

The day after: effects of vocal interactions on territory defence in nightingales

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Summary

1. Models on territory acquisition and tenure predict that territorial animals benefit by adjusting territorial defence behaviour to previous challenges they had experienced within the socially complex environment of communication networks.
2. Here, we addressed such issues of social cognition by investigating persisting effects of vocal contests on territory defence behaviour in nightingales *Luscinia megarhynchos* (Brehm).
3. Using interactive playback during nocturnal song of subjects, a rival was simulated to countersing either aggressively (by song overlapping) or moderately (by song alternating) from outside the subjects' territory. Thereby, the time-specific singing strategy provided an experimentally controlled source of information on the motivation of an unfamiliar rival.
4. Expecting that nightingales integrate information with time, the same rival was simulated to return as a moderately singing intruder on the following morning.
5. The results show that the vigour with which male nightingales responded to the simulated intrusion of an opponent during the day depended on the nature of the nocturnal vocal interaction experienced several hours before.
6. Males that had received the song overlapping playback the preceding night approached the simulated intruder more quickly and closer and sang more songs near the loud-speaker than did males that had received a song alternating playback.
7. This adjustment of territory defence strategies depending on information from prior signalling experience suggests that integrating information with time plays an important part in territory defence by affecting a male's decision making in a communication network.

Key-words: bird song, floaters, social cognition, territorial behaviour, acoustic communication.

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Introduction

Studies on animal communication contribute significantly to our understanding of cognitive processes such as perception, memory and decision making by providing insights on how animals gather and use information in a socially complex environment. Specifically social dyadic

interactions have provided a useful model to advance our understanding of the role of aggression in territorial species (Maynard Smith 1982; Grafen 1987). Such models can predict the winner of a contest depending on resource holding potential (Parker 1974), resource value, and dynamic response strategies individuals use in interactions (Maynard Smith 1982). Recently, theoretical studies on territory acquisition and tenure stressed the potential advantages of an individual acting upon its prior experience (Stamps & Krishnan 2001; Switzer, Stamps & Mangel 2001; Rutte, Taborsky & Brinkhof 2006). Thus, a resident should benefit by adjusting its territory defence behaviour to preceding territorial challenges (Stamps & Krishnan 2001; Switzer *et al.* 2001).

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To experimentally address these issues of social cognition, studies on birdsong have been an important model as birdsong is among the most complex communication systems in vertebrates. Territorial songbirds are usually within signalling and hearing range of conspecifics and therefore can be considered as members of a communication network (McGregor & Dabelsteen 1996). Here, individuals may obtain information on the quality or motivation of conspecifics by interacting directly with each other or by eavesdropping on interactions of other individuals (Naguib 2005). In such intrasexual interactions males may use dynamic singing strategies by varying either the pattern of the signal, e.g. matching the opponent's song type, or the timing of the signal, i.e. overlapping the opponent's song or alternating their own songs with those of an opponent. There is increasing evidence supporting the hypothesis that song overlapping is perceived as a signal of aggression by overlapped males (McGregor & Horn 1992; Dabelsteen *et al.* 1997; Mennill & Ratcliffe 2004b; Naguib & Kipper 2006). Moreover, depending on the immediately preceding experience they have had, territorial males alter their singing strategy in a contest with a persisting intruder (Naguib 1999; Peake *et al.* 2002; Mennill & Ratcliffe 2004a). Thus, territorial males extract information from the time-specific singing strategy of a rival and use this information appropriately within a short time frame. However, little is known about whether vocal contests also have persisting effects on territory defence behaviour in subsequent encounters. Long-term memory plays a role in territorial defence strategies in male hooded warblers *Wilsonia citrina*, which have been shown to individually recognize their neighbours of the previous year (Godard 1991). Regarding encounters with unfamiliar rivals, however, only a few recent studies have addressed long-lasting effects of territorial intrusions on territory proclamation or territory defence strategies (Amrhein & Erne 2006; Hall, Illes & Vehrencamp 2006). Being confronted with an intrusion as a strong immediate threat to territory tenure, resident males may assess the intruder by gathering auditory information as well as by visual inspection combined with spatial movement towards the intruder. Yet, it remains to be shown whether songbirds adjust territory defence based on previous singing interactions with nonintruding rivals, i.e. based exclusively on auditory information. Long-range singing interactions, as an established model for studies in social cognition (Naguib 2005; Peake 2005), provide an important source of information in a communication network (Peake 2005), specifically at early stages of the breeding cycle during territory establishment and mate attraction.

