

University of Groningen

Acoustic signalling in Eurasian penduline tits *remiz pendulinus*

Pogany, A.; van Dijk, R. E.; Menyhart, O.; Miklosi, A.; DeVoogd, T. J.; Szekely, T.

Published in:
Acta zoologica academiae scientiarum hungaricae

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2013

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Pogany, A., van Dijk, R. E., Menyhart, O., Miklosi, A., DeVoogd, T. J., & Szekely, T. (2013). Acoustic signalling in Eurasian penduline tits *remiz pendulinus*: Repertoire size signals male nest defence. *Acta zoologica academiae scientiarum hungaricae*, 59(1), 81-96.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

ACOUSTIC SIGNALLING IN
EURASIAN PENDULINE TITS *REMIZ PENDULINUS*:
REPERTOIRE SIZE SIGNALS MALE NEST DEFENCE

POGÁNY, Á.^{1*}, VAN DIJK, R. E.^{2,3,5}, MENYHÁRT, O.⁴
MIKLÓSI, Á.¹, DEVOOGD, T. J.⁴ AND T. SZÉKELY²

¹Department of Ethology, Eötvös University

H-1117 Budapest, Pázmány Péter sétány 1/c, Hungary, E-mail: apogany@ludens.elte.hu

²Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK

³Behavioural Ecology and Self-Organization, Centre for Ecological and Evolutionary Studies
University of Groningen, 9700 CC, Groningen, The Netherlands

⁴Department of Psychology, Cornell University, Ithaca, NY 14853, USA

⁵Present address: Department of Animal and Plant Sciences, University of Sheffield
Sheffield S10 2TN, United Kingdom

Elaborate male song may restrain competitors in various songbirds, although the exact mechanism, information content and information flow of acoustic signals are not completely understood. Here we focus on the interactions between resident and intruder males using the Eurasian penduline tit *Remiz pendulinus*. The breeding system of this small passerine bird is unusually variable including sequential polygamy by both sexes, and appears to be driven by intense sexual selection and sexual conflict over parental care. We had two objectives in this study: (i) to investigate whether male repertoire size is an intra-sexual signal in penduline tits, and (ii) to determine emitter and receiver roles during acoustic communication between residents and intruders. We tested these objectives in a natural population by recording the resident males' song and then challenging them by song playbacks. The residents' approach to intruder stimuli and behavioural responses were monitored. We found that intruder repertoire size did not evoke different responses by the residents. Rather, the resident's approach distance and behavioural response was predicted by his own repertoire size, suggesting that song in penduline tits is involved in male-male communication, and repertoire size may function as a reliable signal of the resident male's ability and willingness to defend his nest and avert possible intruders.

Key words: acoustic communication, repertoire size, sexual selection, penduline tit, dominance.

INTRODUCTION

Acoustic signals are among the most common sexually selected traits in many taxa, especially in insects, anurans and birds (ANDERSSON 1994, NAGUIB *et al.* 2009). Male songbirds communicate vocally with females, with other males, or both (SEARCY & ANDERSSON 1986, CATCHPOLE & SLATER 2008). The inter-sexual function of male song includes attraction of females, advertising various male characteristics which increase female fitness when choosing the male ('male quality', including genetic quality, ability to defend territory, breeding site or resources, and parenting ability) and stimulating females to

mate (CATCHPOLE *et al.* 1984, SEARCY 1992, CATCHPOLE & SLATER 2008), but see BYERS & KROODSMA (2009) for alternative explanations of the evolution of repertoire size). In inter-sexual acoustic communication, the emitter is usually the male and the receiver is the female, although in some species these roles are reversed or, as in duetting monogamous species, both sexes are emitters and receivers simultaneously (HALL 2004).

When song functions as an intra-sexual signal, however, the direction of communication is less obvious and multiple ways may evolve. In many songbirds the male sings around his nest, or within his territory ('resident' male, henceforth). It is generally assumed, therefore, that song signals the owner's presence, quality and ability to defend territory boundaries against other males ('intruder' males, henceforth). Indeed, two main lines of field experiments support these intra-sexual functions of male song (KREBS *et al.* 1978, SEARCY & NOWICKI 2000, CATCHPOLE & SLATER 2008). First, when conspecific male song was played in a suitable 'vacant' breeding site (i.e. one without a resident), this territory was occupied later than other suitable vacant territories in which no playback was played. Second, removal of a resident male from his territory resulted in more likely and faster take-over from new males. In addition to resident signalling, intruders may also communicate their presence, their intention to intrude and their quality to resident males by their song (cf. male quality hypothesis, LAMBRECHTS 1992). Finally, male-male communication may be bi-directional, so that song characteristics bear information for both the resident and the intruder, and both are emitters and receivers, simultaneously or sequentially. The rationale behind the latter type of male-male communication is that resident and intruder roles are interchangeable, and it is in the common interest of both the resident and the intruder to settle male contests by harmless means – a function of male song that has been revealed by muting and playback experiments (MCDONALD 1989, BALLENTINE *et al.* 2008).

