ORIGINAL ARTICLE

Effects of previous intrusion pressure on territorial responses in Nightingales

Philipp Sprau · Tobias Roth · Valentin Amrhein · Marc Naguib

Abstract In territorial animals, establishing and defending a territory against rivals is commonly a prerequisite for successful reproduction. Yet, often, non-territorial males that are seeking to establish their own territory may intrude into occupied territories and persistently challenge residents in order to test their resource-holding potential. Such challenges may have long-term consequences for the territorial behaviour of the residents. Here, we tested whether territorial Nightingales (*Luscinia megarhynchos*) use information on the intrusion pressure of rivals in territory defence. Using multi-loudspeaker playback experiments, we simulated rivals that either sang from different song perches within occupied territories (persisting intruders) or that immediately left territories after previous intrusions (retreating intruders). In a final playback 1 h later, we then simulated the same rivals singing from a different location at the territory boundary. During this final playback, residents approached the loudspeaker closer, changed their song perches more often, sang more songs in close proximity to the loudspeaker, and sang more trills when rivals were previously simulated as having stayed in their territory without retreating. Our findings show that songbirds can integrate the level of threat from rivals differing in their intrusion behaviours in subsequent territorial encounters. This study emphasises the importance of considering how territorial defence is affected by previous intrusion pressure from rivals.

Keywords Animal communication · Birdsong · Prospecting behaviour · Intrusion persistence · Intrusion pressure · Spatial movement

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Zusammenfassung

Einflüsse früherer territorialer Konflikte auf die Revierverteidigung von Nachtigallen

Um sich erfolgreich fortzupflanzen, sind territoriale Tiere meist auf die Besetzung und die Verteidigung eines Reviers gegenüber Rivalen angewiesen. Oftmals versuchen nicht-territoriale Männchen während der Etablierung eines eigenen Revieres in besetzte Reviere einzudringen und die Fähigkeiten der Revierinhaber zu testen. Solche Eindringversuche können langfristige Auswirkungen auf das territoriale Verhalten der Revierinhaber haben. Ziel der vorliegenden Studie war es, zu untersuchen, ob territoriale Nachtigallen (*Luscinia megarhynchos*) Informationen über das Verhalten von Rivalen während der Eindringversuche

in ihr späteres Revierverhalten einbeziehen. In einem Playback-Experiment haben wir mit mehreren Lautsprechern Rivalen simuliert, die entweder von unterschiedlichen Positionen in einem besetzten Revier sangen (beharrliche Eindringlinge), oder die sofort nach dem Eindringen das fremde Revier wieder verließen (zurückweichende Eindringlinge). In einem abschließenden Playback wurde eine Stunde später der gleiche Rivale von einer anderen Position an der Reviergrenze simuliert. Während des letzten Playbacks näherten sich Revierinhaber dichter an den Lautsprecher an, veränderten ihre Gesangsposition öfter, sangen mehr in unmittelbarer Nähe zum Lautsprecher und sangen mehr Trills wenn die simulierten Rivalen zuvor im Revier blieben ohne sich zurückzuziehen. Unsere Ergebnisse zeigen, dass Singvögel den Grad der Gefahr, die von Rivalen ausgeht welche sich in ihrem Eindringverhalten unterscheiden, in späteren territorialen Auseinandersetzungen berücksichtigen können. Diese Studie unterstreicht die Bedeutung früherer territorialer Konflikte für die Revierverteidigung.

Introduction

Territorial animals defend and maintain resources such as food, mates and space against others, and occupancy of a territory is usually essential for reproduction (Grant 1993; Maher and Lott 2000). In many animals, territoriality is a seasonal phenomenon with the consequence that, at the onset of the breeding season, space has to be divided up between individuals (Stamps 1994). Early in the breeding season, territorial systems are thus characterised by residents, i.e. males that already have established a territory, and by non-territorial individuals that have not yet settled (Smith 1978; Stamps 1994; Amrhein et al. 2004; Piper et al. 2006; Roth et al. 2009; Hughes and Hyman 2011).

