Communication networks in nightingales: singing and territory defence in relation to mating success and settlement

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Table of Contents

Abstract

Communication is an essential component of all social behaviour performed by animals, and social relations between animals often are mediated by elaborate signals. Among the various signalling modalities, birdsong has proved to be an excellent model to study principles in animal communication. As birdsong serves in territorial defence and in mate choice, sexual selection acts on song structure and singing behaviour leading to striking variation within and across species. Choosy females often favour those features that reliably reflect a male's condition, such as his repertoire size, song complexity or the performance of specific song structures. In dyadic interactions among males, rival assessment, in contrast, seems to be based mainly on the use of dynamic singing strategies as they reflect the motivational state of the interacting singers. Song overlapping, for example, has been shown to be perceived and used as signal of aggression, indicating the willingness to escalate a contest, whereas song alternating has been shown to be a less aggressive singing strategy. Whether or not territorial defence strategies used in dyadic interactions also convey information on the males' quality is the main question I address in this thesis, using the socially monogamous common nightingale *Luscinia megarhynchos* as model species.

Males that became paired later in the season responded stronger to simulated rivals in nocturnal long-range interactions than males that remained unpaired throughout the season, irrespective of the level of threat posed by the amount of songs the simulated rival overlapped (Chapter I). The nature of this nocturnal interaction had long-lasting effects, as males adjusted their territory defence behaviour to the prior signalling experience when confronted again with the simulated rival during the following day. Males that already had experienced the rival to be aggressive the night before (by being overlapped) responded more vigorously to the simulated intrusion during the day than did males that had experienced the rival to be less aggressive (Chapter II). These results suggest that integrating information with time plays an important role in territorial defence by affecting a male's decision making in a communication network. Furthermore, I found that males assessed the competitive abilities of rivals based on variation in a conspicuous and physically challenging song structure that is characteristic for many bird species, the rapid broadband trill (Chapter III). The males even attended to the fine structure of trills as subsequently mated males increased their response intensity with increasing performance of presented trills, whereas males that remained unpaired responded in the opposite direction. Finally, based on my findings in Chapters I and III, I investigated whether eavesdropping conspecifics in a communication network appropriately use the information on a challenged male's quality and on a trespasser's aggressiveness that may be conveyed by the signalling strategies the interacting singers use during a territorial conflict. My findings in Chapter IV suggest that resident males that are confronted with a persisting rival use their neighbours as early warning system as they adjusted their territorial defence behaviour to the level of aggressiveness the simulated rival had interacted with beforehand during a vocal contest with the neighbour. Furthermore, residents also include the individual quality of their neighbours, measured as their mating success, in decisions on how to respond to rivals.

To conclude, my experiments provide new insights into the adaptive function of signalling strategies that are used in the competition over resources and in mate choice in territorial animals. The information on a male's subsequent mating success that is reflected in his response strategy may be used by conspecifics in their decision making on where to establish a territory. Thus, my studies demonstrate that individual differences between competing animals and the adjustment to past territorial challenges should be taken into account to understand strategies in territory settlement and tenure.

Résumé

La communication est un élément essentiel du comportement social des animaux et les relations sociales entre animaux sont souvent régies par la transmission de signaux élaborés. Parmi les diverses modalités de signalisations, le chant des oiseaux est un excellent modèle pour étudier les principes de la communication. Puisque le chant des oiseaux sert à défendre un territoire et à attirer une femelle, la sélection sexuelle influence la structure du chant et le comportement lié au chant. Ceci conduit à une variation énorme entre les mâles d'une même espèce ou entre espèces différentes. Les femelles favorisent souvent les caractéristiques d'un chant qui indique la condition d'un male, par exemple la dimension du répertoire des strophes, la complexité des strophes ou la performance des structures spécifiques du chant. En revanche, au cours d'interaction dyadiques entre mâles, les différentes stratégies temporelles semblent être importantes pour juger de la condition de motivation d'un mâle particulier. Par exemple, il est connu que chanter une strophe pour recouvrir la strophe d'un rival sert à signaler l'agression et la propension à faire naître un conflit. En revanche, alterner ses strophes avec celles d'un rival est une façon moins agressive de chanter. La question principale que j'adresse dans cette thèse est si les interactions vocales entre deux mâles peuvent indiquer des informations sur la qualité des mâles. Comme espèce modèle, j'ai étudié le Rossignol philomèle (*Luscinia megarhynchos*) qui est socialement monogame.

Au cours d'interactions nocturnes longue distance pendant la période précédent l'accouplement, les mâles qui arrivent à attirer une femelle répondent plus fortement à des rivaux simulés par playback que les mâles qui restent célibataires pendant toute la saison de reproduction. Ceci, quel que soit le nombre de strophes recouvertes par le playback (chapitre I). La nature de cette interaction nocturne a des effets à long terme, car les mâles s'adaptent à l'expérience de l'interaction nocturne quand ils reçoivent à nouveau le même playback le matin suivant. Les mâles dont les strophes ont été recouvertes pendant la nuit (playback agressif) répondent plus fortement pendant le playback matinal que les mâles qui ont eu un playback nocturne moins agressif (chapitre II). Ces résultats indiquent que l'intégration temporelle des informations est importante pour la défense d'un territoire. Ces informations affectent les décisions d'un mâle dans un « réseau de communication ». De plus, j'ai trouvé que les mâles évaluent la compétitivité des rivaux en se basant sur la variation d'une caractéristique du chant qui est frappante et difficile à chanter pour les mâles de différentes espèces : les trilles rapides à large bande (chapitre III). De même, les mâles répondent à la structure fine des trilles, car les mâles qui arrivent à attirer une femelle répondent plus fortement si la qualité de trilles est augmentée alors que les mâles qui restent célibataires répondent moins fortement. Finalement, en me basant sur les données des chapitres I à III, j'ai examiné si d'autres individus qui entendent des interactions entre deux mâles peuvent utiliser des informations gagnées sur les mâles pendant une interaction avec un de ces mâles. Les résultats du chapitre IV indiquent que des mâles territoriaux adaptent leur réponse à un intrus selon le niveau d'agressivité avec lequel l'intrus avait interagit avec un voisin auparavant. Les mâles territoriaux prennent aussi en considération la qualité de ces voisins, mesuré par le stade d'appariement, pour répondre à l'intrus de manière différenciée.

En conclusion, mes expériences apportent de nouvelles connaissances sur le fonctionnement adaptif des stratégies de communication qui sont utilisées dans la compétition pour les ressources et le choix des femelles chez des espèces territoriales. L'information sur le succès reproductif futur inféré par la stratégie de réponse pourrait être utilisée par d'autres Rossignols pour le choix du lieu où établir un territoire. Ainsi, mes études démontrent que les différences individuelles entre animaux et les intrusions territoriales antérieures devraient être prises en considération afin de comprendre les stratégies de choix et de défense d'un territoire.

Zusammenfassung

Kommunikation ist ein wichtiger Bestandteil des Sozialverhaltens von Tieren, und oftmals dienen auffällige Signale der Vermittlung sozialer Beziehungen zwischen Tieren. Der Gesang von Vögeln hat sich als ausgezeichnetes Modell zur Untersuchung von Grundlagen der Kommunikation und der sexuellen Selektion bewährt. Vogelgesang, der in der Revierverteidigung und zur Partnersuche eingesetzt wird, stellt ein sexuell selektiertes Merkmal dar, so dass er innerhalb einer Art und über Arten hinweg in auffallender Variabilität zu finden ist. Weibchen scheinen bei ihrer Wahl eines geeigneten Männchens oftmals auf solche Gesangsmerkmale zu achten, die in ehrlicher Weise die Kondition eines Männchens widerspiegeln, wie dies zum Beispiel bei der Größe des Repertoires, der Komplexität des Gesangs oder bei der Darbietung bestimmter struktureller Gesangsmerkmale der Fall zu sein scheint. Im Gegensatz dazu scheinen Männchen ihre Gegner eher während direkter Auseinandersetzungen anhand von dynamischen Gesangsstrategien, welche die Motivation oder Erregung widerspiegeln, abzuschätzen. So wurde beispielsweise nachgewiesen, dass das Überlappen der Strophen des Gegenübers (*song overlapping*) ein aggressives Signal darstellt, welches die Bereitschaft zur Eskalation eines territorialen Konflikts anzeigt, wohingegen der abwechselnde Gesang (*song alternating*) eine weit weniger aggressive Gesangsstrategie zu sein scheint. Die Hauptfrage meiner Dissertation ist, ob eingesetzte Strategien zur Revierverteidigung auch Rückschlüsse auf den späteren Paarungserfolg eines Männchens zulassen, was ich an der sozial monogamen Nachtigall *Luscinia megarhynchos* untersucht habe.

Ich fand heraus, dass Männchen, die später in der Brutsaison zur Verpaarung kamen, während nächtlicher Gesangsinteraktionen vor der Verpaarung stärker auf simulierte Rivalen reagierten, als solche Männchen, die während der gesamten Brutsaison hindurch unverpaart blieben (Kapitel I). Dabei war das Ausmaß der Bedrohung, die der simulierte Rivale durch die Anzahl überlappender Strophen darstellte, unerheblich. Die Dynamik dieser Interaktion hatte eine lang anhaltende Wirkung, da die Männchen ihr Territorialverhalten noch am folgenden Tag an diese Vorerfahrung anpassten, wenn sie mit demselben Rivalen konfrontiert wurden. Männchen, die in der Nacht zuvor mit einem gesangsüberlappenden, also aggressiven, Gegner konfrontiert wurden, reagierten am folgenden Morgen stärker auf die erneute Bedrohung, als solche Männchen, die zuvor mit einem moderat singenden, also nicht überlappenden Gegner konfrontiert wurden (Kapitel II). Vorerfahrungen scheinen somit in der Revierverteidigung eine wichtige Rolle zu spielen, da sie über einen längeren Zeitraum hinweg Entscheidungen eines Territoriumsbesitzers beeinflussen können. Außerdem nutzen Männchen besondere Strophenstrukturen, nämlich schnelle und breitbandige Triller (*trills*), die für den Gesang vieler

Singvogelarten charakteristisch sind und als schwer zu produzieren gelten, um die Stärke ihrer Gegner einzuschätzen (Kapitel III). Die Männchen passten ihre Reaktion dabei sogar der Feinstruktur der *trills* an, da später zur Verpaarung kommende Männchen um so stärker auf die *trills* reagierten, je breitbandiger diese waren, wohingegen Männchen ohne späteren Paarungserfolg in gegenteiliger Richtung reagierten. Schließlich untersuchte ich, aufbauend auf den Ergebnissen der Kapitel I und III, ob Männchen innerhalb eines Kommunikationsnetzwerkes einer Interaktion zweier Artgenossen zuhören und Informationen, die durch die Wahl der spezifischen Gesangsstrategien deutlich wird, adäquat nutzen. Die Ergebnisse in Kapitel IV zeigen, dass Revierbesitzer ihre Nachbarn als eine Art „Frühwarnsystem“ nutzten. Sie passten ihr Territorialverhalten gegenüber einem Eindringling an die Information über dessen Aggressivität, die sie aus einer vorhergehenden Interaktion dieses Eindringlings mit ihrem Nachbarn gewonnen hatten, an. Außerdem berücksichtigten die Revierbesitzer in der Wahl ihrer Antwort auf den Eindringling auch die Qualität des Nachbarn, gemessen anhand seines Verpaarungserfolges.