Here, we tested whether male territorial nightingales, *Luscinia megarhynchos*, use information obtained during a nocturnal vocal interaction subsequently in decision making in territory defence. In a previous study, we showed that territorial males responded differently to unfamiliar rivals countersinging from outside their territory either aggressively (by song overlapping) or moderately (by song alternating, Schmidt *et al.* 2006).

The present study investigates whether this experience has long-lasting effects on decision making when responding to an intrusion by the same rival on the following morning. Assuming that it pays animals to integrate information with time in strategies to defend resources, we predicted that the vigour with which subjects responded to the intruder would depend on the nature of the vocal challenge the subjects had experienced in the signalling interaction during the previous night.

Materials and methods

STUDY SITE AND SUBJECTS

The study was conducted in a population of nightingales in the Upper Rhine Valley in France, about 10 km north of Basel (Switzerland). In this area, in the vicinity of the Petite Camargue Alsacienne, nightingales usually settle in patches of woods or dense bushes along rivers and footpaths. Territories often border grasslands or fields, resulting in clear territory boundaries. The territories we chose for playback were sufficiently isolated to avoid interference with responses from neighbours. Therefore, subjects were individually recognizable by territory location.

Noninteractive diurnal playbacks on 40 territorial male nightingales were conducted at the beginning of the breeding period in 2004 (18 April–3 May, 21 playbacks) and 2005 (19 April–2 May, 19 playbacks). Each subject male had been exposed to an interactive playback conducted from outside the subject's territory (30–40 m from the subject's song post) the preceding night (23.45–03.30 h CEST). During this nocturnal playback we had either overlapped or avoided overlapping the subjects' songs ($n = 20$ in each treatment group, Schmidt *et al.* 2006). In the present study, we simulated the same rivals intruding into the subjects' territories during the day (07.00–10.00 h CEST), 7.2 ± 0.75 h (mean \pm SD) after subjects encountered the rival the first time.

As usually nightingales sing regularly at night only until a female has settled in their territory (Amrhein, Korner & Naguib 2002), nocturnal singing activity of all subjects was determined regularly over the breeding season to assess their mating status. All subjects had been singing for at least two nights before they received the playback and they sang for at least another two nights after playback. Thus, subjects were considered to be unmated at the time of playback. Later in the season, 18 males ceased nocturnal song and were therefore regarded as mated males, whereas the other 22 males were considered to remain unmated because they were singing at night throughout the breeding season (Amrhein *et al.* 2002).

PLAYBACK STIMULI

The stimulus songs were derived from nocturnal recordings of 40 different colour-banded male nightingales made between 2002 and 2005 in the study population. Nocturnal song was recorded on a Sony TC-D5M or

WM-D6C tape recorder (SONY Ltd, Japan) with a Sennheiser ME66/K6 microphone (Sennheiser electronic GmbH, Germany) and digitized using Cool Edit 2000 (Syntrillium Software Cooperation, USA, sample frequency: 44.1 kHz, resolution: 16 bit). From each recording, 22 different songs were selected (Avisoft SASLab Pro 3.5, R. Specht, Berlin, Germany), which were then randomly arranged in one wave file (Cool Edit) with silent intervals of 3 s between two consecutive songs, corresponding to a naturally singing nightingale (Kunc, Amrhein & Naguib 2005). Each file of about 2 min duration was normalized to the peak amplitude to standardize playback amplitude across trials and recorded on tapes with a standardized record level. Thus, each stimulus tape consisted of a set of 22 songs from one source male, and each tape was used in only one playback trial. For each subject, we used the same stimuli songs that we had used the previous night (Schmidt *et al.* 2006) to simulate the same rival returning as an intruder.