Sexually selected traits are predicted to be more expressed in animals with polygamous mating systems and intense sexual conflict (ANDERSSON 1994, ARNQVIST & ROWE 2005, VAN DIJK *et al.* 2010a). We investigated acoustic signalling in one such species, the Eurasian penduline tit *Remiz pendulinus*. The breeding system of this small songbird is sequential polygamy by both males and females, so that each sex may obtain up to six mates in a single breeding season (PERSSON & ÖHRSTRÖM 1989, SZENTIRMAI *et al.* 2007). Parental care (incubation and offspring provisioning) is carried out by one parent only, which can be either the male or the female. Approximately one-third of the clutches are deserted by both parents (PERSSON & ÖHRSTRÖM 1989, SZENTIRMAI *et al.* 2007). In line with this highly variable breeding system, previous field and laboratory studies identified the sizes of the black facial mask and the

nest built by males as sexually selected traits (HOI *et al.* 1994, GRUBBAUER & HOI 1996, POGÁNY & SZÉKELY 2007, KINGMA *et al.* 2008). In addition, males sing elaborate songs apparently to advertise the location of their nest for females, they restrict singing to reproductive periods, and repertoire size varies between males (VAN DIJK *et al.* 2010a), suggesting that song is also involved in sexual selection.

Although penduline tits do not defend territories, they do defend the immediate surroundings of the nest so that nest-building males chase away intruders near their nests (BLEEKER *et al.* 2005, SZENTIRMAI *et al.* 2007, KINGMA *et al.* 2008). Based on our observations, the resident male's response at his nest is probably driven by competition between males for suitable nest sites, nest material (nests of other males provide an easy source of material) and females. The latter assumption is further supported by our data; 5.5% of nests (46 out of 838) were taken over by intruder males in our population, and approximately one-third of such takeovers (32.6%; 15 out of 46) took place after pair formation. When an intruder took over the nest of a mated pair, the female frequently stayed with the new male (71.4%; 11 out of 14 nest takeovers), so that the resident male lost both his nest and his mate to an intruder. Additionally, using nine polymorphic microsatellite loci, van Dijk *et al.* (2010b) showed that 60.0% of nests contained at least one extra-pair young, and that 23.5% (39 out of 166) of offspring were sired by extra-pair males. This may to some extent be associated with nest takeovers.

Here we report a field study in which we investigated the roles of male repertoire size (i.e. the number of different syllable types a male sings) as a means of communication in male contest situation. Furthermore, we aimed to determine the direction of communication between resident and intruder males. After recording the song of nest-building males, we challenged these residents at their nest using song playbacks to test whether the repertoire size of intruder and/or resident male song is involved in intra-sexual acoustic communication of penduline tits. In addition to the main questions of our study, in a subset of males that acquired mate we investigated how mating status influenced response towards intruders.

MATERIALS AND METHODS

Fieldwork

The study was carried out in Fehértó (46°19'N, 20°5'E), an extensive fishpond system in southern Hungary, between 22 May and 6 July in 2006. This period coincided with the peak breeding season of penduline tits (SZENTIRMAI *et al.* 2005). We searched for new and checked existing nests, and trapped and ringed penduline tits following the standard methods described in our field protocol (VAN DIJK *et al.* 2007a), resulting in N = 22 male

penduline tits involved in this study. We carefully avoided nest abandonment by trapping only at nests which were in advanced stages of building (at least in stage C, see our field protocol, VAN DIJK *et al.* 2007a). In each case, the trapping trial lasted for less than 30 min; if trapping was not successful, we repeated the trial on the next day. Males were caught in mist nets after they had built their first nest, using a male penduline tit dummy (Fig. 1) and a short song playback that consisted of a mix of penduline tit calls and song syllables. Penduline tits were ringed with a numbered metal ring from the Hungarian Ornithological Institute and three colour rings (A. C. Hughes, Middlesex, UK) for individual identification. To minimise disturbance the nests were observed using binoculars from at least 15 m every other day to establish the breeding status of the male. Nest abandonment (leaving the nest before mating) of the unmated male, or nest desertion (leaving the nest, offspring and mate) by the mated male was established if the male was not observed at the nest for at least 15 min on two consecutive nest checks following VAN DIJK *et al.* (2007b). We observed no nest abandonment related to trapping, or to the presentation of stimuli at the nest. Penduline tits can be sexed reliably according to their behaviour (nest building and singing), and their sexually dimorphic plumage traits (VAN DIJK *et al.* 2008); males have large black masks, and their colours are brighter than those of females (CRAMP *et al.* 1993, KINGMA *et al.* 2008).

Repertoire size of resident males

By repertoire size we refer to syllable repertoire size, i.e. the total number of different syllable types sang during various song bouts (KROODSMA 1982). Since Eurasian penduline tits have small repertoires (range 11–20 in our population, see sonograms of some of the



Fig. 1. Resident male penduline tits react to playback song and a dummy penduline tit around their nest. Behavioural responses included attacking, i.e. pecking at the dummy, as seen on this photo (credit: Csaba Daróczy).

most frequently sung syllable types in Fig. 2), we used simple enumeration to count syllable types in a song sample (BOTERO *et al.* 2008). We recorded and analysed the songs of all resident males ($N = 22$) included in this study (mean \pm SD, 4.2 \pm 1.1 h of recording per individual that contained 28.2 \pm 12.8 song bouts), in order to estimate repertoire size ('resident repertoire size' henceforth). This recording time is sufficient to assess repertoire size in penduline tits, since repertoire size does not change (i.e. no new syllable types) after 3.1 \pm 1.4 h of recording (or 17.7 \pm 10.1 song bouts) in our sample. Recording took place before the first experimental trial of a given male. During these recordings, a parabolic microphone (Telinga) and a digital song recorder (Marantz PMD 660) were hidden in the vegetation at a fixed distance (approximately 8 m) from the male's nest.