Zusammenfassend lässt sich somit sagen, dass die Ergebnisse meiner Studien neue Einblicke in die Funktion von Kommunikationsstrategien gewähren, die in der Revierverteidigung und der Partnersuche territorialer Tiere eingesetzt werden können. Informationen über den zukünftigen Verpaarungserfolg eines Männchens, die durch spezifische Kommunikationsstrategien in vokalen Interaktionen übermittelt werden, könnten Artgenossen auch bei der Wahl eines geeigneten Revieres beeinflussen. Somit verdeutlichen meine Ergebnisse, dass individuelle Unterschiede zwischen Rivalen und die Auswirkungen von Revierkämpfen über einen längeren Zeitraum hinweg zu der Evolution bestimmter Muster in der Bildung und Aufrechterhaltung von Revieren beitragen könnten.

General Introduction

Territoriality is a widespread phenomenon in the animal kingdom. A territory can be defined as ‘a more or less exclusive area defended by an individual or group’ (Davies & Houston 1984) and often males are the sex defending territories. Territorial behaviour may be linked for example to the distribution of food (Zahavi 1971; Johnson et al. 2002), the availability of suitable breeding sites, the protection afforded from predators (Davies & Houston 1984; Barnard 2004), and also to the access to females. Females can be seen as a limited resource for males due to their often higher initial investment into reproduction (Bateman 1948; Trivers 1972). Based on this asymmetry in initial investment, the two sexes can be predicted to follow different strategies to maximise their reproductive success. Signals that serve in the acquisition and defence of limited resources may be evaluated differentially by the two sexes in the assessment of males as potential mates or rivals. Thus, elaborate animal signals provide important models in the study of sexual selection.

Birdsong and its striking variation within and across species, one of the most complex communication systems in vertebrates, has often been investigated in the context of sexual selection during the past decades (Catchpole 1987; Kroodsma & Byers 1991; Searcy & Nowicki 2000; ten Cate et al. 2002; Searcy & Nowicki 2005; Naguib & Riebel 2006). Birdsong has evolved under both inter- and intrasexual selection, as an individual’s song may function in territorial defence as well as in mate choice (Catchpole & Slater 1995; Searcy & Nowicki 2000; Collins 2004). One of the first published studies that provided evidence for the territorial defence function of birdsong was an experiment conducted by (Peek 1972) showing that muted red-winged blackbirds, *Agelaius phoenicus*, experienced more territorial intrusions by rival males than did non-muted control males. Less invasive ‘speaker replacement experiments’, in which resident males were removed and replaced by loudspeakers, revealed that territories from which a control stimulus or no song was played were occupied sooner or more often by rival males than territories in which a resident male was simulated to be present by playing back its song (great tits, *Parus major*: Krebs 1976; Krebs 1977; white-throated sparrow, *Zonotrichia albicollis*: Falls 1988; song sparrow, *Melospiza melodia*: Nowicki et al. 1998b). These experiments indicate that song as a ‘keep-out signal’ directed to other males serves in territorial defence and thus may be subject to intrasexual selection. A similar experimental design has been used to demonstrate the function of song in male-female communication, namely the attraction of females to the male and his territory. Here, a comparison of female visitation rates to nestboxes from which male song was broadcast or to control nestboxes revealed that it is the male song that attracts females (Eriksson & Wallin 1986; Mountjoy & Lemon 1991; Johnson & Searcy 1996; Mountjoy & Lemon 1996; Martin-Vivaldi et al. 2000). Furthermore, male seaside sparrows (*Ammodramus maritimus peninsulae*) muted early in the breeding season, in contrast to sham-operated controls, failed to attract females (Mcdonald 1989).

As song is to serve these dual functions, females and males may use different song traits in their assessment of a male as potential father of their offspring or as a rival, respectively. We can expect factors such as a male's age, his condition, ability to feed the offspring, or the quality of his territory to influence his attractiveness as a social mate for a choosy female. Regarding male rivalry, we can expect factors such as the aggressiveness or the competitive abilities of a male to be of importance concerning whether or not a rival should consider to establish a territory nearby or even to attack. In the following I will describe how these factors may be reflected in a male's singing performance.

There is accumulating evidence that females prefer to mate with males that have larger repertoires or sing more complex songs, for instance in great tits (Mcgregor et al. 1981; Lambrechts & Dhondt 1986), different *Acrocephalus* species (Catchpole 1980; *A. arundinaceus*: Catchpole 1986; *A. paludicola*: Catchpole & Leisler 1996; *A. schoenobaenus*: Buchanan & Catchpole 1997), or pied flycatchers (*Ficedula hypoleuca*, Lampe & Espmark 1994). Females may also prefer males that sing at higher rates (willow warbler, *Phylloscopus trochilus*: Radesater et al. 1987; pied flycatcher: Alatalo et al. 1990; European starling, *Sturnus vulgaris*: Eens et al. 1991; white-throated sparrow: Wasserman & Cigliano 1991). In general, these aspects of male singing performance seem to be affected by male age and survival (Cosens & Sealy 1986; Hiebert et al. 1989; Alatalo et al. 1990; Lampe & Espmark 1994; Hasselquist et al. 1996; Gil et al. 2001). Older and more experienced males may also feed the offspring better than younger, less experienced males (Saetre et al. 1995), and repertoire size may be negatively correlated with parasite infection rates (Buchanan et al. 1999). Thus, choosy females may get more experienced, healthier males or those in a better physical condition. Recently, it has been argued that a male's singing performance may reflect the condition experienced during early development as males that experience nutritional stress early in live may develop smaller song repertoires or may learn songs only poorly (Nowicki et al. 1998a; Nowicki et al. 2002a, b; Spencer et al. 2003, 2004; but see Gil et al. 2006). In some species, females have been shown to assess males based on their performance of specific song structures such as rapidly repeated short notes, the so called trills (canary, *Serinus canaria*: Vallet & Kreutzer 1995; Vallet et al. 1998; Leboucher & Pallot 2004; swamp sparrow, *Melospiza georgiana*: Ballentine et al. 2004). Since the production of trills appears to be physically challenging (Westneat et al. 1993; Podos 1996, 1997; Hoese et al. 2000; Podos & Nowicki 2005), a female preference for high performance trills may again lead to mating with a male of good physical condition.

Contest among males may be over mates or resources that attract females, such as a nest, a feeding site or a territory in general. As the outcome of a vocal contest may depend on body condition and the motivation to engage in countersinging or even in a physical fight. factors determining physical strength, such as body size, weight, body condition or energy reserves may become important in the assessment of rivals. Across species, lower frequencies may indicate a larger body size (Ryan & Brenowitz 1985), and within a species individuals of the larger sex may sing at lower frequencies (Ballintijn & ten Cate 1997). However, it is not clear whether within a species larger or heavier individuals produce lower frequency vocalizations than smaller individuals, some studies found a positive correlation, for example in barn swallows (*Hirundo rustica*, Galeotti et al. 1997) or grey partridges (*Perdix perdix*, Beani & Dessi-fulgheri 1995), whereas other studies did not (Shy 1983; Genevois & Bretagnolle 1994). Thus, relationships between frequency and male competitive abilities are not always clear and it remains to be tested whether frequency differences convey significant information in male-male communication (Shackleton & Ratcliffe 1994; ten Cate et al. 2002).

Similarly, the functional significance of repertoire size or song complexity in male rivalry still remains to be investigated. On the one hand, male great tits and red-winged blackbirds, for example, are less likely to intrude into territories where males have larger repertoires (Krebs et al. 1978; Yasukawa 1981), and in European starlings males with a larger repertoire more effectively keep rivals away from the nestbox (Mountjoy & Lemon 1991) and are more likely to win encounters (Eens 1997). But on the other hand great reed warblers, for example, produce shorter, less complex songs during encounters with rival males (Catchpole 1983).

In addition to general traits such as phonological and temporal singing traits, it has been shown that the strategies with which males use their song in a vocal interaction with a rival play a significant role in their social behaviour (reviewed in Naguib 2005). Directly interacting animals often respond to the counterpart's signals in a very specific way to express their motivation, social status or their competitive abilities. Furthermore, each of the interacting animals usually takes the role of both a signaller and a listener. In such intrasexual interactions males use dynamic singing strategies by varying the pattern of the signal or the timing of the signal in relation to the opponent (McGregor & Dabelsteen 1996; McGregor & Peake 2000; Todt & Naguib 2000).