PLAYBACK PROTOCOL

To simulate an intrusion during the day, the loudspeaker was placed inside the territory, within 10 m to the subject's previous nocturnal song post, at a height of about 2 m. By positioning markers at distances of 4, 8 and 16 m in the vicinity of the loudspeaker, the subjects' approach to the loudspeaker could be measured. To standardize playback context, we started playbacks only when subjects were singing.

Songs were played from a Sony WMD 6M tape recorder, which was connected to a Blaupunkt MPA 2 amplifier (Blaupunkt GmbH, Germany) and a Canton Plus X passive speaker (Canton Elektronik GmbH, Germany). Volume of broadcast songs was adjusted prior to playback to 90 dB at 1 m distance (Brumm 2004), measured with a Brüel & Kjær precision SPL meter 2233 (C-weighting, fast response). Singing of a focal male and the distance to the speaker after each subject's song were recorded on the left channel of a Sony TCD 5M stereo tape recorder with a Sennheiser ME 66/K6 directional microphone. On the right channel we recorded the songs broadcast by the loudspeaker with another microphone of the same kind. This allowed us to assess the on- and offset of playback for analysis. Subjects' response behaviour was recorded on tape during the time songs were played, and for an additional period of 10 min after playback terminated.

RESPONSE MEASURES AND STATISTICAL ANALYSIS

We measured the following nonsinging responses: (1) the closest approach to the speaker (metres); (2) the latency to the closest approach (seconds); and (3) the percentage of songs the subjects sang within 4 m to the speaker. Moreover, we calculated the following singing responses: (4) the song rate (number of songs per minute); (5) the percentage of whistle songs, which vary substantially

among males (Kunc *et al.* 2005) and in the way they are used in vocal interactions (Naguib *et al.* 2002; Kunc, Amrhein & Naguib 2006); and (6) the percentage of songs containing rapid broadband trills that are interpreted as a song trait indicating arousal in singing interactions (Kunc *et al.* 2006).

We applied a multivariate general linear model (SPSS 12.0) with the six response parameters (1)–(6) as dependent variables. The initial model included three factors: nocturnal playback treatment (song alternating or song overlapping), the males' subsequent pairing status (mated or unmated male), and the year of study (2004 or 2005). In our sample, 13 of 20 males in the song alternating treatment group and nine of 20 males in the song overlapping group remained unpaired. As neither the subsequent mating status nor the year were significantly related to the responses in the MANOVA (both $P > 0.12$), these factors were removed in the final model. The influence of nocturnal playback treatment on the particular response parameters was then considered using univariate general linear models. Data on closest approach and song rate were log-transformed to meet assumptions of the model. Results are given as mean \pm SE; all reported tests are two-tailed.

Results

Taking all measures together, the territory defence behaviour of the subjects during the day was significantly influenced by the nature of the preceding nocturnal playback treatment (MANOVA, $F_{6,33} = 3.7$, $P = 0.007$). Males whose songs had been overlapped the previous night approached the loudspeaker significantly closer ($F_{1,38} = 7.3$, $P = 0.01$, Fig. 1a) and more quickly ($F_{1,38} = 8.5$, $P = 0.006$, Fig. 1b) than did males that had received a song alternating playback the previous night. Moreover, males that had been exposed to a song overlapping playback the preceding night sang a significantly higher percentage of songs near the speaker than did males of the song alternating treatment ($F_{1,38} = 17.3$, $P < 0.001$, Fig. 1c).

Nocturnal playback treatment did not significantly influence any of the three measures of singing response, indicating that subjects did not alter their song rate, nor the proportion of whistle songs or that of trilled songs (all $F_{1,38} < 0.9$, all $P > 0.35$, Fig. 1d–f).