Non-territorial individuals that are seeking to establish their own territories may gather information about possible breeding sites by actively prospecting different territories before deciding where to settle (Reed 1999; Amrhein et al. 2004). In songbirds, prospecting of occupied territories usually occurs silently (Arcese 1987; Grant 1993; Amrhein et al. 2004), but at some point, prospecting males may challenge residents by advertising within occupied territories (Arcese 1987). Such challenging of residents may have important consequences for prospecting individuals (Parker and Rubenstein 1981) and residents alike (Hixon 1980; Norton et al. 1982; Arcese 1987). For prospecting males, challenging a resident through frequent intrusions or high resilience to resident aggression can be necessary to settle in or near an occupied territory. Indeed, the number of intrusions into an occupied territory has been shown to

predict the success of non-territorial males in gaining control over nesting sites (Arcese 1987; Stutchbury 1991). Therefore, motivation and quality of intruders may be reflected in their persistence. Likewise, residents that have been challenged by persistent intruders may be more alerted and thus behave differently in future challenges than residents that were less challenged by previous intruders. In fact, territory owners may lose their territories to persistent intruders and thereby lose the basis for survival and reproduction (Hixon 1980; Norton et al. 1982; Arcese 1987).

Despite a number of observational studies on the effects of persistence during intrusions (Parker and Rubenstein 1981; Arcese 1987; Stutchbury 1991), there is little experimental evidence that the persistence of intruders, as indicated by the time spent within the territory or the number of song posts used within or outside the territory, affects current and future territorial behaviour in residents. Recently, studies have demonstrated long-term effects of singing behaviour of rivals on territorial behaviour of residents. In Banded Wrens (*Thryothorus pleurostictus*) (Hall et al. 2006), and also in Nightingales (*Luscinia megarhynchos*) (Schmidt et al. 2007), it was shown that rivals simulated as behaving vocally more aggressively in a first vocal encounter elicit stronger responses in subsequent simulated vocal encounters the following days than less aggressive rivals. Further, Amrhein and colleagues showed in a series of studies on Winter Wrens (*Troglodytes troglodytes*) that territorial intrusions and spatial movements by intruders can affect dawn singing behaviour 1 day after the intrusion (Erne and Amrhein 2008; Amrhein and Lerch 2010). In Hooded Warblers (*Wilsonia citrina*), it was further shown that repeated intrusions of familiar neighbours lead to increased territorial defence against the neighbours compared to strangers (Godard 1993). All these studies have shown that rivals returning to a territory after a previous intrusion affect territorial behaviour of residents. One open question is whether intrusion pressure, and more specifically the persistence during intrusions by unfamiliar rivals, has lasting effects on territorial behaviour of residents. Examining how residents respond to intruders depending on intrusion pressure during previous encounters is necessary to understand the complexity of territorial systems and the possible consequences for strategies used by territory-seeking males.

In the present study, we tested whether rivals singing from different locations within and outside a resident's territory affect subsequent territorial behaviour of resident male Nightingales. Using multi-loudspeaker playback experiments, we simulated rivals that either moved within the territory of a resident or that retreated from the territory after an intrusion. We then simulated the same rivals singing from a different location at the territory boundary 1 h later and compared the residents' behaviour depending

on the previous intrusion behaviour. We expected that rivals that had been switching song perches within the territory boundaries in the previous encounter would be perceived as more threatening and consequently evoking stronger responses in residents than rivals that immediately left the territory after an intrusion. Such behaviour would indicate that males use information on perceived threat levels or intrusion pressure during previous challenges in their subsequent territorial responses.

Methods

Study site and subjects

We conducted playback experiments in a population of Nightingales in the nature reserve Petite Camargue Alsacienne (47°37′20N, 7°32′13E; France) between 12 April and 11 May 2009. In this area of approximately 18 km^2, about 200–240 males occupy distinct territories each year (Amrhein et al. 2007). Most territories are characterised by dense bushes or groves that border rivers, footpaths, grasslands or fields, so that territory boundaries are usually well defined by the habitat. For the experiments, we chose only males whose territories allowed clear distinction of boundaries. Throughout the breeding season, we conducted census rounds at midnight and at dawn to map the occupancy of the territories and the residents' singing activity and to determine residents' pairing status. Like other songbird species, male Nightingales sing at dawn throughout the breeding season. At night, however, male Nightingales cease nocturnal singing as soon as they become paired, whereas unpaired (bachelor) males continue to sing at night (Amrhein et al. 2002, 2004). Thus, pairing status can be assigned to the territory holders by examining their nocturnal singing activity (Roth et al. 2012). Subsequent pairing success of male Nightingales has previously been shown to predict territorial behaviour (Kunc et al. 2006; Schmidt et al. 2006; Sprau et al. 2010a, b, 2012a, b). Therefore, we used subsequent pairing status as a trait indicating male quality in the analysis. Playbacks were conducted during the day on 45 territorial males. These males were also singing at night prior to the playbacks and thus were considered to be unpaired at the time of playback (Amrhein et al. 2002, 2007). Of the 45 males, 32 ceased nocturnal song over the course of the breeding season but maintained singing at dawn, and were thus considered as males that became paired. 13 males continued singing at night (and at dawn) throughout the breeding season and were thus considered as having remained unpaired (bachelors).