As a pattern-specific response, an individual can respond to a conspecific by singing either the same type of vocalization (song type matching, Krebs et al. 1981; McGregor et al. 1992; Burt et al. 2001; Vehrencamp 2001; Mennill & Ratcliffe 2004b), by using a song that is shared by the two interacting singers (repertoire matching), or by using a different type of vocalization that is not shared. By matching either way, a male directly addresses the counterpart. As a male needs to know beforehand which song he shares with the opponent to match his repertoire, it is assumed that repertoire matching may be used among well-known neighbours in non-aggressive situations, in which a responder signals his presence to the neighbour (Beecher 1996). Song sparrows, for example, acquire the songs of their neighbours after dispersal to sing a shared song in almost 90% of all songs during playback trials (Beecher et al. 2000). If male song sparrows cannot engage in repertoire matching they will suffer more frequently from aggressive encounters with neighbours (Wilson et al. 2000; Wilson & Vehrencamp 2001) suggesting that the ability to match the neighbour's repertoire partly stabilizes the relationship between neighbouring males in this species. Song type matching seems to be used in response to intruders and is assumed to be an agonistic signal that often is associated with a subsequent approach (Krebs et al. 1981; Falls et al. 1982; Kramer et al. 1985; Falls et al. 1988; Shackleton & Ratcliffe 1994; Nielsen & Vehrencamp 1995; Vehrencamp 2001). Moreover, males may also match only part's of the opponent's song such as the frequency (Morton & Young 1986; Shackleton & Ratcliffe 1994; Otter et al. 2002) or specific song components (Burt et al. 2002).

Varying the timing of song delivery, a male may alternate his songs with those of an opponent or overlap the opponent's songs (McGregor & Dabelsteen 1996; McGregor & Peake 2000; Todt & Naguib 2000). It is widely accepted that song alternating enables both interacting singers to get their message across as acoustic interference is avoided and thereby broadcasting range is increased (Ficken et al. 1974; Popp et al. 1985; Popp 1989; Dabelsteen et al. 1996; Todt & Naguib 2000). During song overlapping, in contrast, males start to sing a song before the counterpart has finished its song. While song alternating seems to be a moderate singing strategy in less intense contexts, song overlapping has been shown to serve as a directed agonistic signal in close-range male-male vocal contests as overlapped males often show strong responses characteristic of a high level of arousal (Brindley 1991; Collins 2004; Naguib 2005). Playback studies have shown that overlapped males may rapidly approach the overlapper (Dabelsteen et al. 1997; Osiejuk et al. 2004) or sing more irregularly by showing a higher variability in their song length and song timing (Dabelsteen et al. 1996; Langemann et al. 2000; Mennill & Ratcliffe 2004b) or by changing to an almost continuous pattern of singing (Dabelsteen et al. 1997). Moreover, in escalating vocal contests increasing song overlap causes males to interrupt their regular song (Naguib & Kipper 2006).

To summarize, characteristics of song components or song delivery have predominantly been investigated in an intersexual context aiming to reveal which song characteristics honestly reflect male quality to be shaped by female choice. In contrast, studies on male rivalry have focussed on short-term motivation and aggression of males by investigating the signal value of time- or pattern-specific singing strategies that are used in male-male interactions.

However, we can hypothesize that the way males use their song during vocal interactions does not only depend on the perceived level of threat signalled by the opponent but may also be affected by the quality of a challenged male. The song rate of a male, as mentioned above, has been shown to be an important factor in female choice (Radesater et al. 1987; Eens et al. 1991; Wasserman & Cigliano 1991). Males provided with extra food may sing more (Alatalo et al. 1990) and attract a female earlier (Tobias 1997a) suggesting that a high food availability, and thereby an aspect of territory quality (Gottlander 1987), positively affects a male' condition. In male-male interactions, an increase in song rate is considered to be related to the increase in arousal and aggressive motivation (e.g. Kramer et al. 1985; Weary et al. 1988). Taking these results together, the ability of a male to increase his song rate during a vocal interaction may reflect his level of arousal as well as his general condition, his resource holding potential or his quality. Therefore, the general question whether territorial defence strategies also convey information on the challenged males' quality is the main question I addressed in the present work. Using subsequent mating success as an indicator of male quality (see section 'The Nightingale as a Model Species', p. 15, and Appendix, p. 91), I investigated the territorial defence behaviour of nightingales, *Luscinia megarhynchos*, in response to simulated rivals that differed in the amount of song overlap (**Chapter I**) or in the amount of a specific song structure (**Chapter III**), respectively.

As 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), information on competitive abilities and male quality exchanged in a dyadic interaction may also be conveyed to conspecifics in the neighbourhood (Peake 2005). Indeed, males eavesdropping on a dyadic interaction between other males have been shown to distinguish between aggressive, i.e. overlapping individuals and moderate, i.e. alternating singers in two-loudspeaker playback experiments (Naguib & Todt 1997; Naguib et al. 1999; Peake et al. 2001, 2002; Mennill & Ratcliffe 2004a). However, it is not clear whether males also attend to differences in quality of conspecifics that may be inherent in the singing strategies the conspecifics adopt during territorial challenges. In **Chapter IV**, I addressed this question in another playback experiment based on the results of **Chapters I** and **III**.

So far, I have reviewed and investigated short-term consequences vocal interactions may have on the territorial defence behaviour of male birds. However, for a long-term territory tenure, males may benefit by adjusting their response strategies in a contest with an opponent to the preceding experience they have had with that opponent. This may be of particular importance early in the breeding season during periods of territory establishment and mate attraction during which disputes over territory boundaries are likely to occur frequently. Models on territory acquisition assume that for a floater the likelihood to establish a territory at a given area increases with the amount of obtained information on that area (Ens et al. 1995; Stamps & Krishnan 1999, 2001). This information may be obtained by prospecting and an evaluation of the quality of an area by assessing environmental cues (Doligez et al. 2003) or public information, e.g. on the density of conspecifics (Doligez et al. 2002; Doligez et al. 2004), and of course also by repeated intrusions into other territories (Arcese 1987, 1989). Experimental studies confirmed these assumptions by showing that local information gained by frequent intrusions is essential for territory acquisition, e.g. in purple martins (*Progne subis*, Stutchbury 1991) and oystercatchers (*Haematopus ostralegus*, Bruinzeel & van de Pol 2004). Accordingly, if this argumentation is turned around and looked at from the perspective of a resident male then a resident is likely to benefit by acting upon its prior experience (Stamps & Krishnan 2001; Switzer et al. 2001; Rutte et al. 2006). In **Chapter II**, I tested this hypothesis by investigating whether territorial defence strategies are influenced by the nature of a preceding vocal contest.

The Nightingale as a Model Species

The nightingale (*Luscinia megarhynchos*) and the sister species, the thrush nightingale (*Luscinia luscinia*) are well known for their nocturnal song which seems to be an extraordinary singing behaviour occurring only in a few species, for instance in several warblers, such as the marsh warbler (*Acrocephalus palustris*), the sedge warbler, and the reed warbler (*A. scirpaceus*), or in the nightjar (*Caprimulgus europaeus*, Cramp & Simmons 1998). In the last decades, nightingale song has been the subject of extensive research, for example on song organisation and song learning (Hultsch & Kopp 1989; Hultsch & Todt 1989; Hultsch 1991; Hughes et al. 2002; Geberzahn & Hultsch 2003; Hultsch & Todt 2004; Kipper et al. 2004), on the costs of singing (Thomas 2002a, b), on the functions of song at different times of the day and at different times in the season (Amrhein et al. 2002; Amrhein et al. 2004b; Kunc et al. 2005a, b), or on the use and function of song in vocal interactions (Naguib 1999; Naguib et al. 1999; Naguib et al. 2004; Naguib 2005; Naguib & Kipper 2006). In the nightingale, nocturnal song is assumed to serve in mate attraction because males cease nocturnal song after pair formation, and up to 49% of territorial males remain unmated throughout the breeding season (Amrhein et al. 2002; Amrhein et al. 2004b; Amrhein et al. 2007). Males usually arrive earlier from migration on the breeding ground than females (Amrhein et al. 2007) which provides the opportunity to compare singing behaviour or territorial defence strategies of males early in the breeding season in relation to their subsequent mating success which can be determined based on the presence or absence of regular nocturnal song over the course of the breeding season (see Appendix, p. 91; Amrhein et al. 2002; Amrhein et al. 2007). Thereby, a standardized situation at the time of investigation can be maintained with males having already established a territory but still being unpaired. As only about 8% of nestlings are sired by an extra-pair male (Amrhein 2004), a male's capability to attract a social mate can be seen as the main factor limiting a male's reproductive success. Thus, potential differences in territorial behaviour can be linked to fundamental individual differences. This makes nightingales a good candidate species to investigate male-male interactions from the perspective of the challenged males in order to evaluate whether territorial defence strategies differ with the males' quality.

Chapter I

Responses to interactive playback predict future pairing success in nightingales

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Responses to interactive playback predict future pairing success in nightingales

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Birdsong is a sexually selected trait that serves in territory defence and mate choice. Individual song traits can be affected by the body condition of the male and thus may reflect his quality. Such relations between male quality and general singing performance raise the question whether differences in male quality also affect response strategies used in dyadic interactions. To address this question, we studied the relation between pairing success of male common nightingales, *Luscinia megarhynchos*, and their responses to rivals posing different levels of threat. Using interactive playback, we exposed males prior to mating to either aggressively or moderately singing rivals (by song overlapping and song alternating, respectively). Males that remained unpaired throughout the season (bachelors) interrupted their singing significantly more often after being overlapped than after alternating playback, whereas subsequently mated males kept the number of singing interruptions more constant across playback treatment. This suggests that subsequently paired males are less discriminative than are bachelors when challenged by rivals varying in aggressiveness. Regardless of playback treatment, males that later became paired responded significantly more strongly than did bachelor males. Thus, an increase in singing after a vocal interaction prior to mating predicted future mating success.

