

Cautious response of inexperienced birds to conventional signal of stronger threat

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Several studies demonstrated that bird song functions as a first line of territorial defence. The efficiency of deterring rivals depends strongly on the strategy of singing used (e.g. alternating/overlapping singing, singing with low/high rate, matching song type of a rival or singing different type). Causes of between males variation during countersinging are still not fully understood, especially when different signals have similar production costs and their meaning is assigned by arbitrary convention (conventional signalling). We tested whether an oscine bird with small repertoire size, the ortolan bunting *Emberiza hortulana*, differentiate strategy of responding to song of an intruder in relation to its age and threat value of signals. We performed playback experiments to measure response of second year (SY) and after second year (ASY) males to a song of low (eventual variety singing) and high (immediate variety singing) threat value. We found substantial differences in response to playback, which were related both to the type of stimuli used and age of responding males. Both SY and ASY males gave more calls than songs in response to immediate variety playback, which suggest stronger vocal response to the signal of higher threat value. Approaching loudspeaker was similar for both age classes when lower threat value signal was played back, while simultaneously SY males clearly avoided approaching loudspeaker when stronger threat values signal was played back. We conclude that ortolan bunting differentiate response to signal of different threat value and that the strength of response depends on the age of a male. This study provides experimental evidence that age of receiver affects its response to a territorial intruder. It also demonstrates that observed in many studies variation in response to playback may be an effect of age differences between males, which rarely is controlled.

Bird song is a sexually selected trait and some song characteristics like repertoire size, song rate or specific syllable contents has been shown to be honest indicators of male quality (Gil and Gahr 2002). Songs are also used by males in conflict situations, especially during territorial contests, which may have characteristics of conventional communication in some species (Vehrencamp 2001). In such a system the meaning of signals used is associated with their structure through arbitrary convention (Guilford and Dawkins 1995, Vehrencamp 2000).

Territorial defence using song is one of the best examples of animal communication networks (McGregor 2005). Maintaining a territory is often a prerequisite of attracting females, but, signals aimed at repelling rivals and attracting mates usually differ in their

characteristics (Catchpole and Slater 1995). Defending territories by song is often connected with very sophisticated use of repertoires, e.g. birds may direct the signal towards specific individuals or grade aggressive motivation by delivering particular song types: e.g. the same as the receiver at the moment (Beecher et al. 2000). Such a differentiation of signal output is considered not to bear extra costs. Repeating single song types (eventual variety singing) or switching between different types (immediate variety singing) is usually treated as opposite singing strategies of different songbird species (Kroodsma 1977). On the other hand, some species seems to use different singing modes depending on the context (Molles and Vehrencamp 1999). These strategies are likely conventional signalling because switching or repeating are assumed to have

equivalent costs of production, but may differ in possible costs generated by signal receivers, e.g. retaliation (Vehrencamp 2000). Using such strategies does not demand large or even medium size repertoires, as two song types are enough to change song type switching mode. Moreover, song type switching and song type diversity may encode different messages and be act somewhat independently (Molles 2006) There is a lack of knowledge concerning how conventional acoustic signalling is related to males' quality and age, and how these arbitrary conventions of signalling arise. Simultaneously, several studies revealed that quality of males is positively related to age and that some song characters are reliable cues of quality (e.g. Gil et al. 2001, Garamszegi et al. 2005, 2007).

In this study we conducted playback experiments with ortolan buntings *Emberiza hortulana* from an isolated and small Norwegian population. In this population males have average repertoires of ca. 5 song types, and seem to be eventual variety singers switching between different types every 5–15 songs in very repeatable order (Osiejuk et al. 2003, 2005). Eventual variety singing is typical for solo song and countersinging between males interacting at a larger distance, especially between neighbours when territory borders are well established. The other type of singing, when males increase switching rate and present different song types one after another is much less frequent; however, it is also typical for a specific context-territorial intrusion. It is mostly observed when rivals are very close to each other and is often connected with slowing down song rate, intensive calling and chasing, i.e. behaviour suggesting escalated conflict (Cramp and Perrins 1994; own unpubl. data). Similar increase of switching rate in agonistic contexts was also found in other eventual variety singers like, e.g. western meadowlark *Sturnella neglecta* (Horn and Falls 1991).