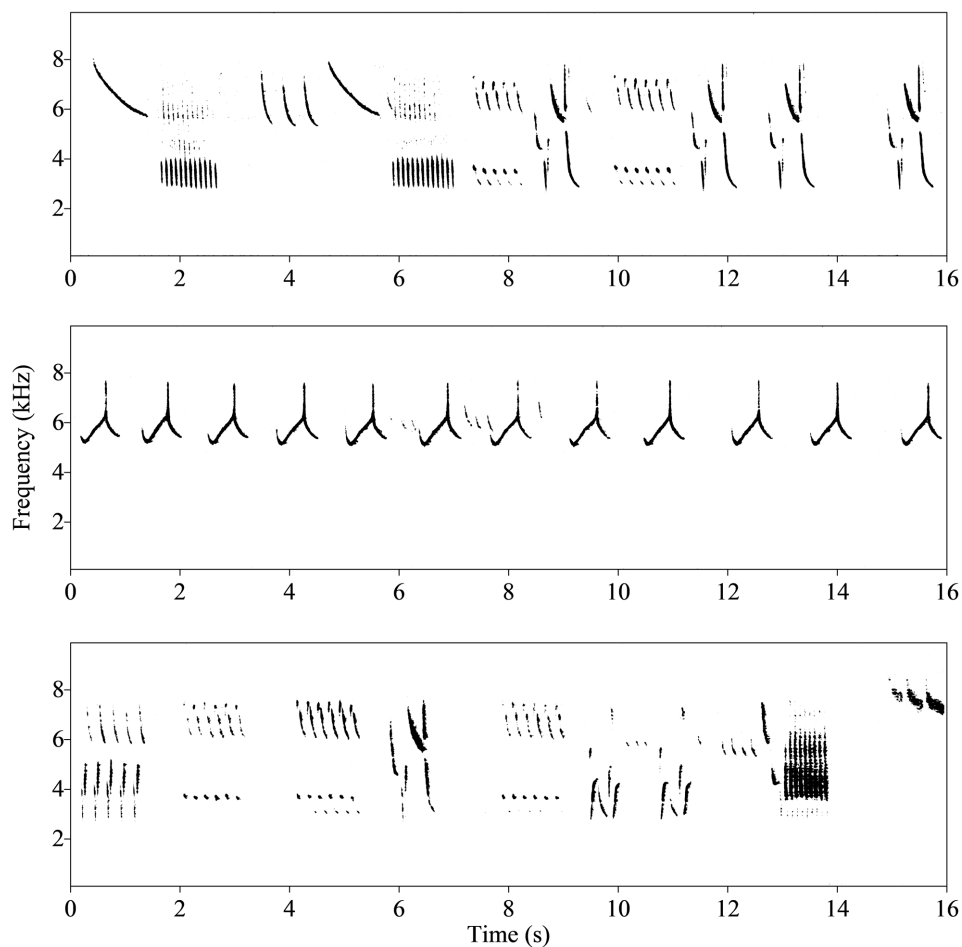


Fig. 2. Sonograms of some typical syllable types of Eurasian penduline tits. Song bouts may consist of various syllables (top and bottom sonograms) or may include monotone repetitions of a certain syllable (middle sonogram).

Song playbacks

Playbacks were compiled from male penduline tit songs recorded in 2003 in Fehértó, using a Sony digital recorder (TCD-D7 DAT) and a Telinga parabolic microphone. We recorded 25 males, each for 1.6 ± 0.6 h. The number of clean recordings without significant background noise for playback purposes was limited due to the nature of song recording in the field. Therefore, syllables for stimulus playbacks were drawn from recordings of six males. From these recordings, syllable types were extracted and mixed so that each stimulus playback contained some syllables from all six males. We compiled two sets of stimulus playbacks (set 1 and set 2) that we selected randomly before the first trial of a resident and then used consistently in his subsequent trial (see below). Each set included two playback treatments: a small and a large repertoire size playback treatment ('intruder repertoire size' henceforth). Each playback treatment within a given set lasted for 10 min and the number of syllable types within playbacks was calculated based on the natural range of repertoire size in penduline tits. In our 2006 song samples the repertoire size of individual males ranged between 11 and 20 (mean = 16) syllable types. In set 1 and set 2 playbacks, we included 8 and 9 syllable types in small repertoire size playback treatments, and 16 and 17 different syllables in large repertoire size playback treatments. Therefore, we used less syllable types in both small and large repertoire playback treatments than the natural minimum and maximum, respectively. This was to create realistic playbacks; our playback treatments lasted for only 10 min and individuals reach their repertoire maximum only within hours (see above).

The length of penduline tit song bouts is 23.88 ± 9.33 s. When analysing natural songs we defined the end of a song bout as when the silent interval lasted for at least 5 s between two syllables. Therefore, in each 10-min playback a 30 s song bout was followed by a 30 s break to mimic natural singing behaviour of penduline tits, which is within the natural range of singing activity. This series was repeated 10 times, so that no two song bouts were identical in the given playback treatment (syllable types or their order within the song bout was different). Stimulus playbacks (small and large repertoire treatments in set 1 and set 2 playbacks) were similar in other parameters, such as the total number of syllables, the number and length of song bouts, and the intensity of songs. Playback songs were compiled using GoldWave software (v 5.13, GoldWave Inc., Canada).

Experimental design

Unmated resident males ($N = 22$) were tested for small and large intruder repertoire size treatments on two consecutive mornings or afternoons, either between 06:00 and 10:00 h (Central European Time, 'morning session' henceforth), or between 16:00 and 20:00 h ('afternoon session'). The first experimental playback of a given male took place 11 ± 3 days (mean \pm SE) after trapping, so trapping is unlikely to have influenced the outcome of the experiment.