Elaborate animal signals serve in the acquisition and defence of resources, and thus provide important models in studies on sexual selection. Among the various signalling modalities, acoustic signals have been investigated most intensely in several taxa to assess principles in communication (Greenfield 1994; Catchpole & Slater 1995; Grafe 2005). Birdsong and its striking variation within and across species is among the most complex communication systems in vertebrates. It has evolved under both inter- and intrasexual selection, as an individual's song may function in territory defence as well as in mate choice (Kroodsma & Byers 1991; Catchpole & Slater 1995). Individual song traits, such as song rate (Alatalo et al. 1990), or specific song elements, such as trills (Vallet & Kreutzer 1995; Ballentine et al. 2004; Leboucher & Pallot 2004), can be affected by constraints in production or by male body condition and thus may advertise the quality or condition of a singer. Furthermore, dyadic singing interactions can provide information on relative differences in motivation or social status between rival males to the interacting singers themselves as well as to eavesdropping conspecifics in a communication network (McGregor & Dabelsteen 1996; Peake 2005). Males of various songbird species have been shown to extract information from vocal interactions to assess rivals (Naguib et al. 1999, 2004; Peake et al. 2001, 2002; Mennill & Ratcliffe 2004a), and females also use information obtained from interactions in mate choice (Otter et al. 1999; Mennill et al. 2002, 2003; Leboucher & Pallot 2004).

In such intrasexual interactions males use dynamic singing strategies by varying the pattern of the signal or the timing of the signal in relation to that of the opponent (Todt & Naguib 2000). By varying the timing of song delivery, a male can either alternate his songs with those of an opponent or overlap the opponent's songs. Several studies support the hypothesis that song overlapping is perceived as a signal of aggression during vocal interactions, as males sing more irregularly during overlapping than during alternating playback (Dabelsteen et al. 1996; Naguib 1999; Mennill & Ratcliffe 2004b; Naguib & Kipper

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2006) or stop singing when their songs are overlapped (Langemann et al. 2000).

Given the possible relations between male quality and general singing performance in 'undirected' territorial song and the importance of song in territory defence, differences in male quality or condition can also be predicted to affect response strategies used in male–male interactions occurring in territorial conflicts (see also Poesel et al. 2004). For instance, the singing performance of common nightingales, *Luscinia megarhynchos*, during interactions conveys information that may be used in female choice (Kunc et al. 2006): males that are successful in attracting a social mate overlap more songs of a noninteractive playback during mate attraction than males that remain unpaired throughout the breeding season ('bachelors'). Such findings and the above considerations raise the question whether males that differ in their subsequent pairing success respond differentially to rivals varying in aggressiveness (see also Mennill & Ratcliffe 2004b).

If males that are attractive to females are more aggressive or have a higher resource-holding potential than males that remain unpaired throughout the season, then we predict that these attractive males should also be less likely to lose a contest against aggressive unfamiliar rivals. Therefore, we expect subsequently paired males to respond similarly regardless of the threat posed by an unfamiliar rival. In contrast, we predict bachelor males to be more discriminative because although they may succeed against moderate rivals they are less likely to succeed against aggressive rivals.

To study the relation between pairing success of males and their responses to rivals posing different levels of threat, we simulated unfamiliar rivals singing near the territory boundaries of subsequently mated and unmated nightingales as being either aggressive (by song overlapping) or moderate (by song alternating). Playbacks were conducted with nocturnal song during which nightingales interact intensively, with communication being restricted to acoustic signalling. We predicted that subsequently mated and unmated males would respond differently to playback treatments. Nightingales form socially monogamous seasonal pair bonds, but in our study population up to 49% of territorial males remain unpaired throughout the breeding season (Amrhein et al. 2004a), which gave us the opportunity to compare singing behaviour of males with respect to their future success in attracting a female. To investigate changes in singing behaviour elicited by playback, we compared singing behaviour before and after playback. We analysed singing behaviour by quantifying temporal singing patterns and structural song components.

METHODS

Subjects and Study Site

We conducted playbacks on 42 nocturnally singing nightingales in the vicinity of the Petite Camargue Alsacienne (France) within a population of about 240 singing males in an area of 18 km^2 in the Upper Rhine Valley north of Basel, Switzerland (Amrhein & Zwygart 2004). Here, nightingales usually settle in patches of woods and dense bushes along rivers or footpaths. In addition, territories often border grasslands or fields, resulting in clear territory boundaries. For playback, we chose territories that were sufficiently isolated that playbacks did not interfere with responses from neighbours. Thus, our subjects were individually recognizable by territory location. Song posts of subjects were mapped on at least 2 days and nights prior to playback to determine territory boundaries. Playbacks were conducted at the beginning of the breeding period in 2004 (20 playbacks between 18 April and 3 May) and 2005 (22 playbacks between 19 April and 2 May). Grüll (1981) found that 76% of males return to the same territory of the preceding year or to a directly neighbouring one. Since we used different, non-neighbouring territories in both years, it is unlikely that we used the same subject twice.

Male nightingales sing regularly at night for a prolonged period only until a female has settled in their territory (Amrhein et al. 2002, 2004b). Thus, we monitored nocturnal singing activity of all subjects after playback every second night throughout the breeding season to assess their mating status. All subjects had been singing during the nights before they received playback and they sang during at least 2 nights after playback. Later in the season, 20 males ceased nocturnal song indicating that they became paired, whereas the other 22 males sang at night throughout the breeding season indicating that they remained unpaired.

Playback Stimuli

We created stimuli from nocturnal recordings of 42 male nightingales made between 2002 and 2005 in the study population. These males were different from those used as subjects in the present study. Nocturnal song was recorded on a Sony TC-D5M or WM-D6C cassette recorder with a Sennheiser ME66/K6 microphone and digitized using Cool Edit 2000 (Syntrillium Software, Scottsdale, AZ, U.S.A.; sample frequency: 44.1 kHz; resolution: 16 bits). To generate stimuli, we selected 22 songs from each recording (Avisoft SASLab Pro 3.5, R. Specht, Berlin, Germany). In line with the natural proportion of different song categories in nocturnal song (Kunc et al. 2005), each set of 22 songs consisted of three whistle songs, i.e. songs starting with a series of mostly unmodulated whistles (Hultsch & Todt 1996), and 19 nonwhistle songs (Fig. 1a). Three of these 19 nonwhistle songs contained a rapid broadband trill (Fig. 1b). Male nightingales have been shown to vary these song traits during vocal interactions with rival males (Todt & Naguib 2000; Kunc et al. 2006). We generated a new set of stimulus songs for each playback and used the songs of each source male only once. The 22 songs were randomly merged into one wave-file in Cool Edit and normalized at once to the peak amplitude to standardize playback amplitude across trials. Then, we arranged the 22 single songs in one file of SyrinxPC version 2.3s (J. Burt, http://www.syrinxpc.com), on a Toshiba Satellite notebook (S2210CDT), to enable the experimenter

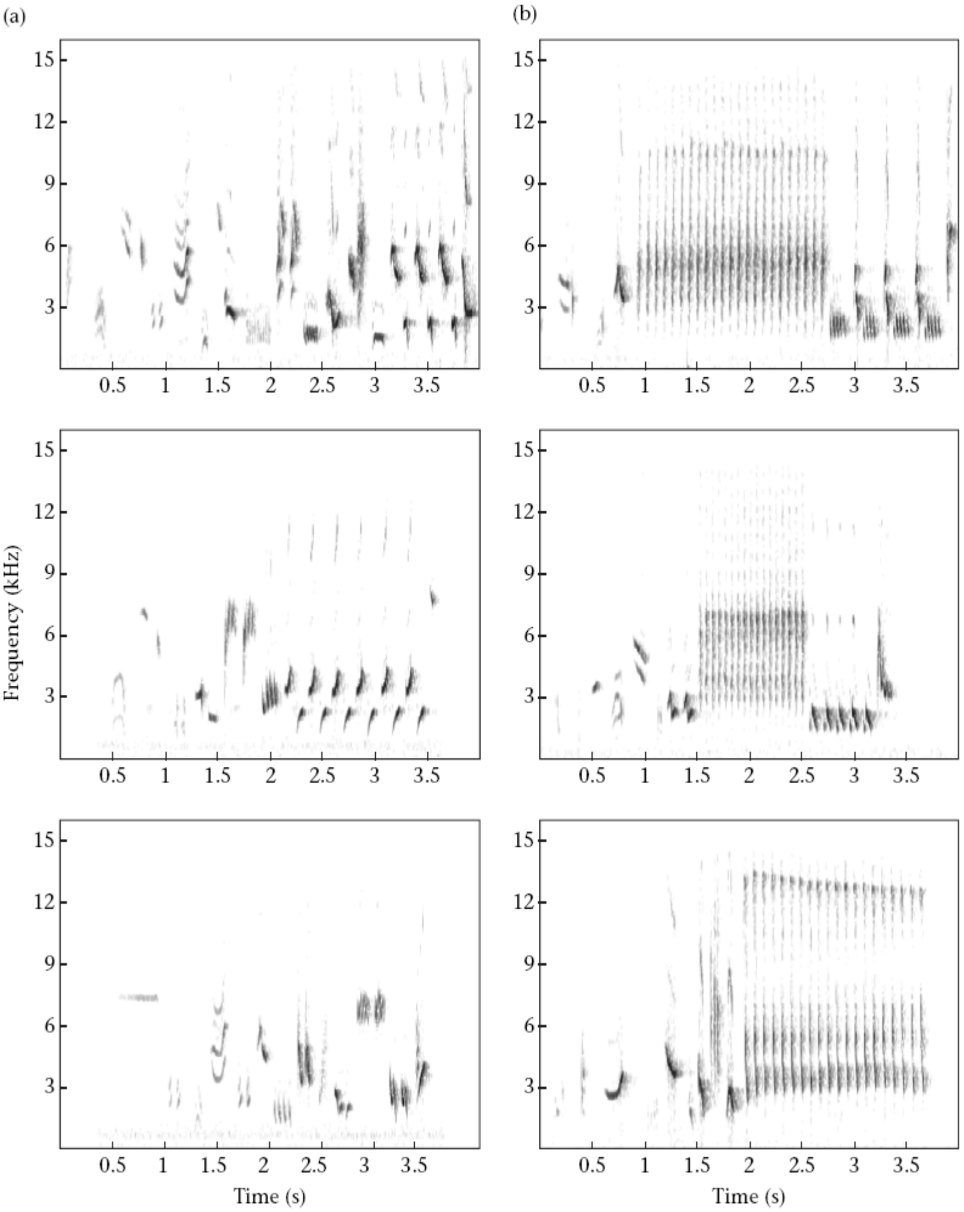

Figure 1. Spectrograms of six different song types of male nightingales' song: (a) nonwhistle songs without rapid broadband trills and (b) nonwhistle songs containing such trills.

to play each song separately during playback. Thereby, each song could be played on demand, but no song was played twice during a playback trial.