In this paper we experimentally test if young (i.e. inexperienced) and old ortolan bunting males respond differently to playback of song of distinct threat value as indicated by conventional signalling rules (eventual variety playback – lower threat value vs immediate variety playback – higher threat value). We assume that second year (SY) and after second year (ASY) males differ in their self assessment of fighting ability, and therefore we would expect differences between those groups in response to signal of different threat value, with ASY males responding stronger than SY males.

Methods

Study area and species

The ortolan bunting is a territorial, monogamous bird. The species is long-distance migrant wintering in

sub-Saharan Africa. In the breeding season it defends small territories covering ca. 1–3 ha. The species usually nests on the ground in a variety of open to semi-open habitats: from farmland to forest clearings. Pairs produce a single brood each year, although replacement clutches can occur after nest failures (Cramp and Perrins 1994). The study was carried out in Hedmark County (60.29–60.53°N; 11.40–12.18°E), Norway, in a relict population (up to 50 habitat patches have been used by ortolan buntings during the study period). Most of the 150 (in 2004) and 115 (in 2005) ortolan bunting males in this area were individually marked with colour rings and only such birds were subject of experiments. For more details on study sites and methods see references (Osiejuk et al. 2003, Dale et al. 2005, 2006, Osiejuk et al. 2007, Skierczyński et al. 2007).

Field procedures

We carried out the experiments in May 2004 and 2005 (between 04:00 and 10:00 h local time). All subject males were unpaired and defended territories for 1–3 d before the experiments were conducted. Before each treatment the loudspeaker was placed in a tree about 1.5–2 m above the ground. The place was always within the subject male's territory and at a distance of 30–50 m to his song post observed during equipment setup. We used a Philips Magnavox ESP25 compact disc player with a wireless HAMA SP433 amplified loudspeaker (linear frequency response within species-specific frequency range, i.e. 1.8–6.6 kHz) for the playback experiments. Each experiment consisted of two stages: a one-minute playback followed by a one minute of observation period after playback of the behaviour of the focal male. Six songs were played back during one minute, which is a typical song rate for the species and the playback was started ca. 2 s after the last song of the focal male. This allowed the focal male to avoid overlapping with the following playback songs, especially as an increase of song rate during a playback phase is not a typical response (Osiejuk et al. 2007, Skierczyński et al. 2007). Observations of the behaviour of the males' were dictated to a microcassette recorder and notes were transcribed later the same day. The following measures of focal males response were noted: flight latency towards loudspeaker (s), 5 m loudspeaker approach latency (s), closest distance to the loudspeaker (m), number of flights over 1 m during and after playback, and number of songs and calls given during the playback and after playback stages.

Each subject was tested only once, and 13 males responded to eventual variety song playback (i.e. six same songs: A A A A A A) and 12 males to immediate variety playback (i.e. alternating playback of two song

types: A B A B A B). In both seasons we tested birds with eventual and immediate variety playback in alternate random order. In case of eventual variety treatment, five males were young i.e. ringed one year earlier as nestlings (later SY) and eight males were older (later ASY). For immediate variety playback, we tested seven SY and five ASY males. For each experiment we used different song stimuli from randomly chosen ASY non-neighbours of the subject males. All the songs used were of good quality and were digitally prepared (2 kHz high-pass filter, amplified or attenuated) to match 86 dB signal pressure level (SPL) at 1 m from the loudspeaker (measured with a CHY 650 sound level meter). The SPL value was set on the basis of ortolan bunting regular song amplitude level, which had been measured in the field earlier. We used Avisoft SASLab Pro 4.34 software (Specht 2002) to prepare playback cuts.