Playback stimuli

We used nocturnal song recordings of 45 different male Nightingales made between 2004 and 2008 to create playback stimuli. Songs were recorded with a Sony TC-D5M or WM-D6C tape recorder (Sony, Tokyo, Japan) or a Marantz PMD 660 digital solid state stereo recorder (Marantz, Kenagawa, Japan) connected to a Sennheiser ME66/K6 microphone (Sennheiser Electronic, Wedemark, Germany). Tape recordings were digitised using Cool Edit 2000 (Syntrillium Software, Phoenix, AZ, USA), and for all recordings we used a sampling frequency of 44.1 kHz and a resolution of 16 bit. The recordings of the 45 Nightingale males were cut into single songs using the sound analysis software Avisoft SASlab Pro 4.4 (R. Specht, Berlin, Germany). We then randomly chose 20 different songs from each of the recordings. Thus, we obtained 45 playback stimuli that were each composed of 20 different songs. Songs were normalised in peak amplitude (i.e. the amplitude of each song was adjusted to the maximum amplitude) and arranged in a sequence of songs with 3.25 s pause duration between the songs (mean ± SD nocturnal pause duration measured for 10 males and 50 songs: 3.25 ± 1.12) using Adobe Audition (Adobe Audition 1.0; Adobe Systems, San Jose, CA, USA). The average duration of the playback stimuli was 160 ± 4 s (mean ± SD), and playback duration did not significantly differ between the two treatments (see below; Welch t test: $t = 1.58$, $df = 40.59$, $P = 0.12$). Playbacks were obtained from nocturnal recordings made in territories differing from the territories chosen for the experiments, and we also did not use recordings obtained from neighbouring territories. Furthermore, recordings from stimulus birds were obtained in a different year than the study was carried out. Thus, a subject was most likely unfamiliar with the male whose songs were used for playback. Sound pressure of the stimulus songs was adjusted to 90 dB at 1 m distance measured with a Voltcraft digital sound level measuring meter SL-300, which is within the range of the sound pressure of singing male Nightingales (Brumm 2004).

Playback protocol

Playbacks were conducted on 45 territorial males between 0800 and 1300 hours Central European Summer Time (CEST). For the non-interactive playbacks, we used uncompressed WAV files stored on Foxpro FX5 remote controlled speakers (Foxpro, USA) that were positioned on tripods at a height of 1.5 m. Before the playbacks, we measured and noted the distances between potential song posts (trees and bushes) and the loudspeakers on maps of the territories, using a Leica DISTO™ A5 laser distance meter (Leica Geosystems, Germany). The 45 males each received four successive playbacks using the same playback stimulus. The first playback was broadcast from 10 m outside the territory boundary to simulate an approaching rival and to draw attention to the site (Fig. 1). Residents were considered as
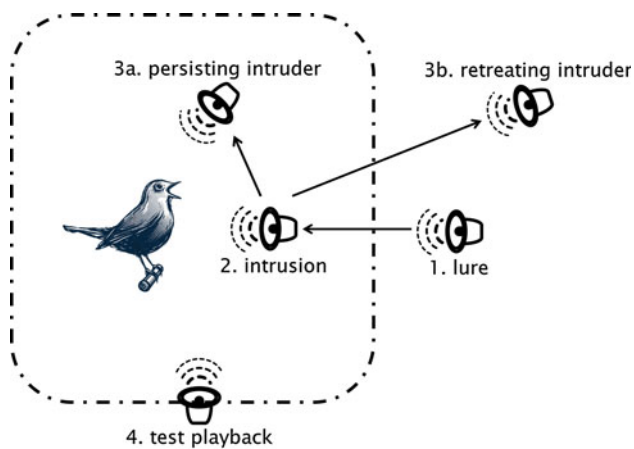
Fig. 1 Experimental setup. The *dashed black line* indicates the territory boundary of a resident male Nightingale (*Luscinia megarhynchos*). *Arrows* indicate the sequence in which loudspeakers were broadcasting. Resident males were first lured to the playback site using a speaker placed 10 m outside the territory boundary (*lure*). Then, 5 min after termination of the first playback, residents received a second playback simulating an intruder singing from 10 m inside the territory boundary (*intrusion*). Another 5 min after the termination of the second playback, residents were exposed to a third playback simulating intruders that were either singing from another location inside the territory boundary 10 m from the second loudspeaker (*persisting intruder*), or from 20 m outside the territory boundary (*retreating intruder*). One hour after the third playback, residents received a fourth playback from the territory boundary (*test playback*). For all four playbacks in a given territory, the same stimuli were used to simulate the same rival