Playback Protocol

All nocturnal playbacks were conducted between 2345 and 0330 hours CEST. We only used males that were already singing when the playback started. The subjects received either an overlapping playback ($N = 21$), in which a mean ± SD of 86.0 ± 11.1% of playback songs overlapped the subjects' songs, or an alternating playback ($N = 21$) in which we avoided song overlapping (0.4 ± 1.3% of playback songs overlapped the subjects' songs). The order of playback treatments was alternated systematically across the males. To simulate a newly arrived rival singing near the subject males' territory boundaries, we positioned the loudspeaker outside the males' territories at distances of 30–40 m from their nocturnal song posts.

The notebook used for playback was connected to a Blaupunkt MPA 2 amplifier and a Canton Plus X passive speaker. The sound pressure of broadcast songs was adjusted before playback to 90 dB at 1 m distance, measured with a Brüel & Kjær precision SPL meter 2233 (C-weighting, fast response). This sound pressure level lies within the range of naturally singing nightingales

(Brumm 2004). Singing of focal males was recorded on the left channel of a Sony TCD 5M stereo tape recorder with a Sennheiser ME66/K6 directional microphone. On the right channel we recorded the songs broadcast by the loudspeaker with another microphone of the same kind. Before we started playbacks, we recorded the subjects' nocturnal song for 10 min, and we continued recordings for 10 min after playbacks terminated.

Response Measures and Statistical Analysis

To investigate influences of playback treatment, we focused on the changes in singing after playback compared to the singing before playback. Therefore, we first measured the song parameters for the 10-min intervals before and after playback separately. Then, we calculated the difference between these two measures for each subject to assess the change in singing behaviour elicited by the playback treatment.

Our subjects occasionally interrupted their regular nocturnal singing, leading to between-song intervals that differed markedly from the mean intersong intervals ± SD of 3.2 ± 3.7. As the occurrence of such nonvocalizing elicited by playback confounds the analysis of regular intersong intervals, we analysed these interruptions separately. By defining a singing interruption as a silent interval exceeding 10 s (Fig. 2), we followed previous studies which showed such interruptions to be characteristic responses to overlapping playback (Naguib 1999; Mennill & Ratcliffe 2004b).

We measured (1) the number of singing interruptions (number of silent intervals longer than 10 s) and (2) the total duration of these interruptions (s). Measures unaffected by singing interruptions were (3) the percentage of songs containing trills, (4) the percentage of whistle songs and (5) the song duration (s). Furthermore, for the effective signalling time, i.e. after excluding the duration of interruptions, we measured (6) the pause duration (duration of intersong intervals < 10 s) and (7) the song rate (number of songs/min).

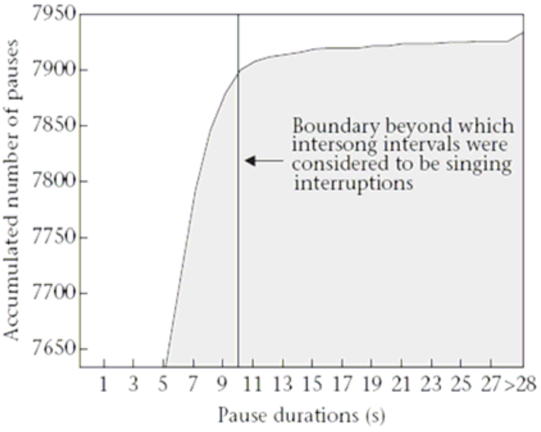

Figure 2. Distribution of silent intersong intervals of all male nightingales during 10 min before and 10 min after playback. We defined a singing interruption to be a silent interval of more than 10 s.

We applied a multivariate general linear model (SPSS 12.0, SPSS Inc., Chicago, IL, U.S.A.) with the seven song parameters as dependent variables, measured as changes after playback in comparison to before playback (i.e. the difference between them). The initial model included three fixed factors: the playback treatment (song alternating or song overlapping), the males' subsequent pairing status (mated male or bachelor) and the year of study (2004 or 2005). Since the year of study did not have a significant influence in the MANOVA ($P > 0.2$), this factor was removed in the final model. The influence of playback treatment on each song parameter and the relation between subsequent mating status and song parameters were then assessed with univariate general linear models. Data on the number of singing interruptions were log transformed to meet requirements for parametric statistics. In our sample, 13 of 21 males in the song-alternating treatment group and nine of 21 males in the song-overlapping group remained unpaired throughout the season. Results are given as mean ± SE; all reported tests are two tailed.

RESULTS

With all measures taken together, the subjects' singing was influenced by playback treatment, although not quite significantly so (MANOVA: $F_{7,32} = 2.23$, $P = 0.058$), and responses differed significantly between subsequently mated males and bachelors ($F_{7,32} = 2.95$, $P = 0.017$). The interaction between treatment and pairing success overall was significant ($F_{7,32} = 3.28$, $P = 0.010$), indicating that subsequently mated males and bachelors responded differently to overlapping and to alternating playback.

Effects of Playback Treatment

Across subsequently mated and unmated males, subjects whose song had been overlapped interrupted their singing for significantly longer than subjects that had received the song-alternating treatment (Fig. 3a, Table 1). Playback treatment did not significantly affect the number of singing interruptions across all males, but the interaction between playback treatment and mating status was significant (Table 1). In other words, bachelors interrupted their singing more often after the overlapping treatment than after the alternating treatment, whereas subsequently mated males kept the number of interruptions at similar levels in both treatments (Fig. 3b).

The structural song components (whistle songs and trills) and the three measures of singing activity (song duration, pause duration and song rate) did not differ significantly in response to the two types of interactive playback; nor did we find significant interactions between playback treatment and pairing success with regard to these five variables (Table 1).

Effects of Subsequent Pairing Success

Males that later in the season attracted a female sang at significantly higher rates (Fig. 4) and increased the proportion of trills after playback, although not significantly so (Fig. 5, Table 1), regardless of playback treatment. These males increased their song rate by shortening the intersong intervals significantly by about 14% (Fig. 4), whereas song duration remained similar in subsequently mated males and bachelors (Fig. 4). Pairing success was not significantly related to the duration or number of singing interruptions, nor to the use of whistle songs (Table 1).

DISCUSSION

The singing behaviour of male nightingales in response to playback suggests an influence of playback treatment, i.e. whether the simulated rival overlapped or alternated with the subjects' songs. Compared to the time before playback, males interrupted their singing for significantly longer after they had been overlapped by playback than they did after playback songs had alternated with them. Furthermore, those males that remained unpaired throughout the breeding season interrupted their singing significantly more often after the song-overlapping treatment than after the song-alternating treatment. In contrast, subsequently mated males showed a similar number of singing interruptions, regardless of whether their songs had been overlapped by playback. These males increased their song rate more than did bachelors, regardless of playback treatment. Furthermore, subsequently mated males, which prior to playback sang fewer songs containing rapid trills than did bachelors, tended to increase the use of such songs after playback and sang as many as did bachelors, which did not vary the use of trills.

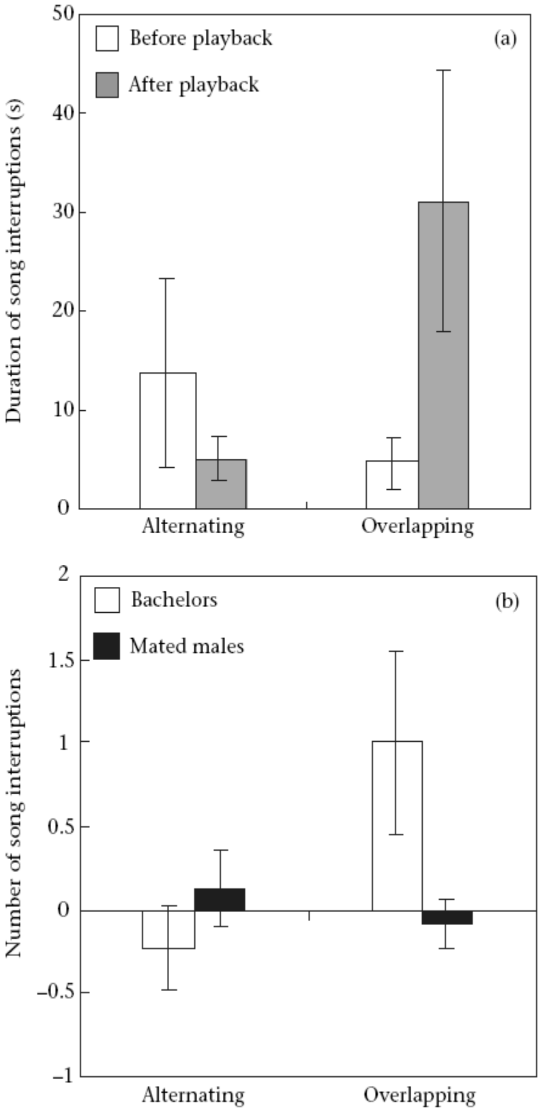

Figure 3. (a) Duration of all singing interruptions ($\overline{X} \pm SE$) within 10 min before playback and within 10 min after playback depending on playback treatment. (b) Difference between the number of singing interruptions after and before playback ($\overline{X} \pm SE$) for subsequently unmated and mated males depending on playback treatment. Negative values indicate fewer, and positive values more, interruptions after than before playback.

The increased duration of singing interruptions after being overlapped by a simulated opponent is in line with previous findings (Naguib 1999; Mennill & Ratcliffe 2004b; Naguib & Kipper 2006) and supports the hypothesis that song overlapping is a signal of aggression in male–male vocal interactions (Dabelsteen et al. 1996, 1997; Langemann et al. 2000; Mennill & Ratcliffe 2004b). These studies focused on the singing strategies of males responding during playback to the perceived level of aggression of a simulated opponent. Expanding on these findings, our results show that singing interruptions persisted even after playback had terminated, i.e. when residents could not localize the rival. The persistence of singing interruptions after a song contest supports the idea that singing irregularities in response to overlapping playback are not necessarily only the result of avoiding being overlapped, but rather more generally reflect male arousal as a consequence of being challenged or intimidated by a threatening opponent.