Data and statistical analyses

We measured altogether ten response variables which describe the response of the males to playback, and these measures were partly correlated with each other. As separate tests on original variables would not be statistically independent and would not reveal the multivariate character of the response we combined all original variables into three (PC1 to PC3) orthogonal principal components (Rice 1989, McGregor 1992). All of song- and call-related response measures were strongly correlated with PC1 and higher values of this compound measure corresponded with cease of singing and strong call response (Table 1). PC1 indicated mode of vocal response, which may be song- (high PC1 values) or call-biased (low PC1 values). PC2 was positively correlated with latency to approach and

Table 1. Eigenvalues, variance explained and weightings of the original variables in the first three principal components extracted from the ten original variables of the response to the playback. Significant values are in bold.

Statistics and original response variable	Component		
	PC1	PC2	PC3
Eigenvalue	3.26	2.35	1.79
Percent of variance	32.6	23.5	17.9
Cumulative percent	32.6	56.1	74.0
Flight latency	-0.23	0.09	-0.72
Approach latency	-0.03	0.80	-0.19
Closest distance	0.04	0.90	-0.15
Flights during playback	0.08	-0.47	0.80
Flights after playback	0.11	-0.54	0.01
Song latency	0.82	0.11	0.28
Songs during playback	-0.86	-0.13	-0.29
Songs after playback	-0.73	0.18	0.56
Calls during playback	0.85	-0.24	0.13
Calls after playback	0.72	-0.51	-0.28

closest distance to the loudspeaker a focal male reached. The larger PC2 value the lower was also number of flights done by a male. Therefore PC2 could be called approaching as it indicated willingness of a focal male to approach or to keep away from a simulated intruder. PC3 correlated negatively with the latency of first flight after playback was started and with the number of flights during playback. Therefore, PC3 was related to movements, and it describes promptness of first flight decision and mobility of males during playback. It is worth noticing that elevated mobility was not necessarily connected with approaching and staying consistently close to the loudspeaker.

We used General Linear Model (GLM) to test for differences in response between males of different age (fixed factor) and to different type of song stimuli (fixed factor). Statistical tests were two-tailed.

Results

We found substantial differences in response to playback, which were related both to the type of stimuli used and age of responding males. According to vocal response, both age classes used calls significantly more than songs, when exposed to immediate variety playback ($F_{1,22} = 5.23$, $P = 0.033$, Fig. 1). We found that the approaching response was affected both by the age of males ($F_{1,22} = 7.73$, $P = 0.012$) and kind of stimulus used ($F_{1,22} = 10.31$, $P = 0.004$). In case of eventual variety song playback, both young and older males showed similar tendency to approach the loudspeaker (Fig. 1). A different pattern was found in response to the immediate variety song playback. Inexperienced males clearly avoided approaching the loudspeaker, whereas older males exhibited only a higher variation in the approaching response than in the case of eventual variety playback. The type of stimuli \times age interaction almost significantly affected the approaching response ($F_{1,22} = 3.59$, $P = 0.072$). We found no significant differences in mobility response (age: $F_{1,22} = 1.24$, $P = 0.277$; playback stimuli: $F_{1,22} = 0.02$, $P = 0.893$), but males from both age classes tended to respond with higher variation when eventual playback was conducted (Fig. 1).

Discussion

Territorial males of song birds respond to song of the rivals with very variable pattern. Playback experiments revealed that response of a territorial male depends on who is singing. For example, territory owners usually respond weaker to the known neighbours songs than to the songs of strangers ("dear enemy effects"), which was recently shown also in ortolan bunting (Skierczyński

