MALE SIGNALLING AND DOMINANCE
IN THE PENDULINE TIT *REMIZ PENDULINUS*

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Secondary sexual characters (e.g. badges, ornaments) are involved in many species in male-male competition and/or in female choice. We tested a proposition, the armament-ornament model, in a small passerine bird, the Eurasian penduline tit *Remiz pendulinus*, which exhibits an unusually diverse breeding system that includes sequential polygyny and polyandry, and uniparental care by the male or the female. Previous studies showed that the size of black eye-stripes (masks) in males functions as an ornament, since females prefer males with large masks. Here we report that in an aviary experiment, male mask size did not predict dominance status. Our results are consistent with a field study, and these together suggest that male mask size is a secondary sexual signal in penduline tits: it is a trait used by females in mate choice decisions.

Key words: dominance, dual function, sexual signal, penduline tit, *Remiz pendulinus*

INTRODUCTION

The evolution of secondary sexual signals is one of the most puzzling aspects of evolutionary biology, ever since Darwin (1871) described the theory of sexual selection. Darwin separated two versions of sexual selection to explain how traits not favoured by natural selection may evolve. By intrasexual selection (mostly between males), those males are favoured which bear traits (armaments henceforward) helping them to defeat or intimidate their rivals, and by their means achieve more access to females than other competing males (e.g. black badges of Eurasian siskins *Carduelis spinus* and Great tits *Parus major*; reviewed by Griffith *et al.* 2006, Senar 2006). Whereas *via* intersexual selection (mostly females choosing males), those males are selected that exhibit certain traits that are preferred by females (ornaments henceforward); these traits may indicate direct or indirect benefits for females (e.g. beak colouration of Zebra finches *Taeniopygia guttata*, male pheromones of the Sandfly *Lutzomyia longipalpis*, reviewed by Andersson 1994, Jennions & Petrie 2000, Andersson & Simmons 2006).

Berglund *et al.* (1996) argued that many secondary sexual signals have dual functions, i.e. serve both as armament (such as weapons and status badges) and ornament. The duality might give rise to new theories about signal evolution, and the interaction between competition and mate choice (armament-ornament

model, BERGLUND *et al.* 1996, WILEY & POSTON 1996). However, the latter inter-action, the issue whether competition and mate choice are parallel (or contrasting) processes is controversial (reviewed by WONG & CANDOLIN 2005).

Black masks of male Eurasian penduline tits *Remiz pendulinus* may have dual function. On the one hand, experimental and field studies showed that male mask size is involved in female choice (POGÁNY & SZÉKELY 2007, KINGMA *et al.* 2008, but see SCHLEICHER *et al.* 1996). On the other hand, several features of penduline tits suggest that male mask size may be involved in male-male competition. First, the penduline tit mating system is one of the most diverse of avian breeding systems, with sequential polygamy (up to seven mates in a breeding season by both males and females), and uniparental care by either sex (FRANZ & THEISS 1983, FRANZ 1991, PERSSON & ÖHRSTRÖM 1989, SZENTIRMAI 2005). Second, a recent study focusing on sexual conflict over parental care revealed that either sex may maximize its own reproductive success by deserting their mate and clutch, and breeding with new mates (SZENTIRMAI *et al.* 2007). Therefore, both fe-male choice and male-male competition are expected to be amplified. Third, un-mated males react aggressively towards intruder males, and they attack dummy males presented close to the residents' nests. This behavioural response is ex-ploited for trapping and ringing males during field-work (PERSSON & ÖHRSTRÖM 1989, BLEEKER *et al.* 2005, SZENTIRMAI *et al.* 2007).

Here we report an aviary experiment which was designed to investigate the role of male mask size in male-male competition. Our prediction was that male penduline tits establish dominance rank based on their relative mask size, hence male mask size will predict their dominance status.