In our study, bachelor males interrupted their singing significantly more often after being overlapped than after alternating playback, whereas subsequently mated males kept the number of singing interruptions more constant across playback treatment. This suggests that subsequently mated males did not differentiate between an aggressive and a moderate opponent, whereas bachelors did. Thus, bachelors may be more intimidated by an aggressively singing opponent. However, the small, albeit significant, difference in the absolute number of interruptions raises the question whether these differences reflect biologically relevant differential response strategies of subsequently mated and unmated males, an issue that requires further study.

To increase song rate, a male can either shorten his songs or reduce the pauses between two songs, the latter being the most common pattern in songbird species (Catchpole & Slater 1995), including the nightingale (Naguib 1999; Kunc et al. 2005). Our study revealed that only those males that attracted a social mate later in the season sang at higher rates after playback, regardless of playback treatment. Furthermore, they increased the percentage of songs with trills to the same level as bachelor males after playback, although this was not quite significant. Changes in song output are often discussed in the

Table 1. Changes in singing behaviour elicited by playback treatment (overlapping versus alternating) on subsequently mated and unmated male nightingales as identified by general linear models

	Treatment		Mating success		Interaction	
Dependent variable	$F_{1,38}$	P	$F_{1,38}$	P	$F_{1,38}$	P
Overall effect	2.23*	0.058	2.95*	0.017	3.28*	0.010
Duration of interruptions	6.03	0.019	1.13	0.30	0.04	0.85
Number of interruptions	2.15	0.15	0.25	0.62	4.52	0.040
Percentage of trilled songs	0.07	0.79	3.88	0.056	1.09	0.30
Percentage of whistle songs	1.85	0.18	2.01	0.16	0.19	0.67
Song rate	0.03	0.87	5.53	0.024	0.21	0.65
Pause duration	0.002	0.96	8.93	0.005	0.22	0.64
Song duration	0.11	0.75	0.02	0.90	1.36	0.35

Changes were calculated as differences during 10 min after and 10 min before playback.
*$F_{7,32}$ in the overall analysis.

context of constraints and performance limits acting, for instance, on song rate (Alatalo et al. 1990; Wasserman & Cigliano 1991; Collins 2004) or the production of trills (Podos 1996, 1997; Ballentine et al. 2004; Podos & Nowicki 2005). Our finding that only males that sang at higher rates after playback became paired could be taken to suggest that an increase in singing activity may reflect male condition or quality. On the other hand, the same males, although they sang more trills in response to playback, sang a similar percentage of trills after playback as did bachelor males. Thus, differences between males were reflected more in temporal patterns of song and the change in use of trills in response to playback than in the absolute number of songs containing trills.

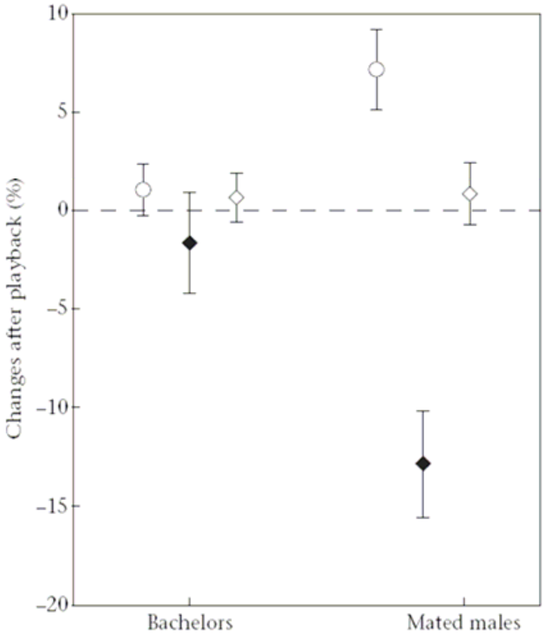

Figure 4. Relative increase (positive values) or decrease (negative vales) of nocturnal singing within 10 min after playback compared to 10 min before playback; mean values ± SE of the song rate (○), the pause duration (◆), and the song duration (◇) are shown separately for bachelors and males that attracted a social mate.

The lack of a differential use of whistle songs is in line with the idea that whistle songs are used as long-range signals (Richards 1981; Todt & Naguib 2000) which are important in female choice (Hultsch 1980; Kunc et al. 2005), rather than being used in close-range vocal contests between males.

Another link between increased performance and aspects of male quality involves the time for which the subjects had already held a territory on the day they received the playback. The value of the territory is likely to increase with time of residency so that males holding a given territory for longer may be more willing to defend it (Davies & Houston 1981; Krebs 1982; Beletsky & Orians 1987; Stamps 1987; Jakobsson 1988; Tobias 1997; Forstmeier 2002). In addition, the time of residency on the day of playback depends on the date on which the males arrived from migration. The differences in response to playback by subsequently mated and bachelor males

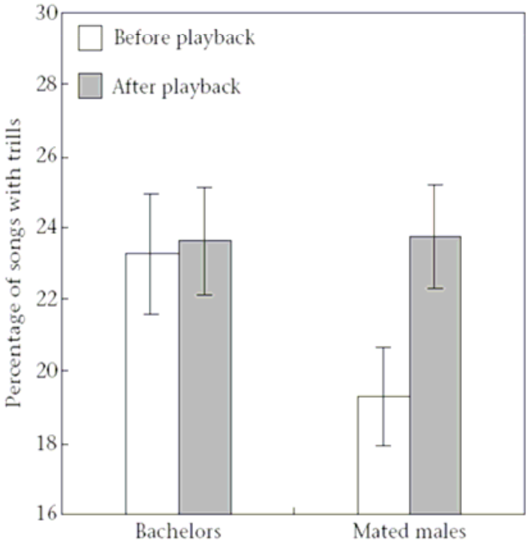

Figure 5. Percentage of songs ($\overline{X} \pm$ SE) containing trills within 10 min before playback and within 10 min after playback with respect to the males' future pairing success.

might have been affected in part by such differences in time of residency. However, an early arrival on the breeding ground may also indicate a male's quality (Forstmeier 2002), and early arriving males in our study population of nightingales are more likely to attract a social mate (Amrhein et al., in press).

Regardless of the mechanisms underlying changes in male song output in response to playback, the singing performance of males after the song contests predicted future pairing success of the singers. These results expand on a previous study by Kunc et al. (2006), which revealed that a male's subsequent pairing success is reflected in the temporal pattern of his song relative to a simulated noninteracting opponent during a vocal interaction. This information on the males' future pairing success may be conveyed not only to the opponent within a dyadic interaction, but also to eavesdropping conspecifics in a communication network. There is accumulating evidence that males assess the level of aggression of rivals from male–male interactions (Naguib et al. 1999, 2004; Peake et al. 2001, 2002; Mennill & Ratcliffe 2004a). Based on our findings, we hypothesize that males also obtain information on their rivals' future mating success from singing interactions. Recently, paired female black-capped chickadees, *Poecile atricapillus*, have been shown to eavesdrop on male singing interactions and to base their choice of extrapair copulations on the performance of their mates in vocal contests (Mennill et al. 2002, 2003). Our results expand on these findings by showing that even at the very beginning of the breeding season, i.e. prior to mating, females could eavesdrop on singing interactions of males and prefer the male that sings more vigorously as a social mate. Thus, direct male–male interactions (Kunc et al. 2006), as well as the singing performance after a song contest, may well be important for female choice during the early stages of the breeding cycle in nightingales. Whether females use information obtained by eavesdropping when choosing a social mate remains to be shown. If they do, they may impose an indirect selection pressure on males to interact vocally with each other, if only those males that perform well in intrasexual conflicts are preferred by females (Wiley & Poston 1996; Naguib 2005).

In conclusion, our study supports previous findings that time-specific patterns in song contests, namely song overlapping versus song alternating, signal socially relevant information, as overlapped males sang more irregularly after contests than did males that were not overlapped. Furthermore, we showed that differences between males as indicated by their subsequent pairing success were reflected in singing responses to challenges posing different levels of threat. Elevated singing after a vocal interaction even at the beginning of the breeding season predicts future mating success, and thus may be used by conspecifics in a communication network to assess the singer's condition or quality.

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Chapter II

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

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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 loudspeaker 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), $7{\cdot}2 \pm 0{\cdot}75$ h (mean ± 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 ± 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