being successfully alerted once they had started singing and approached the loudspeaker. Residents were then exposed to a second playback, 5 min after termination of the first playback, using the same song stimuli broadcast from a second loudspeaker 10 m inside the territory boundary. Another 5 min after termination of the second playback, residents were exposed to a third playback with the same songs, simulating either (1) a rival that remained within the territory but changed the song perch and thus behaved more persistently ('persisting intruder'), or (2) a rival that retreated from the territory and thus behaved less persistently ('retreating intruder'). Songs of simulated intruders that moved inside the territory were broadcast from a third loudspeaker inside the territory and 10 m from the second loudspeaker. Songs of intruders that then retreated were broadcast from 20 m outside the territory boundary. During all playbacks, loudspeakers faced the centre of the resident's territory. We used different speakers for the first three playbacks, which were positioned before the start of the experiment. The two treatments (persisting vs. retreating) were assigned to the residents before the start of the experiment. Territorial males responded to the treatment playbacks as evident from continuous singing of all males throughout the third playback. One hour after the third playback, the same stimulus songs were broadcast in a fourth playback with the speaker positioned at

least 10 m from the remaining speakers at the territory boundary. This fourth playback was the actual test playback addressing the question on how the intrusion pressure of previous encounters affects territorial responses. We recorded each resident's vocal behaviour using a Marantz PMD 660 digital solid state stereo recorder connected to a Sennheiser ME66/K6 microphone throughout the experiments. At the same time, we used binoculars to determine the spatial behaviour of territory holders and recorded in real time the distance of the birds relative to the loudspeaker. Resident identity was assured based on census rounds (see above). Sixteen of the 22 males that were exposed to the playback simulating a retreating intruder became paired later in the season, and 6 remained unpaired throughout the season (bachelors). Seven bachelors and 16 subsequently paired males received the playback simulating a persisting intruder but all males were still unpaired on the day of the playback. All males responded to all playbacks except for one paired male that had received a retreating intruder playback. This male did not respond to the fourth playback and therefore had to be excluded from the analysis.

Response measures and statistical analysis

We analysed each resident's behaviour during and 10 min after the fourth playback that was broadcast from the territory boundary. In order to test for vocal differences in response to playback that followed the two playback treatments, we measured the following song parameters: (1) song rate (songs/min), (2) percentage of songs containing rapid broadband trills (proportion of song with rapid broadband trill from the total number of songs sung within that period), (3) percentage of songs sung within 4 m of the loudspeaker (proportion of songs sung within a range of 4 m to the loudspeaker from the total number of songs sung within that period), and (4) percentage of songs used to overlap playback songs. Furthermore, we measured (5) closest approach (m), as well as (6) number of song perch changes (SPC) in order to obtain measures of the residents' spatial responses to the simulated intruders. The choice for these measurements was based on previous studies showing that they reliably predict aggression and/or motivational state of a bird (Schmidt et al. 2007; Sprau et al. 2012a, b). Songs were defined as containing rapid broadband trills when trills in the final section of the song had a frequency bandwidth larger than 5,000 Hz (measured at −24 dB), at least five repeated elements, and an element repetition rate larger than 8.5 elements/s (Naguib et al. 2008). Previous experiments have shown that rapid broadband trills in Nightingales are predominantly used as an aggressive signal in close-range interactions (Kunc et al. 2006; Schmidt et al. 2006; Sprau et al. 2010a) and can predict qualitative aspects of signalers (Sprau et al. 2013).