At their first trial, a playback set (set 1 or set 2) was assigned to each resident male randomly, and for his second trial the same set was used consistently. For the first trial of a given male, session (morning or afternoon) and treatment level order (small *vs.* large repertoire) was randomised. For the second trial on the following day, treatment level was balanced so that if his first trial was small repertoire size treatment then he was subjected to large repertoire size treatment and *vice versa*. In each morning and afternoon session, maximum four different males were tested: the order of the males within their first morning (or afternoon) session was randomised, and then the same order was kept on the fol-

lowing day. All experimental trials were initiated only when the resident male was present at the nest.

In addition to the main focus of our study, we tested whether the resident male's response is predicted by his mating status using the subset of males that acquired mate during our study ($N = 9$ males). These males received repeated treatments again on two consecutive days (both trials either in the morning or in the afternoon, selected randomly), using the same set of small and large repertoire playbacks that was used when the male was unmated to investigate the change in his response according to change in mating status. In five out of nine nests, the male was tested at a different nest when mated than that of his unmated nest. This was due to frequent nest abandonment of unmated males; males may start building a new nest if they do not acquire a mate at their previous nest (PERSSON & ÖHRSTRÖM 1989, SZENTIRMAI *et al.* 2007, VAN DIJK *et al.* 2007b).

Experimental protocol

Stimulus playbacks were played from a three-way speaker (MKS-58/SW, Monacor GmbH & Co. KG, Germany) connected to an amplifier (PA-100, Monacor GmbH & Co. KG, Germany), a compact disc player (XP-V730, Aiwa Co. Ltd, Japan) and a lead acid battery power supply (LA 1220, Hi-Watt Co. Ltd, China). The speaker was positioned at about 15 m from the nests of resident males together with a hand-painted, plaster male penduline tit dummy (Fig. 1), mounted on a stick. The experimenter hid behind a hide 27 m from the nest, so that only the speaker, the dummy and the speaker's 12 m cord (hidden in the grass or reed) were positioned between the experimenter and the nest, whereas all other technical equipment was located behind the experimenter's hide and thus unlikely to disturb the behaviour of resident males. The direction of speaker from the nest was randomised for the first trial. The exact position of the stimulus speaker and dummy was marked for the first trial of a resident, and these were used during his subsequent trial(s).

The density of vegetation around the nest may influence the responses and/or the visibility of the residents during playbacks. To control for visibility, we estimated vegetation coverage (0–3) for each nest site as follows: 0 – the experimenter could see the nest from the hide; 1 – the experimenter could not see the nest but had a clear view of the stimuli and the tree where the nest was located; 2 – sufficient visibility within a 10 m circle from the stimuli; 3 – sufficient visibility within a 5 m circle from the stimuli.

Data processing and statistical analyses

We recorded the responses of resident males using scan sampling every 20 s of a 10-min trial using two variables: (i) distance to speaker and stimulus dummy, and (ii) behaviour during playback. First, we estimated the position of the male from the speaker along x-y-z coordinates, with 0.5 m accuracy. To increase the accuracy of distance estimation, before the first trial at a given nest the experimenter measured the distance of natural signs (e.g. bushes, reed stems or tree trunks) from the speaker, or placed wood pegs to indicate 5 m and 10 m from the speaker. These markers together with the height of the reed served as reference points, and helped the experimenter to estimate the subjects' distance in 3D from the speaker at each time interval. We then estimated the approach distance (D) from the speaker for each time interval by calculating the length of the hypothetical diagonal of an x-y-z-sided cuboid:

$$D = \sqrt{x^2 + y^2 + z^2} \quad [1]$$

Finally, we calculated the average D over the 10-min trial and used this as a response variable in the analyses.

Second, we recorded the following behavioural responses: calling, singing, attacking the dummy and tail quivering (the latter is a signal of excitement by moving rapidly the tail feathers from side to side) using scan sampling every 20 s during the experimental trials. These four types of behaviour out of all behaviours observed in penduline tits (see the list of behaviours in our field protocol, VAN DIJK *et al.* 2007a) are probably related to sexual and aggressive situations in penduline tits (Á. Pogány, R. E. van Dijk, T. Székely, pers. obs.). Finally, we calculated the percentage of scan intervals when a resident male showed any of these behaviours, as a behavioural response to the intruder situation. All observations were carried out by one observer (Á.P.).

We analysed the responses (approach distance or % behavioural responses) of resident unmated males in Generalized Linear Mixed Models (GLMM) in R 2.13.2 (R DEVELOPMENT CORE TEAM 2011). Approach distance (GLMM 1, response variable) was log-transformed ($\log_{10}(D + 1)$), whereas % behavioural responses (GLMM 2, response variable) were arcsine transformed prior to the analyses. Intruder repertoire size (small *vs.* large) was included as fixed factor in GLMMs, whereas resident repertoire size was a continuous variable and male ID was a random factor. In both initial GLMMs we included the potentially confounding effects of advance of the breeding season (measured as the number of days since 1 April), session (morning *vs.* afternoon), vegetation coverage around the nest and number of trials. Initial GLMM 1, but not GLMM 2, also included visibility of the resident (% of 20 s behavioural scans when the male was seen) as it could potentially influence approach distance but not acoustic behavioural responses; the latter included calling and singing and these were observed even when the male itself could not be seen. Model selection was based on AIC and was carried out in a stepwise manner (with direction of selection set both forward and backward).

Nine out of 22 males acquired a mate, allowing us to compare the responses while a male was unmated and mated using paired-samples t test. In these tests, the two unmated trials for repertoire size treatments were averaged for a given male, and we compared these to the average response of the same male to small and large repertoire trials when mated. For two males the order of stimulus presentation regarding mating status was reversed, since first we recorded the responses of these two males as mated, and then at subsequent nests as unmated. Time span between unmated and mated trials were 8 ± 5 days (mean \pm SE) – a short period relative to the duration of the full breeding season (approximately three months, HARRAP & QUINN 1996).