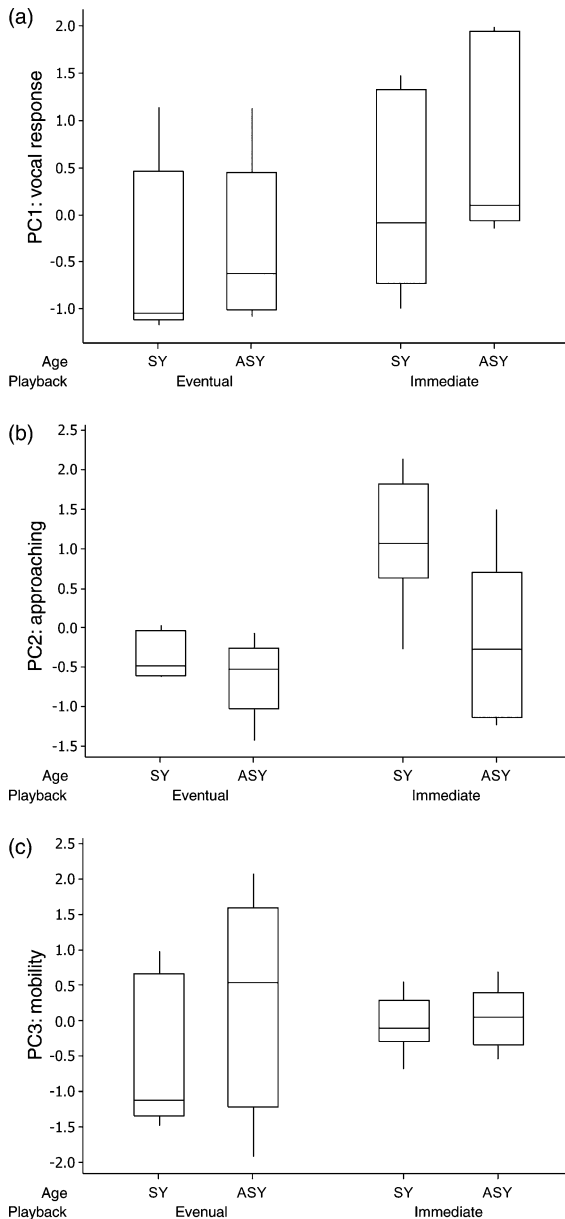


Fig. 1. Responses of young (SY) and old males (ASY) of ortolan bunting to eventual and immediate variety playback as measured by compound reaction measures: (a) vocal response, (b) approaching loudspeaker, and (c) mobility (for methods, see supplementary information). Box plots enclose the interquartile range within the box, the whiskers include the range of values within 1.5 times the interquartile range.

et al. 2007). Response to playback is usually also shaped by the signal threat value, which may be encoded in several ways e.g. by differentiating song rate, song type switching rate or use of specific signals (Vehrencamp 2000). On the other hand, large between individual differences in response to playback are observed even if

the same type of signal is played back. These potentially confounding variation is likely to be a result of variable males' quality, they willingness to defend resources or current needs. For example, the strength of song response to playback in males corn bunting *Miliaria calandra* significantly correlated with their singing activity before playback (Osiejuk et al. 2007). One of the most probable factors underlying such differentiation is age (e.g. Cucco and Malacarne 1999).

Our results indicate that young males of ortolan buntings responded more cautious and avoided approaching the loudspeaker when faced with immediate variety playback (higher threat value), but responded similarly to old males to eventual variety playback (lower threat value). Similarly, Cucco and Malacarne (1999) found that black redstart *Phoenicurus ochruros* males of different age classes differed in they response to playback with adult males showing more rapid response. However, in this case adult and subadult males differed significantly in seven of eleven measured parameters of song structure, and Cucco and Malacarne (1999) suggested that their song is a honest signal of their age. In ortolan bunting, songs of both SY and ASY males do not differ in structure (own unpubl. data) and the differences observed may be assigned only to how (immediate vs eventual variety) different song types were presented during playback.

Our results demonstrate that males which without or little earlier experience in countersinging, discriminated between conventional signals of different meaning. These findings suggest that assigning threat value to a particular form of arbitrary signal may be related to age-biased signal perception. One may assume that experience of males increase during lifetime proportionally to the number of different interactions they have with conspecifics. Thus, if being cautious to unknown (or just infrequent) type of signal is a natural behaviour, we may expect that older (and experienced) males cope better than young with territorial defence and that eavesdropping male-male interactions may be a good source of honest information about a male's age and quality (McGregor 2005). Lack of experience is also mentioned as possible cause of variation in response in other types of acoustic signals in birds (see Rajala et al. 2003). Poesel et al. (2006) found recently, that ASY males of the blue tit *Cyanistes caeruleus* began to sing earlier relative to sunrise than did SY males, which is caused by age-related changes in individual performances. Furthermore, males that began to sing earlier attracted more partners. We suggest that not only variation in such song traits as performance (or repertoire size), i.e. traits which may be measured directly for particular male, are possibly related to age of a bird and may be honest indicators of male's quality. Efficiency of repelling rivals with song may be measured only in relation to the singing strategy applied by those