Data were analysed using R 2.15.1 (R Core Team 2012). We used generalised linear models using the function glm and included the two fixed factors treatment (persisting or retreating intruder) and pairing status (paired or bachelor), as well as the interaction between these factors. We used backward model selection using AIC values and removed terms from the full models with both main factors and the interaction, starting with the interaction (Crawley 2007). The significance of the predictor variables ($P < 0.05$) was assessed using F tests. The response variables song rate and closest approach were fitted with Gaussian error distribution; the response variables songs with trills, songs used for overlapping, and proportion of songs sung within 4 m were fitted with Quasi-binomial error distribution; and the response variable number of song perch changes was fitted with Poisson error distribution. In all models, we visually checked homogeneity of variance and normality of error using plots of the standardised residuals fitted against the explanatory variables and qq-plots of deviance residuals.

Results

When being challenged by rivals from the territory boundary (the fourth and final playback), resident males differed in their response depending on how they were challenged previously. Residents used more songs with rapid broadband trills and tended to sing at higher song rates when simulated intruders had previously moved within the territory rather than having retreated from the territory (Table 1; Fig. 2a, b). Males also sang more songs within 4 m of the loudspeaker when previous simulated rivals stayed within a territory, but they did not overlap the playback significantly more often (Table 1; Fig. 2c, d). Furthermore, males approached the loudspeaker closer and changed their song perches more often when previously simulated intruders stayed within the territory (Table 1; Fig. 2e, f). There was no significant effect of the residents' subsequent pairing success on the responses to the simulated intruders (Table 1).

Discussion

Resident male Nightingales responded differently to simulated rivals depending on how rivals had behaved during previous intrusions. Territorial males responded more strongly to intruders that had previously moved within their territories and thus were simulated as behaving persistently than when they had been challenged by rivals that retreated after the intrusion. Residents sang more songs with rapid broadband trills when simulated rivals had previously been

Table 1 Effects of rival intrusion pressure (persisting versus retreating intruders) and subsequent pairing status on the territorial behaviour of resident male Nightingales (*Luscinia megarhynchos*) using linear and generalised linear models

Response	Predictor	Res. df	F	P
Song rate	Pairing status	42	0.21	0.65
	Treatment	43	3.76	0.06
	Pairing status × treatment	41	<0.001	0.99
Trills	Pairing status	42	<0.001	0.98
	Treatment	43	4.94	0.03
	Pairing status × treatment	41	0.83	0.37
Songs within 4 m	Pairing status	42	0.21	0.65
	Treatment	43	6.38	0.02
	Pairing status × treatment	41	2.99	0.09
Song overlap	Pairing status	43	3.34	0.07
	Treatment	42	0.15	0.70
	Pairing status × treatment	41	0.43	0.52
Closest approach	Pairing status	42	0.26	0.62
	Treatment	43	24.44	<0.001
	Pairing status × treatment	41	1.89	0.18
No. song perch changes	Pairing status	43	0.62	0.43
	Treatment	44	8.91	0.005
	Pairing status × treatment	42	1.26	0.27

The response variables song rate and closest approach were fitted with Gaussian error distribution; the response variables of songs with trills, songs used for overlapping, and proportion of songs sung within 4 m were fitted with Quasi-binomial error distribution; and the response variable number of song perch changes was fitted with Poisson error distribution. Given are residual degrees of freedom (res. *df*), *F* values (*F*) and *P* values (*P*). Parameter values are from minimal models containing the respective response variables

