for duration ( $n = 10$ ,  $W = 14$ ,  $Z = 1.37$ ,  $p = 0.17$ ).

defence, high-rate soft trills, which might be more demanding to produce (see below), have other uses.

Results of our playback experiments are consistent with observations of natural male–male encounters by van Hecke (1979), who described increased production of perched songs after territory intrusion, followed at the end by a flight song. We suggest that the loud trill phrase, included frequently within these perched songs, and modulation of its rate, may play a substantial role in interactions between territorial males and intruders. Increase of trill rate during aggressive encounters, tested by playback experiments, was found for example in Swamp Sparrows (DuBois et al. 2009) or Banded Wrens (Vehrencamp et al. 2013). The latter species uses different trill types (Trillo & Vehrencamp 2005), and Illes et al. (2006) showed that Banded Wren males may use trill rate to assess an opponent's quality. Similarly to that species, Tree Pipit males also use different loud trill types and are to some extent able to vary rate. We thus assume that such trills may also carry information about the singer's quality.

Furthermore, Schmidt et al. (2008) showed that in Common Nightingales, broadband trills are an aggressive signal and their use reflects also future pairing success. The results of Schmidt et al. (2008) are similar to ours – Tree Pipit males with higher loud trill rate were more successful in long-term territory maintenance. They were also successful in pairing, as suggested by observation of courting flights with females, feeding activity, and warning vocalization associated with fledglings within the territory (T. Petrusková, pers. obs.). No such evidence for successful pairing was observed for non-holders.

While the function of loud trills at least in territory defence seems to be supported, it is unclear whether a specific role can be attributed to soft trills. Our results indicate, although indirectly, that these trill structures might be difficult to produce. In particular, males substantially differed in the soft trill rate (Fig. 2a). A similar pattern was found in some species such as Canaries (*Serinus canaria*; Vallet et al. 1998; Drăgănoiu et al. 2002) or Swamp Sparrows (Ballentine et al. 2004), and higher performance trills were preferred by females in those species. Furthermore, Tree Pipit males differed in their ability to produce the soft trill consistently (which was also reflected in trill rate). In Chestnut-sided Warblers (*Dendroica pensylvanica*), Byers (2007) observed a greater extra-pair success in males singing their simple and fast song with higher consistency. Vehrencamp et al. (2013) also demonstrated that consistency and trill performance increased between years in Banded Wrens,

suggesting that these characteristics reflect male age. There are no reliable features to assess age in Tree Pipits; however, we observed in one male a substantial improvement of song performance (including trill consistency and rate) from the first to the second year of the study (I. Pišvejcová, unpubl. data). Further data on returning birds are needed to evaluate whether song performance may also reflect age in our study species.

A comparison of a short stretch within one song, where all syllables should be similarly affected by recording conditions, revealed that loud trills are usually sung at the same or even higher amplitude than the immediately preceding part of the song, while soft trills are significantly quieter. This is another indirect support for soft trills being quality indicators, as structures which are difficult to produce may not be sung at very high amplitudes (Suthers & Zollinger 2004; Ritschard & Brumm 2011). Moreover, trills with similar features were found as signals of quality in various bird species (e.g. Cramer & Price 2007; de Kort et al. 2009; DuBois et al. 2011). Additionally, soft trills are often used in flight songs (Petrusková et al. 2008), which were documented in various species to be directed mostly to females (e.g. Wittenberger 1983; Balsby & Dabelsteen 2003). In Tree Pipits, we may also assume this role, as unpaired males tend to sing more flight songs than paired ones (Loske 1987; T. Petrusková, pers. obs.).

So far, we can conclude that unlike soft trills, loud trills are used in territorial encounters, and their modulation likely reflects the aggressive motivation. Soft trills have several features that suggest them to be performance-demanding song structures, predisposing them to serve as quality indicators. However, it seems that loud trills may also carry information relevant for assessing singer's quality to potential mates or rivals. To test more detailed hypotheses on information carried by specific trills, further fieldwork is needed focusing on mating success in relation to song performance, natural interactions between birds and playback experiments evaluating responses to fast and slow trills presumably reflecting high and low quality of males. The simultaneous use of different trill types and variants makes the Tree Pipit an interesting species to study the role of these structures in bird vocal communication.

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### Supporting Information

Additional supporting information may be found in the online version of this article:

**Figure S1:** Representative examples of distribution of loud trill rates and variants in recordings of analysed males.

**Supplementary Figure 1. Representative examples of distribution of loud trill rates and variants in recordings of analysed males.**

Different loud trill variants are indicated by colour, letters refer to corresponding spectrograms in Fig. 1; male numbers correspond to Table 1. Black arrows delineate trills that were chosen as a “fast” category for the respective bird, and further used in among-male comparisons. In case of bi- or multimodal distribution of loud trills with substantially differing rates, only the fastest distinct group was considered (e.g., males 13, 12, 10, 3); all loud trills sung by the males were evaluated if their distribution followed an unimodal pattern (as in male 11) or the potential fast category had only negligible proportion (as in male 2).