MATERIALS AND METHODS

Birds and aviary conditions

We investigated the dominance behaviour of nine male penduline tits, using two female penduline tits as stimuli. We caught the males and females on 26 September 2003 and 19 October 2004, during their autumn migration in Hortobágy fishpond (47°38'N, 21°05'E), Hungary. Permission to capture and keep penduline tits in captivity were provided by Hortobágy National Park (refs: 84–93/2003; 22–433/2004), and Duna–Ipoly National Park (ref: 6658/2/2003), respectively. Penduline tits were ringed with one metal and two coloured rings (A C Hughes, Middlesex, UK) for identi-fication.

Penduline tits were provisionally sexed based on morphological traits, since adult males and females differ in plumage characteristics (CRAMP *et al.* 1993, KINGMA *et al.* 2008). In addition, all captive penduline tits were sexed using 5–10 μl blood sample and screening the CHD gene at the Uni-versity of Bath (GRIFFITHS *et al.* 1998; see details in van DIJK *et al.* 2008). Molecular sexing produced unambiguous results.

Penduline tits were housed and the experiment was carried out in Göd Biological Station of Eötvös University, Hungary (47°40'N, 19°07'E), in purpose-built indoor and outdoor aviaries. Penduline tits flock outside the breeding season (HARRAP & QUINN 1996), therefore, male and female penduline tits were housed together in two same-sex indoor aviaries (6 m × 4 m, each) between 15 September and 31 March. During the breeding season (1 April to 14 September) males and females were housed in pairs in five indoor aviaries (3.4 m × 3.4 m, each) and six outdoor aviaries (2 m × 3 m, each). Males were monitored before 1 March for possible signs of their breeding stage (chasing, singing and nest building, Á. POGÁNY, personal observation), and they exhibited breeding behaviour (courting for females, singing and nest building) from late April onwards. After 1 April the experimental males did not meet apart from the trials (see below), so that they could not possibly establish dominance rank prior to the experiment.

Food and water were provided *ad libitum* all year round. For a more detailed description on captive husbandry see POGÁNY & SZÉKELY (2007).

Breeding activity

A 7–17 hour regime (dark – light, LAMBRECHTS & PERRET 2000), and Lisovit-R combi vitamin supply (Pentarex Bt, Érd, Hungary) was used for six weeks prior to and during the experiment to facilitate the reproductive activity of males and females.

Between 25 May 2005 and 4 June 2005 the behaviour of five experimental males were monitored to determine whether they were in breeding condition. Each male was observed five times on five consecutive days for 30 min randomly either in the morning (8.00 h – 11.00 h) or in the afternoon (14.00 h – 17.00 h). The observer recorded the frequency of singing and nest building. Then we calculated the number of singing in an hour and the proportion of time the males spent with nest building. Males were in breeding stage prior to the experiment, since the five males we observed in detail exhibited both nest building and singing behaviour (they spent 1.2±0.44 % of their time with nest building, and sang in average 7.6±7.27 times in an hour).

Competitive context of the experiment

Males exhibited sexual behaviour during trials (see below). We noted that four out of nine males started singing, and sometimes males chased each other. Moreover, each of the nine experimental males approached the female's stimulus cage during trials (mean % of time spent on the perch and the mesh: 8.77±1.32%), while without female in the stimulus chamber and during acclimatization, penduline tits have never been observed to stay in the proximity of stimulus chamber (i.e. on perch or mesh, Á. POGÁNY and A. MEREDITH, personal communication).

Morphometric measurements

Three digital photos were taken from each male penduline tit's mask both from the left and right sides. Males were placed on a graph paper. The size of mask was measured using Corel Photo-Paint 9 (Corel Corporation Ltd.). Mask size was determined as the average mask size of six photos; three from each side. We use absolute mask size, because mask size is unrelated to body size in penduline tits (KINGMA *et al.* 2008) Mask asymmetry was also calculated, as the absolute value of (mean left size – mean right size) / mean right size. Tarsus length, wing length, body weights of males

were also measured using standard methodology (BLEEKER *et al.* 2005). Body condition was calculated as residuals from linear regression of body mass on tarsus length.