In all GLMM models and statistical tests two-tailed probabilities are given, and we reject the null-hypothesis at $P < 0.05$ level.

RESULTS

Responses by resident males

All resident males ($N = 22$) responded to the intruder by approaching the dummy, and displaying at least one of four key behaviours (Table 1). Un-

Table 1. Responses of resident unmated and mated male penduline tits to small and large repertoire size song playbacks. Mated males is a subset of unmated males that acquired mate and was repeatedly tested, and we show their responses separately before (UM) and after (M) they got their mate. Mean±SE and statistics of paired samples t-tests are provided.

	Unmated males (N = 22)	Mated males (N = 9)			
		UM	M	t_s	P
% presence during trial	57.3±4.8	63.5±7.6	44.3±9.8	-1.792	0.111
Approach distance (m)	5.3±0.6	6.1±1.5	9.2±2.1	1.533	0.164
% behavioural response	57.6±4.0	55.6±7.2	31.1±8.7	-2.306	0.05
% calling	21.1±2.8	22.6±4.9	11.3±3.7		
% singing	15.4±3.1	18.5±7.4	6.7±2.7		
% tail quivering	19.8±3.3	14.1±3.2	13.0±7.8		
% attacking	1.2±0.7	0.4±0.4	0.2±0.2		

mated males were present around their nest in 57.3±4.8% of time during trials. Attacking (Fig. 1), interpreted as the strongest behavioural response, was displayed by three out of 22 experimental males.

Responses to small vs. large intruder repertoire sizes

Unmated males did not approach the speaker differently depending on whether small or large repertoires were played back, however, the larger their own repertoire size was the closer they approached the stimuli (GLMM 1, Table 2, Fig. 3a). We also found strong individual differences in approach distance (i.e. consistent male response between playback treatments; $\chi^2_1 = 9.246$, $P = 0.002$).

Similarly, behavioural responses of residents were not different between small *vs.* large repertoire playbacks, whereas residents expressed sexual behavioural responses more frequently in relation with their own repertoire increasing (GLMM 2, Table 2, Fig. 3b). In GLMM 2, individual differences between males were not significantly consistent between small and large repertoire playbacks ($\chi^2_1 = 2.068$, $P = 0.150$).

Out of the possible confounding variables, both final GLMM models included session and vegetation coverage (Table 2).

Table 2. Approach distance (GLMM 1, response variable) and % behavioural responses (GLMM 2, response variable) of resident male penduline tits in intruder-mimicking playback experiments. Residents were repeatedly tested with small and large repertoire playbacks. Model selection was based on AIC, and we provide the final models.

GLMM 1 (approach distance)	Parameter estimate±SE	DF	P
Resident repertoire	-0.051±0.022	19	0.032
Time of day	0.130±0.084	21	0.139
Vegetation coverage	-0.142±0.065	19	0.041
GLMM 2 (% behavioural responses)			
Resident repertoire	0.090±0.024	19	0.001
Time of day	-0.200±0.099	21	0.058
Vegetation coverage	0.095±0.070	19	0.188

Male response and resident mating status

Mated males exhibited weaker behavioural responses than unmated males (paired-samples t-test: $t_8 = -2.306$, $P = 0.050$; Table 1), although approach distances were not different between the two mating statuses ($t_8 = 1.533$, $P = 0.164$).

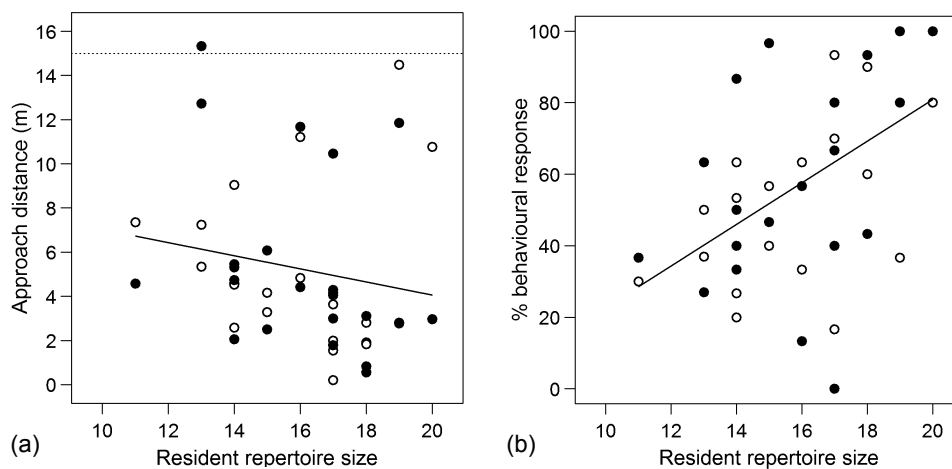


Fig. 3. Approach distance (a) and % behavioural responses (b) towards an intruder in relation to the resident male's own repertoire size. Behavioural responses included calling, singing, tail quivering and attacking. Open circles indicate responses of challenged residents on small repertoire playback, whereas filled circles indicate the same males' responses on large repertoire playback. Note that points shown on the upper half region of (a) represent males that were mostly present very close to their nest (15 m from the stimulus, indicated by dotted line) during the test.

DISCUSSION

Our experiment showed that male penduline tits use acoustic signals in intra-sexual communication. The correlation between resident repertoire size and responses towards intruders suggest that residents may use their repertoire size to advertise their ability (and/or willingness) to defend their nest site. Variation in intruder repertoire size, however, did not elicit different responses, suggesting that resident male penduline tits are emitters and potential intruders are receivers in acoustic male-male communication.