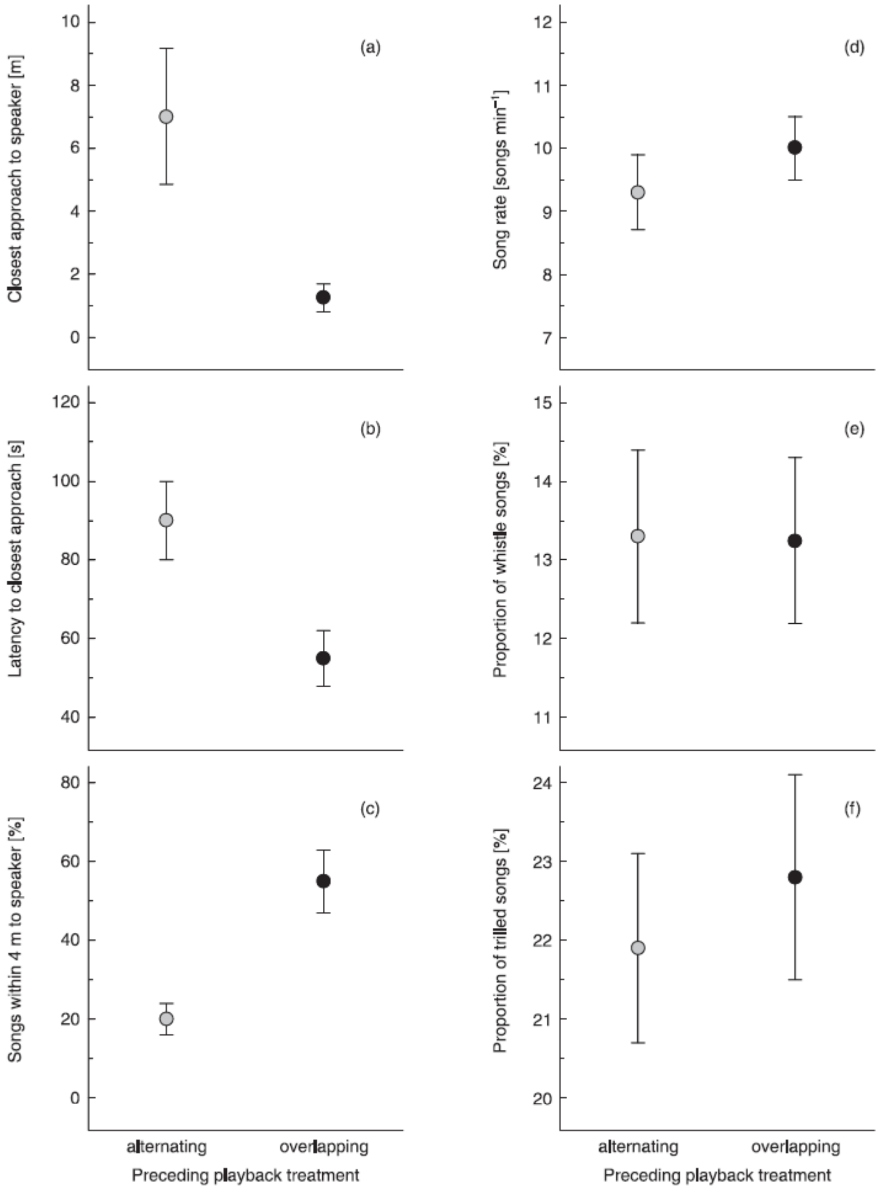

Fig. 1. Mean (± SE) responses of male nightingales to a simulated intrusion during the day in relation to a vocal challenge during the previous night in which subjects received either a song alternating playback (grey circles, $n = 20$) or a song overlapping playback (black circles, $n = 20$): (a) closest 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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Chapter III

Threatening signals in territorial conflicts: responses to trills are related to mating success in nightingales

Submitted

Threatening signals in territorial conflicts: Responses to trills are related to mating success in nightingales

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In songbirds, structural song traits, such as the physically challenging repetitive note production in form of a trill, are well documented to be important in female choice. However, little is known about their signal value in male-male interactions. Here, we investigated whether males assess the competitive abilities of rivals based on variation in trill performance. Using a two-speaker playback experiment, we exposed territorial male nightingales, *Luscinia megarhynchos*, that differed in their subsequent pairing success to a simulated interaction between two rivals varying in the amount of songs that contain rapid broadband trills. Subjects responded more strongly to songs containing trills than to songs without trills. Moreover, they even attended to the fine structure of trills. Males that became paired later in the season (high quality males) increased their response intensity with increasing trill performance whereas males that remained unpaired (low quality males) responded in the opposite direction and decreased response intensity with increasing trill performance. These results demonstrate that rapid broadband trills are interpreted as a signal indicating motivation or aggression and that the nature of response in vocal interactions reflects fundamental aspects of the challenged male's quality.

Animal communication is an essential component of all social behavior as strategies used in communication can determine or reflect an individual's social status, its access to resources and, thereby, its fitness (Searcy and Nowicki, 2005). Specifically elaborate signals and highly complex signaling strategies that often are shaped by sexual selection have intensely been investigated to understand principles in animal communication. Birdsong has been a key model in studies on sexual selection and a variety of song components as well as different singing strategies have been shown to reflect a male's short-term motivation or his fitness, respectively (Searcy and Nowicki, 2005). By focussing on pattern-specific singing strategies (e.g. song type matching vs. switching, McGregor et al., 1992; Burt et al., 2001; Vehrencamp 2001) or time-specific singing strategies (song overlapping vs. alternating, Dabelsteen et al., 1996; Langemann et al., 2000, Mennill and Ratcliffe, 2004b), studies on the function of song in male-male competition have revealed that males assess rivals based on their singing performance in vocal interactions. In contrast, structural song traits such as song complexity or repertoire size (e.g. Catchpole and Leisler, 1996; Hasselquist et al., 1996; Lampe and Epsmark, 1994; Lambrechts and Dhondt, 1986) are well documented to be important in female choice (Gil and Gahr, 2002) Regarding the functional significance of structural song traits in male rivalry, previous experiments have revealed that intruders are less likely to invade territories where males have larger repertoires (Krebs et al., 1978; Yasukawa, 1981) or sing complex songs with large syllable repertoire (Mountjoy and Lemon, 1991). However, surprisingly little is known about effects of structural song components in direct male-male signaling interactions, (Illes et al., 2006; ten Cate et al., 2002).

One conspicuous song structure characteristic for many songbird species is the trill, i.e. a song section consisting of rapidly repeated short notes (Podos and Nowicki, 2005). The production of trills appears to be constrained by a tradeoff between how fast a bird can repeat the trill units and the frequency each unit can span (Podos, 1996; Podos and Nowicki, 2005) because repetitive note production seems to require a precise coordination of vocal tract movement and airflow (Hartley and Suthers, 1990; Hoese et al., 2000; Podos, 1996; Westneat et al., 1993). This tradeoff between trill rate and bandwidth is assumed to result in a performance limit (Podos, 1996), so the production of these physically challenging trills may reflect male quality. Indeed, females have been shown to prefer males that perform trills closer to the production limit (Ballentine et al., 2004; Draganoiu et al., 2002; Vallet and Kreutzer, 1995).

Recently, Illes et al. (2006) found that male banded wrens, *Thryothorus pleurostictus*, which were allowed to choose between a fast and a slow trill stimulus in a two-speaker playback experiment, approached the fast stimulus first, suggesting that banded wren males attend to the fine structure of trills and perceive high performance trills as being more threatening than low performance trills. Interestingly, the males then spent less time at the loudspeaker from which fast trills were presented with increasing performance scores of trills. Illes et al. (2006) concluded that the highest performance trills posed a threat so extreme that they effectively repeled the territory owners.

One important variable determining whether more threatening signals repel territorial animals or incite approach and attack, is the resource holding potential or quality of the challenged male. The response strategy a male adopts during an agonistic interaction will not only depend on the level of threat signaled by the rival but can also be affected by the male’s own condition or quality. Black-capped chickadee, *Poecile atri-*

capilla, males' responses to a simulated song overlapping opponent, for example, differ with their dominance status (Mennill and Ratcliffe, 2004a) and in the nightingale, *Luscinia megarhynchos*, the strategies males use during vocal interactions early in the breeding season predict their subsequent pairing success which can be seen as an indicator of male quality (Kunc et al., 2006; Schmidt et al., 2006).

Previous studies found that male nightingales sing an increased proportion of songs containing rapid broadband trills when they are confronted with a countersinging rival compared to their spontaneous song (Kunc et al., 2006; Schmidt et al., 2006) suggesting that trills are of importance in male-male communication. To address the question whether trills are perceived as aggressive signal, we exposed unmated male nightingales to a simulated vocal interaction between one male singing rapid broadband trills in 50% of all songs and another male singing exclusively songs without such rapid broadband trills. We predicted that subjects would respond preferentially to the loudspeaker from which songs containing trills were presented. Furthermore, we also predicted that responses to trills vary with subjects' quality, measured by their subsequent mating success.

METHODS

Subjects and study site

At the beginning of the breeding season 2005 (between 23 April and 6 May), we conducted two-loudspeaker playback experiments on diurnal song of male territorial nightingales in the vicinity of the Petite Camargue Alsacienne in France, about 10 km north of Basel (Switzerland). In this agriculturally used area of the Upper Rhine Valley, nightingales settle in patches of dense bushes and woods along roads, rivers or fields. As subjects we chose 21 male nightingales holding a territory that was sufficiently isolated by clear territory boundaries to avoid interference with neighbours.

Since nightingales have been shown to cease regular nocturnal song upon attracting a social mate (Amrhein et al., 2002, 2004), we determined nocturnal singing activity of subjects every night over the entire breeding season to assess their mating status as a measure of male quality. All subjects had been singing for at least two nights before they received the playback and thus were considered to be unmated at the time of playback. Later in the season, 10 males ceased nocturnal song indicating that they attracted a social mate (mated males), whereas the other 11 males were considered to remain unmated because they were singing at night throughout the breeding season (bachelors). Subjects were different from those used in another study conducted in the same year (Schmidt et al., 2006).

Playback design and stimuli

We used a two-speaker playback design to simulate a vocal interaction between two males (Fig. 1). On one channel we presented one male singing 10 songs containing rapid broadband trills and 10 songs without trills (trill speaker in Fig. 1) in a randomised order, whereas on the other channel another male singing 20 songs without trills was presented (no-trill speaker). Both such sequences are within the range of naturally singing nightingales (unpubl. data).

Stimulus songs were derived from nocturnal song recordings of 22 color-banded male nightingales made between 2002 and 2005 in the study population. These males were different from the subjects of the present study. Nocturnal song was recorded on a Sony TC-D5M 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 of 20 recordings, two sets of 20 different songs were selected to build both the trill and the no-trill stimulus (Avisoft SASLab Pro 3.5, R. Specht, Berlin, Germany, http://avisoft.com). In each playback trial, songs of two different males were used.