Dominance apparatus

The experiment was carried out between 14 June 2005 and 19 June 2005, in a purpose-built dominance apparatus (Fig. 1). The size of the apparatus was 3.4 m × 3.4 m, and it consisted of two regions that were separated by wire-mesh. In the smaller partition (stimulus chamber henceforward), a female was placed to stimulate the males to compete with each other. The larger partition (arena henceforward) was marked into six equal partitions (L1-L3, R1-R3) by reed pots. Wood sticks were fixed to the floor at the edges of each partition for assisting the localization of males. We distinguished five distance categories: mesh (0 cm) – when male settled down on the part of wire-mesh that separates stimulus chamber and arena, perch (12 cm) – this perch was fixed on the wire mesh, L1-R1
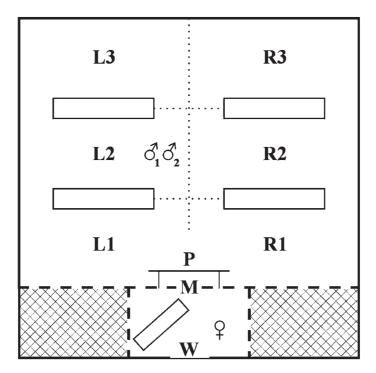

Fig. 1. Schematic view of the apparatus used in dominance tests for male penduline tits. A female penduline tit was placed in the stimulus cage which was separated from the males by wire-mesh (dashed lines). In each trial two males were released in the arena. The arena was divided into six equal squares (three on the left side L1-L3 and three on the right side R1-R3) by pot reeds (rectangles). Trials were observed through a one-way window (W). Five distance categories were used to score the males' distances from stimulus female: M = mesh (0 cm), P = perch (12 cm), L1/R1 (45 cm), L2/R2 (135 cm), L3/R3 (225 cm). Dotted lines indicate wood sticks on the floor that assisted the allocation of distance categories. Food and water was provided during experimental trials in pots placed in the middle point of the arena, i.e. between L2 and R2. Size of room was 3.4 m × 3.4 m.

(middle point: 45 cm) – first partition of arena both on the left and right sides, concordantly, L2-R2 (middle point: 135 cm), L3-R3 (middle point: 225 cm) partitions of the arena.

Trials were monitored through a one-way detective window by adjusting light conditions properly, therefore the experiment could be followed without disturbing the birds. This setup allowed us to distinguish and identify males unambiguously using their colour rings.

Experimental design

We investigated male dominance in a fractional factorial design (BOX *et al.* 2005). Nine males were randomly allocated into three groups. In each group all males were tested once with his other two group-mates separately, using a single female as stimulus. The order of trials within blocks was randomized. After all males in a group were tested (three trials), the female was replaced by a new female, and trials were repeated to control for the possible female-driven effect. Thus each pair of males was tested in two trials using two females in total, and we took the average of their responses.

In each day one group was tested with one stimulus female, so that we had three trials per day. Twenty minutes before each trial, the two males were released in the arena to let them acclimatize. Then the female was released into the stimulus chamber, and the two males' distance from the female was estimated in every 20 second for one hour. The positions of the two males were noted in the larger partition at each time interval. Neither the males nor the female were familiar with the experimental apparatus before their first trial.

Statistical analyses

For each pair of males we calculated their difference in mask size, mask asymmetry, tarsus and wing length, and body condition. We used two response variables: proximity was calculated as the relative average distances of the males from the stimulus female, whereas endurance was estimated by the relative times of males spent in the vicinity of female. In endurance 'vicinity' was defined as the total time spent on the perch and mesh, see Figure 1. We assumed that a lower value in proximity and/or a higher value in endurance indicate higher dominance status.

The relationships between morphometric traits and proxy variables of dominance were tested using SPSS 11 (SPSS Inc., Chicago, USA). We provide mean ± SE, the Pearson correlation coefficients (r), and two-tailed significance levels.