Resident males with large repertoires approached the intruder-mimicking stimuli more closely and gave stronger behavioural responses. Resident repertoire size may thus function to advertise competitiveness to defend the nest (or mate). This proposition, however, needs to be corroborated using an experimental approach, for instance by manipulating resident repertoire size and observing real encounters between residents and intruders. Repertoire size may be associated with other male characteristics that are relevant for intruder-resident encounters, such as age, body size or personality (e.g. MCGREGOR *et al.* 1981, RIVERA-GUTIERREZ *et al.* 2010). Whether this association is used for assessing the quality of the resident by possible intruders needs further investigation.

Previous studies raised questions about the biological relevance of repertoire size (particularly in species with large repertoires) as males (and females) may not have the time, opportunity and brain capacity to observe the full repertoire of a male (GARAMSZEGI *et al.* 2005, BOTERO *et al.* 2008, RIVERA-GUTIERREZ *et al.* 2011). These studies suggest that other song characteristics, such as song consistency, which may also be correlated with syllable repertoire size, may be more important cues in male-male interactions. To reveal the exact signalling function of various song traits, further studies are needed in light of these recent findings. Nevertheless, if repertoire size is a reliable signal in male-male competition, the traditional view of a dichotomous split between female attention to song repertoire and male attention to song intensity may not be true in case of penduline tits (CATCHPOLE & SLATER 2008).

Our findings are in line with the classical functional view of acoustic signals in male-male communication: the resident plays the emitter, whereas the intruder plays the receiver role in penduline tits (KREBS *et al.* 1978, YASUKAWA 1981, NOWICKI *et al.* 1998, BEEBEE 2004). The signalling function of resident repertoire is further supported qualitatively by our behavioural observations; only three of the challenged males attacked the dummy during the experiment and this prevented us from statistically testing correlation between the residents' repertoire and their aggression. Nevertheless, all these attacking residents had large repertoires (sang 18 syllable types) suggesting that resi-

dent repertoire size may reliably signal aggressive intent (SEARCY *et al.* 2006), besides signalling overall nest defence by other, less aggressive means.

Resident males showed less interest in an intruder after they acquired a mate than before. We do not think that this change in male behaviour is explained by advance of the breeding season, since mated trials were taken relatively close in time to unmated trials. Our results suggest that the value of a given nest (or nest site) decreases after a resident male secured matings with a female. The majority (two-third) of nest takeovers happened before mating in our population, so the risk of losing a nest site is higher before than after the male acquired a mate. We have several, non-mutually exclusive explanations to the response difference driven by mating status. First, the nest is a less consistent structure in the early stage of building, so that stealing nest material may be more destructive than when a nest is in a more advanced stage (e.g. when the male is already mated). Second, females spend approximately 20% of their time around the nest of their mate (Á. Pogány, R. E. van Dijk & T. Székely, unpubl. data), and mated males and females appear to avoid each other at the nest due to intense sexual conflict (VAN DIJK *et al.* 2010a). Nest guarding and mate guarding, therefore, appear to be traded off against each other in this species (SCHLEICHER *et al.* 1993; Á. Pogány, R. E. van Dijk & T. Székely, pers. obs.). These observations suggest that resident mated males loose paternity more likely through their mates encountering other males far from the nest than from the males trying to take over their nest. Third, as the time of desertion approaches, males may take a 'transitional state' between breeding at their old nest and starting their next breeding attempt so that their efforts at their current nest may decrease. The latter explanation, however, seems unlikely in the light of our earlier findings; desertion seems to be a very fast process making a slow transition less likely to take place (VAN DIJK *et al.* 2007b).

The lack of a differential response to large and small repertoire intruder playbacks suggests that resident penduline tit males do not acquire information about variation in intruder male quality by means of their repertoire size (i.e. we found no support for the male quality hypothesis, CATCHPOLE 1989, LAMBRECHTS 1992, BALSBY & DABELSTEEN 2001). However, we acknowledge that resident males might have reacted differently to treatments in responses that we did not quantify. For instance, residents frequently sang back to our playbacks. Song type matching (KREBS *et al.* 1981, BEECHER *et al.* 2000), or song type variant switching (SEARCY *et al.* 2000) were not investigated, but are plausible forms of reaction to intruders with different song characteristics.

Male song, besides its function in male communication, appears to attract females to the nest site (Á. Pogány, R. E. van Dijk and T. Székely, pers. obs.). Repertoire size, therefore, likely has a dual function in penduline tits

(cf. BERGLUND *et al.* 1996), so that this trait may evolve by both intra- and inter-sexual selection. Further studies may reveal whether males with more complicated song, which are better able to defend their nests, are selected for by females. In addition, there is an example for a sexually selected morphological trait (mask size, POGÁNY & SZÉKELY 2007, KINGMA *et al.* 2008) to be associated with parental care decisions in penduline tits (VAN DIJK *et al.* 2010a). In the latter study we reported that attractive males were more likely to desert, although females mated to attractive males were not more likely to care for their offspring. This line of research could be followed up by investigating the link between male repertoire size, mate choice and parental care decisions. In penduline tits, mate choice appears to be based on multiple cues, and by linking sexual selection and sexual conflict over parental care, this species provides an excellent opportunity to understand the complex interactions of breeding system evolution.

In conclusion, we showed that acoustic signals are involved in communication between male Eurasian penduline tits. Our results suggest that repertoire size may be a reliable signal of resident males to advertise their ability and willingness to defend their nest against intruders, but experimental studies are needed to corroborate this finding. The individual characteristics signalled by the resident male towards potential intruders, and whether penduline tit song has a dual function and is also involved in inter-sexual communication deserve further investigation.