Discussion

The vigour with which male nightingales responded to the simulated intrusion of an opponent during the day depended on the nature of a nocturnal vocal interaction experienced several hours before. Males whose songs had been overlapped the previous night responded by approaching the loudspeaker more quickly and closer and by singing more songs near the speaker than did males that had received a song alternating playback the previous night. Thus, our findings on approach behaviour demonstrate how experience in signalling after several

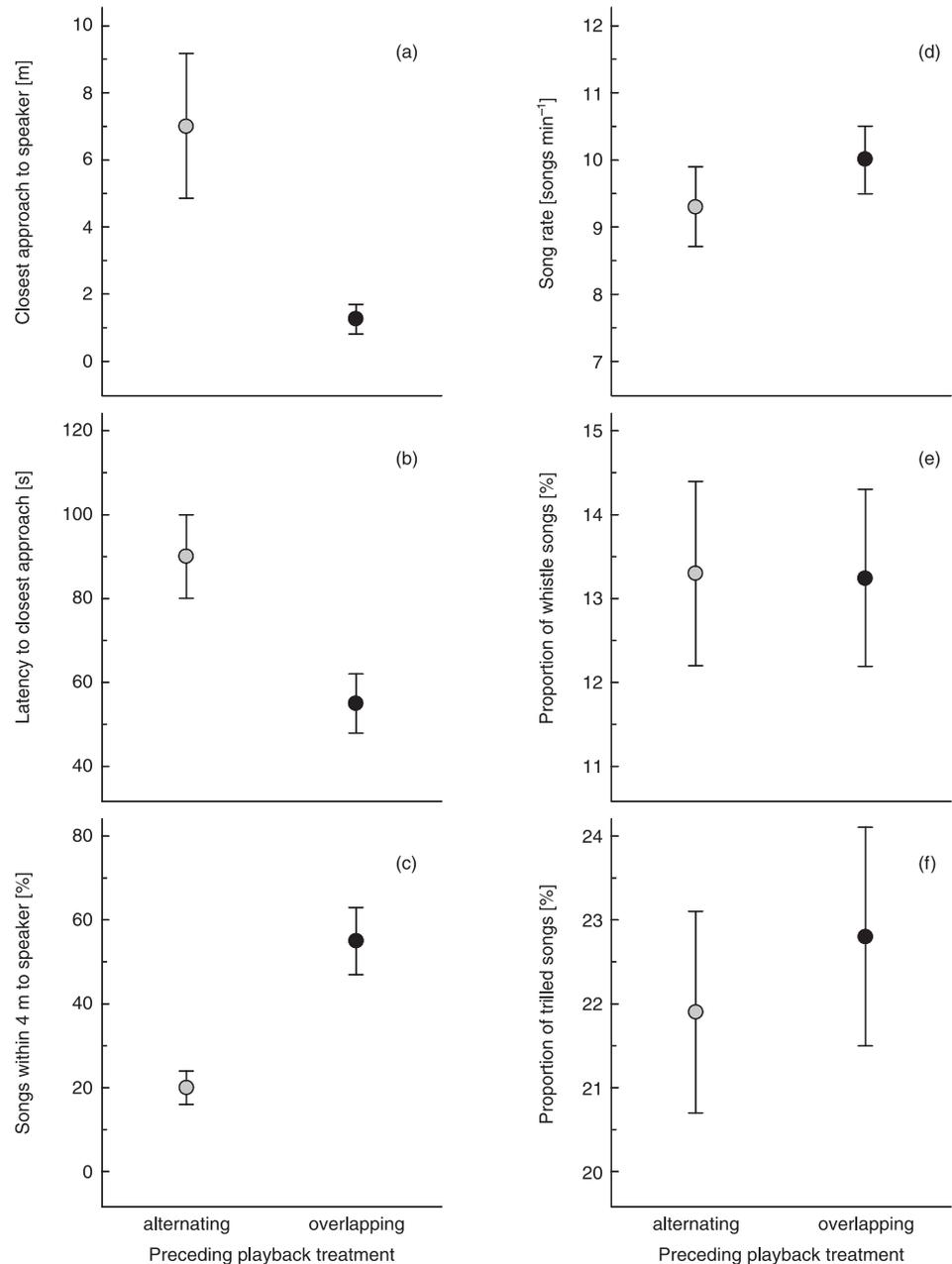


Fig. 1. Mean (\pm SE) responses of male nightingales to a simulated intrusion during the day in relation to a vocal challenge during the previous night in which subjects received either a song alternating playback (grey circles, $n = 20$) or a song overlapping playback (black circles, $n = 20$): (a) closest approach to the loudspeaker (metres); (b) latency to the closest approach (seconds); (c) percentage of songs sung within 4 m to the loudspeaker; (d) song rate (songs min^{-1}); (e) percentage of whistle songs; (f) percentage of songs that contain rapid broadband trills.