Fig. 2 Effects of playback treatment (persisting intruder: $n = 22$; retreating intruder: $n = 22$) on vocal (**a–d**) and spatial (**e**, **f**) response parameters. **a** Song rate (no./min), **b** songs with rapid broadband trills (%), **c** songs sung within 4 m (%), **d** songs used to overlap the playback (%), **e** minimum approach distance, and **f** number of song perch changes; all mean ± SE. *Significant differences ($P < 0.05$) between treatments
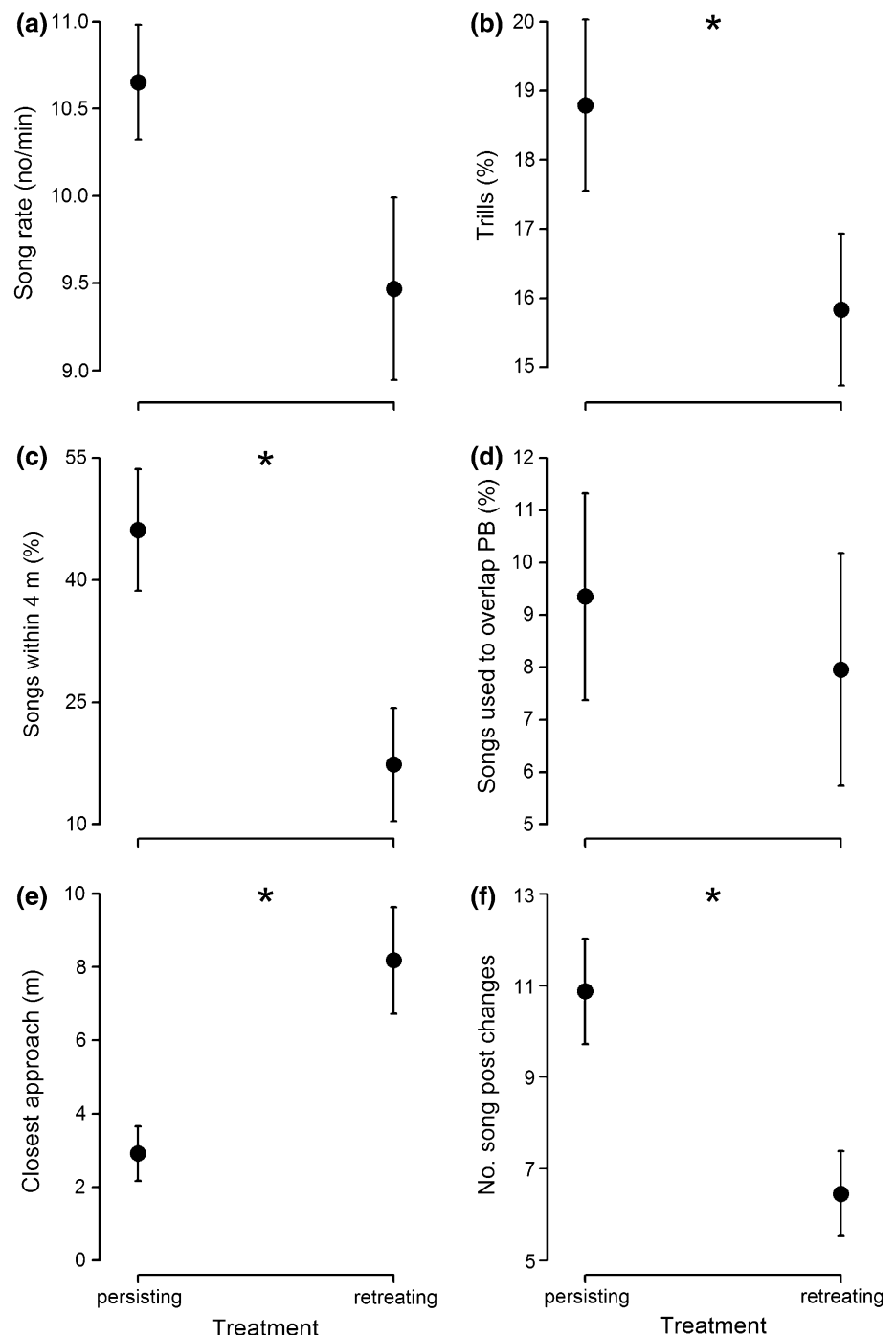
moving within the territory. Moreover, they sang more songs in proximity to the loudspeaker, approached the loudspeaker more closely, and changed their song perches more often when they had been previously challenged by a more persistent intruder. Overall, the stronger response of residents depending on the intrusion pressure during previous encounters suggests that Nightingales perceived previously persistent rivals as more threatening.

Whereas many studies have examined the consequences of prior contest information for contests under laboratory

conditions, experimentally testing the effects of previous experience on territorial contests in the wild is often a challenging task (Hsu et al. 2006). In songbirds, for instance, the effects of dominance during interactions on subsequent territorial encounters have been studied using two-speaker designs in which two unfamiliar individuals were simulated to interact with each other (Naguib and Todt 1997; Mennill et al. 2002), and the dominant/subdominant male subsequently intruded into the territory of a resident (Peake et al. 2001, 2002). We here simulated a

different situation, in which a resident was challenged with only one unfamiliar rival that either immediately retreated from its territory after prior intrusion or not. Even though our study did not explicitly aim to test the effects of winning or losing territorial encounters (for a review on the winner/loser effect, see Hsu et al. 2006), residents may have experienced different levels of a winner effect, dependent on whether or not rivals were simulated to have been immediately expelled from the territory. Thus, having apparently repelled a rival within a short period of time may lead to decreased defence behaviour in subsequent territorial encounters, perhaps because intrusion pressure may give information on the fighting ability of rivals.