*

Acknowledgements – Permission to work at Fehértó fishpond was provided by Kiskunság National Park (Hungary; permit number: 23864–2–3/2006) and Szegedfish Kft. (Hungary). We are grateful to Gábor Bakacsi, Miklós Beke, Béla Tokody, Lídia Mészáros and Zsófia Gergely for their assistance in the field. We thank András Kosztolányi for his comments on statistics in R and Csaba Daróczi for the photograph in Figure 1. The research leading to these results has received funding from the European Community's Sixth Framework Programme FP6 NEST GEBACO (under contract no. 28696), and INCORE (under contract no. 43318). Further support was received from grants to TS (BBSRC BBS/B/05788) and from the Hungarian Scientific Research Foundation (OTKA T043390, K081953).

REFERENCES

- ANDERSSON, M. (1994) *Sexual selection*. Princeton University Press, Princeton, 624 pp.
ARNQVIST, G. & ROWE, L. (2005) *Sexual conflict*. Princeton University Press, Princeton, 360 pp.
BALLENTINE, B., SEARCY, W. A. & NOWICKI, S. (2008) Reliable aggressive signalling in swamp sparrows. *Animal Behaviour* **75**: 693–703.
BALSBY, T. J. S. & DABELSTEEN, T. (2001) The meaning of song repertoire size and song length to male whitethroats *Sylvia communis*. *Behavioural Processes* **56**: 75–84.

- BEEBEE, M. D. (2004) The functions of multiple singing modes: experimental tests in yellow warblers, *Dendroica petechia*. *Animal Behaviour* **67**: 1089–1097.
- BEECHER, M. D., CAMPBELL, S. E., BURT, J. M., HILL, C. E. & NORDBY, J. C. (2000) Song-type matching between neighbouring song sparrows. *Animal Behaviour* **59**: 21–27.
- BERGLUND, A., BISAZZA, A. & PILASTRO, A. (1996) Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biological Journal of the Linnean Society* **58**: 385–399.
- BLEEKER, M., KINGMA, S. A., SZENTIRMAI, I., SZÉKELY, T. & KOMDEUR, J. (2005) Body condition and clutch desertion in penduline tit *Remiz pendulinus*. *Behaviour* **142**: 1465–1478.
- BOTERO, C. A., MUDGE, A. E., KOLTZ, A. M., HOCHACHKA, W. M. & VEHRENCAMP, S. L. (2008) How reliable are the methods for estimating repertoire size? *Ethology* **114**: 1227–1238.
- BYERS, B. E. & KROODSMA, D. E. (2009) Female mate choice and songbird song repertoires. *Animal Behaviour* **77**: 13–22.
- CATCHPOLE, C. K. (1989) Responses of male sedge warblers to playback of different repertoire sizes. *Animal Behaviour* **37**: 1046–1047.
- CATCHPOLE, C. K., DITTAMI, J. & LEISLER, B. (1984) Differential responses to male song repertoires in female songbirds implanted with estradiol. *Nature* **312**: 563–564.
- CATCHPOLE, C. K. & SLATER, P. J. B. (2008) *Bird song: Biological themes and variations*. Cambridge University Press, Cambridge, 348 pp.
- CRAMP, S., PERRINS, C. M. & BROOKS, D. J. (1993) *Handbook of the birds of Europe, the Middle East and North Africa – Birds of the Western Palearctic*. Oxford University Press, Oxford, pp. 376–396.
- GARAMSZEGI, L. Z., BALSBY, T. J. S., BELL, B. D., BOROWIEC, M., BYERS, B. E., DRAGANOIU, T., EENS, M., FORSTMEIER, W., GALEOTTI, P., GIL, D., GORISSEN, L., HANSEN, P., LAMPE, H. M., LEITNER, S., LONTKOWSKI, J., NAGLE, L., NEMETH, E., PINXTEN, R., ROSSI, J. M., SAINO, N., TANVEZ, A., TITUS, R., TÖRÖK, J., VAN DUYSE, E. & MÖLLER, A. P. (2005) Estimating the complexity of bird song by using capture-recapture approaches from community ecology. *Behavioral Ecology and Sociobiology* **57**: 305–317.
- GRUBBAUER, P. & HOI, H. (1996) Female penduline tits (*Remiz pendulinus*) choosing high quality nests benefit by decreased incubation effort and increased hatching success. *Ecoscience* **3**: 274–279.
- HALL, M. L. (2004) A review of hypotheses for the functions of avian duetting. *Behavioral Ecology and Sociobiology* **55**: 415–430.
- HARRAP, S. & QUINN, D. (1996) *Tits, nuthatches and treecreepers*. Christopher Helm, London, 464 pp.
- HOI, H., SCHLEICHER, B. & VALERA, F. (1994) Female mate choice and nest desertion in penduline tits, *Remiz pendulinus* – the importance of nest quality. *Animal Behaviour* **48**: 743–746.
- KINGMA, S. A., SZENTIRMAI, I., SZÉKELY, T., BÓKONY, V., BLEEKER, M., LIKER, A. & KOMDEUR, J. (2008) Sexual selection and the function of a melanin-based plumage ornament in polygamous penduline tits *Remiz pendulinus*. *Behavioral Ecology and Sociobiology* **62**: 1277–1288.
- KREBS, J. R., ASHCROFT, R. & VANORSFOLD, K. (1981) Song matching in the great tit *Parus major* L. *Animal Behaviour* **29**: 918–923.
- KREBS, J. R., ASHCROFT, R. & WEBBER, M. (1978) Song repertoires and territory defence in great tit. *Nature* **271**: 539–542.