hours affects decision making in resource defence. The males did not alter the use of the structural song traits we analysed (i.e. whistle songs and songs with rapid broadband trills). This suggests that the use of these songs is more important in nocturnal vocal interactions, as shown previously (Naguib *et al.* 2002; Kunc *et al.* 2006), than during situations of high arousal in diurnal territory defence, in which, in contrast to nocturnal interactions, also approach behaviour and visual inspection are involved.

There is growing evidence that song overlapping may reflect aggressiveness (reviewed in Naguib 2005) and presumably also male quality as males that respond more

vigorously during intrasexual singing interactions are more likely to become paired (Kunc *et al.* 2006; Schmidt *et al.* 2006). Furthermore, eavesdropping females seem to base their choice of extra-pair mates on the performance of males in vocal contests (Mennill, Ratcliffe & Boag 2002). As agonistic interactions with other males may increase circulating levels of testosterone (Wingfield *et al.* 1990), which is correlated with increased aggression (Silverin 1998), such differential responses we found could be explained by long-lasting effects of male arousal resulting from being intimidated by a threatening opponent that presumably is also attractive to females.

Another explanation for differential responses depending on prior experience may be that the subjects individually recognized the intruder to be the same they had encountered the previous night. So far, the benefits of using such prior information on rivals in territory defence have been hypothesized to be a key argument why individuals should discriminate between neighbours and strangers and individually recognize different neighbours (Stoddard 1996). As the relationship between neighbours is likely to be stabilized as a result of numerous previous encounters, familiar neighbours usually pose less of a threat than strangers. Neighbours as 'dear enemies' may even function as 'border guards' against less predictable strangers and thus as an early warning system that allows effective maintenance of territorial integrity (Eason & Stamps 1993; Naguib, Amrhein & Kunc 2004). However, in our study the subjects had encountered the intruder only once and briefly during the preceding night, and it remains to be tested whether nightingales individually recognize an unfamiliar rival based on a single encounter in subsequent encounters.

The issue of how to respond to strangers that may challenge territory tenure repeatedly generally is important in territorial animals as nonterritorial individuals seeking to establish a territory can challenge resident individuals repeatedly. In general, prospecting individuals of territorial species may sample several territories (Amrhein, Kunc & Naguib 2004) and evaluate the quality of a site by assessing environmental cues (Doligez *et al.* 2003) or public information, e.g. on the density of conspecifics (Doligez *et al.* 2004). Accordingly, dynamic modelling indicates that the likelihood of territory acquisition at a site may increase with the amount of obtained information, i.e. with the time spent at a site or with the number of intrusions into other territories (Ens, Weissing & Drent 1995; Stamps & Krishnan 2001). Experimental studies on floaters confirmed these assumptions in several bird species by showing that local information gained by frequent intrusions is used in territory acquisition (Stutchbury 1991; Bruinzeel & van de Pol 2004). For residents, it should then be beneficial to act on the basis of previously obtained information on such floaters' behaviour, especially if repeated challenges can be expected (Switzer *et al.* 2001), which may be the case during the periods of territory establishment and mate attraction early in the breeding season. Our experiment supported these predictions by demonstrating that resident males use information on aggression that may reflect quality of a stranger when making subsequent decisions regarding territorial defence.

Irrespective of the underlying mechanisms, i.e. persisting physiological arousal or cognitive skills including individual recognition of the opponent, our study shows that nightingales integrate information with time and use this information appropriately in territory defence behaviour. Evidence of this kind provides new insights into how decision making within the socially complex environment of communication networks in territorial animals is affected by previous experience with rivals.

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