rivals and, in our opinion, results of this study show that observing male-male interaction may provide reliable information about males' age for potential mates. We did not investigate communication network directly in this study, but above-mentioned interpretation of our results is supported by other studies. For example, Peake et al. (2005) found that great tits *Parus major* tested exhibited capability of extracting different kinds of information by eavesdropping and that switching song types clearly lowered approach behaviour of tested males, similarly to our study. More studies which stress the importance of eavesdropping on interactions between animals (social eavesdropping) rather than on signals only (interceptive eavesdropping) are presented in Peake's review (2005).

It is worth to notice, that song of conspecifics could be an important cue in habitat selection in long-distance migrants (Hahn and Silverman 2006). Ortolan bunting males in Norway were found to explore suitable (but unoccupied by other males) patches, where the species specific song was played back for several hours (Svein Dale and Anne Karen Darrud unpubl. data). Therefore, young males in such species are faced in serious decision problems as from the one point of view choosing territory close to other (possible older) male is advantageous (increase chance of choosing good habitat and attracting female), but simultaneously it could be dangerous if territory owner is willing to fight. Therefore, cautious approaching response of young birds, observed in our study, may be alternatively explained as an adaptation towards avoiding conflict with potentially stronger rival.

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References

- Beecher, M. D., Campbell, S. E., Burt, J. M., Hill, C. E. and Nordby, J. C. 2000. Song-type matching between neighbouring song sparrows. – *Anim. Behav.* 59: 21–27.
- Catchpole, C. K. and Slater, P. J. B. 1995. *Bird Song. Biological themes and variation.* – Cambridge Univ. Press, Cambridge.
- Cramp, S. and Perrins, C. M. 1994. *Birds of the western palearctic: handbook of the birds of Europe, the Middle East and North Africa, Vol. 9.* – Oxford Univ. Press, Oxford.
- Cucco, M. and Malacarne, G. 1999. Is the song of black redstart males an honest signal of status? – *Condor* 101: 689–694.
- Dale, S., Lunde, A. and Steiffeten, Ø. 2005. Longer breeding dispersal than natal dispersal in the ortolan bunting. – *Behav. Ecol.* 16: 20–24.
- Dale, S., Steiffeten, Ø., Osiejuk, T. S., Losak, K. and Cygan, J. P. 2006. How do birds search for breeding areas at the landscape level? Interpatch movements of ortolan buntings. – *Ecography* 29: 886–898.
- Garamszegi, L. Z., Heylen, D., Møller, A. P., Eens, M. and de Lope, F. 2005. Age-dependent health status and song characteristics in the barn swallow. – *Behav. Ecol.* 16: 580–591.
- Garamszegi, L. Z., Török, J., Hegyi, G., Szöllösi, E., Rosivall, B. and Eens, M. 2007. Age-dependent expression of song in the collared flycatcher, *Ficedula albicollis*. – *Ethology* 113: 246–256.
- Gil, D. and Gahr, M. 2002. The honesty of bird song: multiple constraints for multiple traits. – *Trends Ecol. Evol.* 17: 133–141.
- Gil, D., Cobb, J. L. S. and Slater, P. J. B. 2001. Song characteristics are age dependent in the willow warbler, *Phylloscopus trochilus*. – *Anim. Behav.* 62: 689–694.
- Hahn, B. A. and Silverman, E. D. 2006. Social cues facilitate habitat selection: American redstarts establish breeding territories in response to song. – *Biol. Lett.* 2: 337–340.
- Horn, A. H. and Falls, J. B. 1991. Song switching in mate attraction and territory defense by western meadowlarks (*Sturnella neglecta*). – *Ethology* 87: 262–268.
- Guilford, T. and Dawkins, M. S. 1995. What are conventional signals? – *Anim. Behav.* 49: 1689–1695.
- Kroodtsma, D. E. 1977. Correlates of song organization among north american wrens. – *Am. Nat.* 111: 995–1008.
- McGregor, P. K. 1992. Quantifying responses to playback: one, many, or composite multivariate measures? – In: McGregor, P. K. (ed.). *Playback and studies of animal communication.* Plenum Press, New York and London, pp. 79–96.
- McGregor, P. 2005. *Animal communication networks.* – Cambridge Univ. Press, Cambridge.
- Molles, L. E. 2006. Singing complexity of the banded wren (*Thryothorus pleurostictus*): do switching rate and song-type diversity send different messages. – *Auk* 123: 991–1003.
- Molles, L. E. and Vehrencamp, S. L. 1999. Repertoire size, repertoire overlap, and singing modes in the banded wren, *Thryothorus pleurostictus*. – *Auk* 116: 677–689.
- Osiejuk, T. S., Ratynska, K., Cygan, J. P. and Dale, S. 2003. Song structure and repertoire variation in ortolan bunting (*Emberiza hortulana* L.) from isolated Norwegian population. – *Ann. Zool. Fenn.* 40: 3–16.
- Osiejuk, T. S., Ratynska, K. and Cygan, J. P. 2007. Corn bunting (*Miliaria calandria*) males respond differently to alternating and overlapping playback of song. – *J. Ethol.* 25: 159–168.
- Osiejuk, T. S., Ratynska, K. and Dale, S. 2007. What makes a 'local song' in a population of ortolan buntings *Emberiza hortulana* without a common dialect? – *Anim. Behav.* 74: 121–130.