RESULTS

Variation in male mask size

In the three experimental groups of males, mask size was 1.29 ± 0.07 cm^2, 1.22 ± 0.07 cm^2 and 0.82 ± 0.07 cm^2 (n = 3 in each group, mean ± SE). Variance in mask size was comparable across groups: 0.015, 0.017, and 0.016 cm^2, respectively.

Morphometrics and dominance

Proximity was unrelated to the differences between males in any trait including mask size (r = –0.315, P = 0.409, N = 9), mask asymmetry (r = 0.243, P = 0.528, N = 9), wing length (r = –0.468, P = 0.204, N = 9), and body condition (r = –0.507, P = 0.164, N = 9).

Endurance was also unrelated to differences between males in mask size (r = 0.301, P = 0.432, N = 9), mask asymmetry (r = –0.084, P = 0.830, N = 9), wing length (r = 0.122, P = 0.755, N = 9), and body condition (r = 0.136, P = 0.728, N = 9).

DISCUSSION

We found no evidence that male penduline tits use mask size as an armament cue in a competitive context. Therefore, in line with our earlier study on the effect of mask size on female preference, we suggest that male mask size is a secondary sexual trait more likely involved in female choice than in male-male interactions.

This experiment and a female choice experiment (POGÁNY & SZÉKELY 2007) contribute to a small number of studies that tested the duality function, and found support only for the ornament function (BERGLUND *et al.* 1996). Other studies however, found results consistent with dual functions (BERGLUND *et al.* 1996, JONES & HUNTER 1999, GRIGGIO *et al.* 2007). For instance, TAROF *et al.* (2005) showed black masks are involved in both male-male competition and female mate choice in Common yellowthroat *Geothlypis trichas*, a small passerine with similar black mask to that of the penduline tits. We propose three explanations for the different results between TAROF *et al.* (2005) and our ones as presented here. First, mask size as a secondary sexual signal may have evolved *via* intersexual selection in penduline tits, and may have no function in male-male competition. This proposition is corroborated by a field study of KINGMA *et al.* (2008) in which mask size of male penduline tits was unrelated to aggressive response to intruder-mimicked situation.

Second, since Eurasian penduline tits appears to be expanded from the Far East (HARRAP & QUINN 1996), where the presumed ancestor species and close relatives exhibit little (or no) sexual dimorphism, it is also possible that mask size as a cue in sexual selection is an evolutionarily recent phenomenon and had no time to evolve into a second function. The armament-ornament model (BERGLUND *et al.* 1996), although suggesting a more likely direction from armaments to ornaments, does not exclude that a sexual signal with dual function evolves by intersexual selection, and only later it gains a role in male-male competition.

Third, we believe our experiment provided a competitive situation for males, although it is difficult to prove this. Note, that our experimental males showed breeding behaviour before and during the experiment, and in each trial both males approached the stimulus female. Behavioural observations of non-experimental males confirmed that some of the males sang and chased each other during the experiment, and the experiment was carried out during their natural breeding season. Males react aggressively towards each other – even without apparent stimuli – both in nature and in aviary during their breeding season (BLEEKER *et al.* 2005, SZENTIRMAI *et al*. 2007, Á. POGÁNY personal observation).

In the current study we only used 9 individuals due to constraints on sample size. If sample size can be increased to above 40 birds, r = 0.3 would reach significance level at p = 0.05, so it is a possibility that a larger experiment may provide a different conclusion.

We propose that male penduline tits may use other than morphological traits (such as mask size) when establishing dominance rank in competitive situations. For instance, different characteristics of song (GRUBBAUER 1995), or behavioural traits are likely candidates for cues involved in dominance relationships.

In conclusion, our aviary experiment found no relation between mask size and dominance between males. These results, coupled with aviary experiments and field studies suggest that in penduline tits, mask size does not predict the outcome of male-male interactions.

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