The persistence of intruders can be used by residents to assess the threat that intruders pose on their territorial integrity (Arcese 1987). Perceived threat levels of intruders are important for residents, as persistent rivals may be more likely to acquire the territory of challenged residents (Parker and Rubenstein 1981). Given that residents responded more strongly to an intruder that previously stayed within the territory boundaries, our experiments show that resident males also integrate perceived threat levels from intruders of previous encounters and use this information in later situations. Songbirds have been shown to memorise information on the vocal behaviour of rivals over time (Parker and Rubenstein 1981; Godard 1991) and may even use that information in different situations (Hall et al. 2006; Schmidt et al. 2007). Moreover, songbirds may also use information on spatial behaviour of rivals during territory advertisement. For example, Amrhein and Lerch (2010) found that male Winter Wrens showed a different long-term singing response to a moving than to a stationary intruder. Godard (1993) showed that Hooded Warblers respond aggressively to neighbours only when the neighbours had intruded their territory, suggesting a tit-for-tat strategy among neighbouring males. Moreover, Blue Tits (*Cyanistes caeruleus*) have been shown to discriminate between intruders that leave or stay within the territory (Poesel and Dabelsteen 2005). Expanding on this, our study shows that Nightingales not only discriminate between intruders that have stayed or left their territory but that they also used this information in subsequent territorial encounters. There may be several explanations for this behaviour: (1) residents may have memorised the previous behaviour of the intruder (memory effects); even though we did not explicitly test whether males perceived the 'returning' male of the last playback as the same individual that was simulated during the previous playbacks, this may well be the case, given that we used the same songs during all four playbacks; (2) because the retreating intruders were simulated from a greater distance (20 m from the territory boundary) than the intruders that stayed within the territory (10 m from the first loudspeaker), the observed effects also suggest that individuals respond

more strongly to proximity of a rival during prior song bouts. Indeed, sender–receiver distance has been shown previously to strongly affect territorial behaviour in Nightingales (Sprau et al. 2010a, 2012a; Bartsch et al. 2012); (3) persisting rivals were simulated singing from two perches inside the subject's territory, whereas retreating rivals were simulated from only one perch inside the subject's territory; thus, subjects may have responded more strongly to rivals who sang from more perches within their territories; (4) challenging residents from perches within an occupied territory reflects a more natural situation compared to singing from an open field outside the territory boundary which was used for the simulated retreating rivals; and (5) these lasting effects may be explained by physiological mechanisms, i.e. residents may be prepared for territorial intrusions because of transient increases in testosterone levels following an aggressive encounter ('challenge hypothesis'; Wingfield 1984; Wingfield et al. 1990; Goymann et al. 2007). In this case, stronger responses to more intrusive rivals may have resulted from carry-over effects due to longer exposure to intruders. Yet, regardless of these specific mechanisms, our study demonstrates that residents perceived persistent rivals, i.e. intruders that stayed within the territory boundary, as more threatening than less persistent rivals. These findings suggest that territorial males integrate experience on the perceived intrusion pressures of rivals in subsequent territorial encounters.

Our findings that residents' subsequent pairing status did not significantly affect the way they defended their territories is in line with a previous diurnal playback study that also did not find differences in response behaviour between bachelors and subsequently paired males (Schmidt et al. 2007). In contrast to those diurnal studies, several nocturnal studies on Nightingales have revealed differences in territorial behaviour between paired males and bachelors (Kunc et al. 2006; Schmidt et al. 2006; Sprau et al. 2010a, b, 2012b). This apparent difference between diurnal and nocturnal responses supports the idea that diurnal song is important for territory defence both in unpaired and paired males, while nocturnal song mostly serves mate attraction and thus is likely to reveal differences between males (Amrhein et al. 2002; Roth et al. 2009; Naguib et al. 2011).

Taken together, our study shows that territorial Nightingales integrate information of rival intrusion pressure in subsequent territorial encounters and perceive persistent rivals as more threatening than less persisting rivals. Our experiments thus suggest that differences in perceived threat levels of prospecting rivals may have different consequences for the residents.

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