- KROODSMA, D. E. (1982) Song repertoires: problems in their definition and use. Pp 125–146. *In*: KROODSMA, D. E. & MILLER, E. H. (eds): *Acoustic communication in birds*. Academic Press, New York.
- LAMBRECHTS, M. M. (1992) Male quality and playback in the great tit. Pp 135–152. *In*: MCGREGOR, P. K. (ed.): *Playback and studies of animal communication*. Plenum Press, New York.
- MCDONALD, M. V. (1989) Function of song in Scott's seaside sparrow, *Ammodramus maritimus peninsulae*. *Animal Behaviour* **38**: 468–485.
- MCGREGOR, P. K., KREBS, J. R. & PERRINS, C. M. (1981) Song repertoires and lifetime reproductive success in the great tit (*Parus major*). *American Naturalist* **118**: 149–159.
- NAGUIB, M., ZUBERBÜHLER, K., CLAYTON, N. S. & JANIK, V. M. (2009) *Advances in the Study of Behavior*, Vol 40. Elsevier Academic Press Inc, San Diego.
- NOWICKI, S., SEARCY, W. A. & HUGHES, M. (1998) The territory defense function of song in song sparrows: A test with the speaker occupation design. *Behaviour* **135**: 615–628.
- PERSSON, O. & ÖHRSTRÖM, P. (1989) A new avian mating system – Ambisexual polygamy in the penduline tit *Remiz pendulinus*. *Ornis Scandinavica* **20**: 105–111.
- POGÁNY, Á. & SZÉKELY, T. (2007) Female choice in the penduline tit *Remiz pendulinus*: the effects of nest size and male mask size. *Behaviour* **144**: 411–427.
- R DEVELOPMENT CORE TEAM (2011) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org/>
- RIVERA-GUTIERREZ, H. F., PINXTEN, R. & EENS, M. (2010) Multiple signals for multiple messages: great tit, *Parus major*, song signals age and survival. *Animal Behaviour* **80**: 451–459.
- RIVERA-GUTIERREZ, H. F., PINXTEN, R. & EENS, M. (2011) Songs differing in consistency elicit differential aggressive response in territorial birds. *Biology Letters* **7**: 339–342.
- SCHLEICHER, B., VALERA, F. & HOI, H. (1993) The conflict between nest guarding and mate guarding in penduline tits (*Remiz pendulinus*). *Ethology* **95**: 157–165.
- SEARCY, W. A. (1992) Song repertoire and mate choice in birds. *American Zoologist* **32**: 71–80.
- SEARCY, W. A. & ANDERSSON, M. (1986) Sexual selection and the evolution of song. *Annual Review of Ecology and Systematics* **17**: 507–533.
- SEARCY, W. A., ANDERSSON, R. C. & NOWICKI, S. (2006) Bird song as a signal of aggressive intent. *Behavioral Ecology and Sociobiology* **60**: 234–241.
- SEARCY, W. A. & NOWICKI, S. (2000) Male–male competition and female choice in the evolution of vocal signalling. Pp. 301–315. *In*: ESPMARK, Y., AMUNDSEN, T. & ROSENQVIST, G. (eds): *Animal signals: Signalling and signal design in animal communication*. Tapir Academic Press, Trondheim.
- SEARCY, W. A., NOWICKI, S. & HOGAN, C. (2000) Song type variants and aggressive context. *Behavioral Ecology and Sociobiology* **48**: 358–363.
- SZENTIRMAI, I., KOMDEUR, J. & SZÉKELY, T. (2005) What makes a nest-building male successful? Male behaviour and female care in penduline tits. *Behavioral Ecology* **16**: 994–1000.
- SZENTIRMAI, I., KOMDEUR, J. & SZÉKELY, T. (2007) Sexual conflict over care: antagonistic effect of clutch desertion on reproductive success of male and female penduline tits. *Journal of Evolutionary Biology* **20**: 1739–1744.
- VAN DIJK, R. E., SZENTIRMAI, I. & SZÉKELY, T. (2007a) Practical field guide for investigating breeding ecology of penduline tits *Remiz pendulinus*. Version 1.2. *Univ. of Bath* http://www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/PT_%20Field%20Guide_21_22.pdf.
- VAN DIJK, R. E., SZENTIRMAI, I., KOMDEUR, J. & SZÉKELY, T. (2007b) Sexual conflict over parental care in penduline tits: the process of clutch desertion. *Ibis* **149**: 530–534.

- VAN DIJK, R. E., KOMDEUR, J., VAN DER VELDE, M., SZENTIRMAI, I., YANG, X., FRENCH-CONSTANT, R. & SZÉKELY, T. (2008) Offspring sex ratio in the sequentially polygamous penduline tit *Remiz pendulinus*. *Journal of Ornithology* **149**: 521–527.
- VAN DIJK, R. E., POGÁNY, Á., KOMDEUR, J., LLOYD, P. & SZÉKELY, T. (2010a) Sexual conflict predicts morphology and behavior in two species of penduline tits. *BMC Evolutionary Biology* **10**: 107.
- VAN DIJK, R. E., MÉSZÁROS, L. A., VAN DER VELDE, M., SZÉKELY, T., POGÁNY, Á., SZABAD, J. & KOMDEUR, J. (2010b) Nest desertion is not predicted by cuckoldry in the Eurasian penduline tit. *Behavioral Ecology and Sociobiology* **64**: 1425–1435.
- YASUKAWA, K. (1981) Song repertoire in the redwinged blackbird *Agelaius phoeniceus*: a test of the Beau Geste Hypothesis. *Animal Behaviour* **29**: 114–125.

Revised version received December 5, 2011, accepted September 27, 2012, published March 28, 2013