- Osiejuk, T. S., Ratyńska, K. Dale, S. and Cygan, J. P. 2005. Isolation, patchiness and dispersal pattern strongly affect singing behaviour in ortolan bunting (*Emberiza hortulana*). – XXIX Int. Ethol. Conf. Abs. Budapest, Hungary, 166.
- Peake, T. M. 2005. Eavesdropping in communication networks. – In: McGregor, P. K. (ed.). *Animal communication networks*. Cambridge University Press, pp. 13–37.
- Peake, T. M., Matessi, G., McGregor, P. K. and Dabelsteen, T. 2005. Song type matching, song type switching and eavesdropping in male great tits. – *Anim. Behav.* 69: 1063–1068.
- Poesel, A., Kunc, H. P., Foerster, K., Johnsen, A. and Kempenaers, B. 2006. Early birds are sexy: male age, dawn song and extrapair paternity in blue tits, *Cyanistes* (formerly *Parus*) *caeruleus*. – *Anim Behav.* 72: 531–538.
- Rajala, M., Ratti, O. and Suhonen, J. 2003. Age differences in the response of willow tits (*Parus montanus*) to conspecific alarm calls. – *Ethology* 109: 501–509.
- Rice, W. R. 1989. Analyzing tables of statistical tests. – *Evolution* 43: 223–225.
- Skierczyński, M., Czarnecka, K. M. and Osiejuk, T. S. 2007. Neighbour-stranger song discrimination in territorial ortolan bunting (*Emberiza hortulana*) males. – *J. Avian Biol.* 38: 415–420.
- Specht, R. 2002. Avisoft-SASLab Pro sound analysis and synthesis laboratory. A PC-software for MS-Windows 95/98/ME/NT/2000/XP. – Avisoft Bioacoustics, Berlin.
- Vehrencamp, S. L. 2000. Handicap, index, and conventional signal elements of bird song. – In: Espmark, Y. O., Amundsen, T. and Rosenqvist, G. (eds). *Animal signals: signalling and signal design in animal communication*. Tapir Academic Press, Trondheim.
- Vehrencamp, S. L. 2001. Is song-type matching a conventional signal of aggressive intentions? – *Proc. R. Soc. B* 268: 1637–1642.