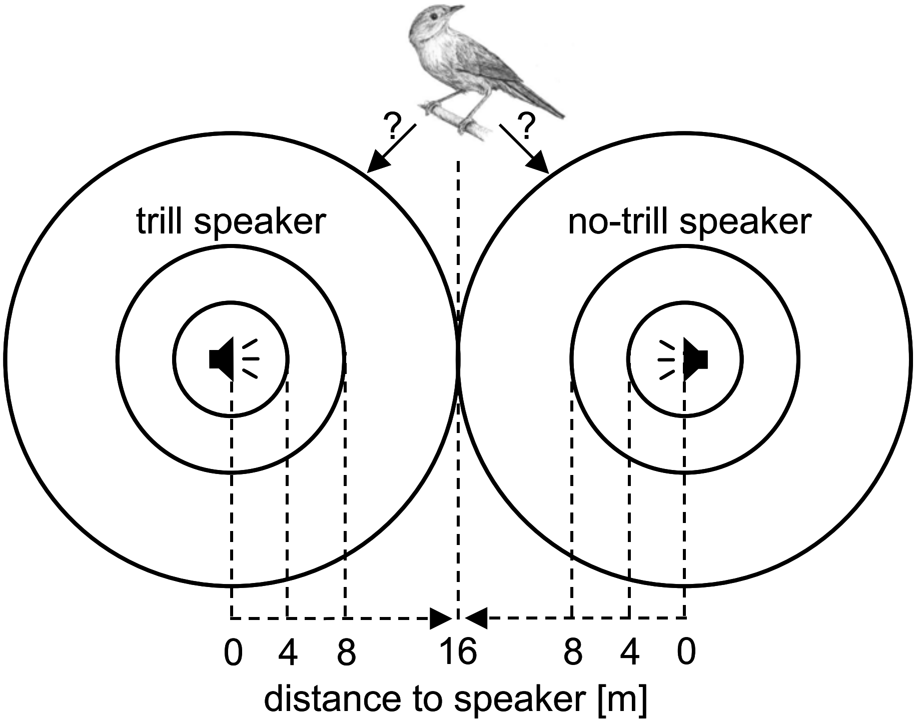

Figure 1

Schematic map of the two-speaker playback setup. Speakers were 32 m apart, circles represent the flagged radii around the speakers, dashed lines show distances to the corresponding speaker.

Across the 21 trill stimulus sets, the trills consisted of 17.1 ± 2.1 elements and trill duration was 1.6 ± 0.2 sec (mean ±SD) as measured from sonagrams and oscillograms using Avisoft (FFT size = 1024, window function = Hamming). These measures resulted in a trill rate of 11.0 ± 0.6 elements/sec (mean ± SD). Power spectra generated from spectograms in Avisoft (FFT size: 512, window function = FlatTop and FFT overlap = 50%, resulting in a frequency resolution of 86 Hz and a temporal resolution of 5.8 ms) were used to identify minimum and maximum frequencies (threshold = -20 dB below frequency at peak amplitude). Trills covered a frequency bandwidth of 6.5 ± 1.8 kHz, ranging from a minimum frequency of 1.8 ± 0.2 kHz to a maximum of 6.8 ± 0.7 kHz (mean ± SD). Trills of different rate and frequency range are illustrated in Fig. 2.

To build the stereo files, we used Cool Edit 2000 to copy the songs of two different males into the separate channels and to normalize the file to the peak amplitude. The order of songs was randomized with the exception of the first song, which was a song without a trill. We arranged alternating renditions of the two stimulus males' songs with a pause of 0.5 sec between consecutive songs so that there was no song overlap between the two channels. The leading stimulus was balanced between trials. Since we used the stimulus male twice (as trill stimulus in one trial and with a different set of songs as no-trill stimulus in another trial), we also balanced the leading stimulus according to individual source males. The whole files were recorded on tapes with a standardized record level.

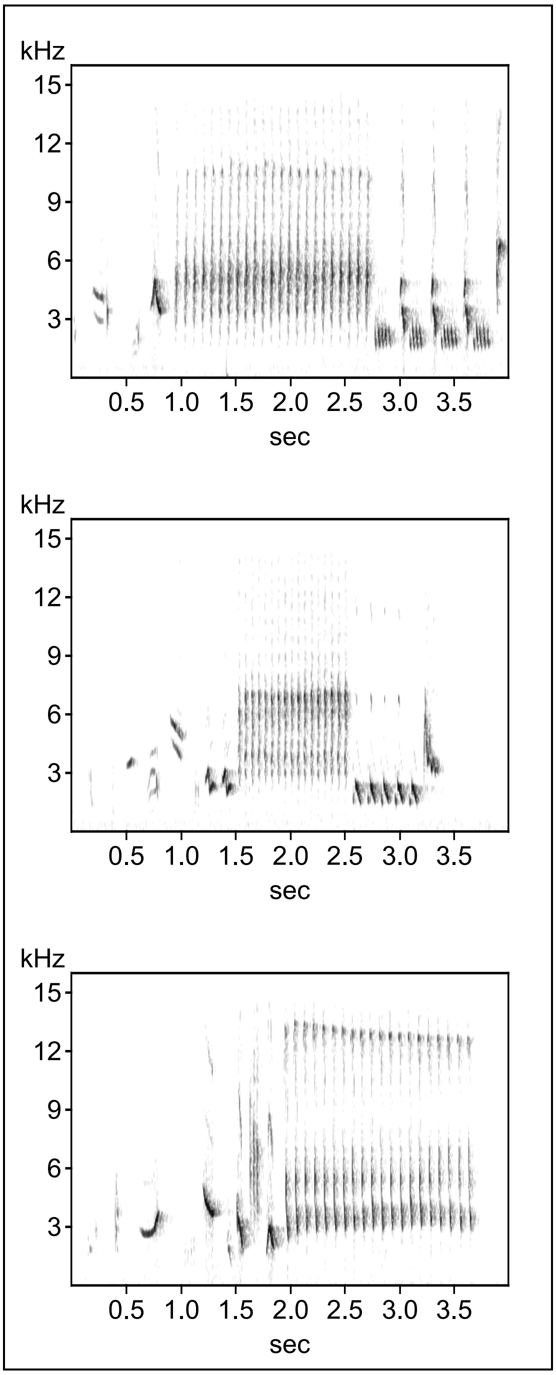

Figure 2

Examples of nightingales' songs containing rapid broadband trills of different repetition rates and frequency ranges.

Playback procedure

All playbacks were conducted during the day, between 0830 and 1100 hours CEST. Diurnal song of nightingales is assumed to serve mainly in territorial defense as the singing activity of both mated and unmated males remain high until late stages of the breeding cycle suggesting that it is less influenced by the reproductive state of females (Amrhein et al., 2004). As Grüll (1981) found that more than 75 % of male nightingales return to the same territory of the preceding year or to a directly neighbouring one, we ensured that our subjects were not familiar with the playback songs by choosing for each subject a stimulus tape built of recordings that were made in non-neighbouring territories at least several hundred metres away. We only used males that were singing already when the playback started.

Playback setup consisted of two loudspeakers (Canton Plus X passive loudspeakers, Canton Elektronik GmbH, Germany) which were placed 32 m apart (Fig. 1), well within the subject's territory. We flagged radii of four, eight, and 16 m around each experimental loudspeaker to measure subjects' approaches. Sound pressure level was calibrated to natural nightingale song amplitudes of 90 dB at 1 m distance (Brumm, 2004), measured with a Brüel & Kjær precision SPL meter 2233 (C-weighting, fast response). Songs were played from a Sony WMD 6M tape recorder, which was connected to a Blaupunkt MPA 2 amplifier (Blaupunkt GmbH, Germany).

Subjects' singing responses and the distance to the loudspeakers 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 to measure the on- and offset of playback for analysis. Subjects' response behavior was recorded on tape during playback and for an additional period of 10 min after the playback had terminated.

Response measures and statistical analysis

We extracted the following behavioral parameters: (i) the closest approach to each loudspeaker [m], (ii) the latency to this closest approach [sec], (iii) the latency to approach each loudspeaker up to at least 4 m [sec]. As singing responses, we calculated (iv) the number of songs sung within the 4 m radius around each loudspeaker, (v) the number of songs sung on each side, i.e. within 16m around each loudspeaker.

We conducted a principal component analysis on these five parameters (SPSS 12.0.1) to summarize male responses to one composite response variable. We then applied a repeated measures ANOVA on the principal component (PC) scores with two levels of the within-subject factor (trill or no-trill speaker) and two between-subject factors: the speaker initiating the simulated interaction and the subject's subsequent mating status (mated male or bachelor). The initial model also included the distance to the speaker from which songs with trills were presented at onset of playback as a covariate. Since the initial distance did not significantly affect the responses ($p = 0.95$), this covariate was removed in the final model. At onset of playback, subjects were singing at mean ± SE distances of 23 ± 4m to the loudspeaker from which trills were broadcast and 22 ± 2m to the other loudspeaker.

To investigate how the vocal performance of stimuli trills affected the subjects' responses, we conducted an ANCOVA on the response intensity (PC score) at the loudspeaker from which rapid broadband trills were presented with the subjects' subsequent pairing success as fixed factor, and two covariates, the rate and bandwidth of stimuli trills. The stimuli trill rate was excluded in the final model as this variable did not significantly affect the responses of subsequently mated and unmated males ($p = 0.5$).

RESULTS

Four males made their first approach before the first song with trill had been played and were, therefore, excluded from the analysis of first approach. Of the remaining 17 birds that heard songs from both loudspeakers and at least one rapid broadband trill before approaching, 14 made their first approach to the loudspeaker from which songs with trills were presented and three subjects first approached the loudspeaker that presented only songs without trills (Binomial test, $p = 0.049$).

Principal component analysis that was applied to the responses of all 21 subjects generated one PC with an eigenvalue >1.0 that explained 62.6% of the variation in subjects' response performance during playback trials (Kaiser Meyer Olkin measure of sampling adequacy: 0.638; Bartlett test of sphericity: $\chi^2 = 113.8$, $p < 0.001$). Measures of approach behavior loaded negatively on this first PC (closest approach: -0.85; latency to closest approach: -0.43; latency to approach up to at least 4 m: 0.90), whereas loadings of singing responses were positive (number of songs within 16 m: 0.85; number of songs within 4 m: 0.82). A pairwise comparison with the scores of this PC as a composite measure of responses indicated significantly stronger responses towards the loudspeaker from which songs with trills were broadcast (rm ANOVA: $F_{1,18} = 6.6$, $p = 0.019$, Fig. 3). Table 1 shows how this stronger response to songs with trills was reflected in the particular response variables: Subjects approached the speaker broadcasting songs with rapid broadband trills more quickly and closer than the speaker from which only songs without trills were presented. Furthermore, subjects sang more songs on the side with the loudspeaker that broadcast trills (i.e. within 16m to the trill speaker) and also close to the trill speaker (i.e. within 4m) than on the side from which exclusively songs without trills were played. Subjects' responses were not notably affected by the speaker that initiated the simulated interaction ($F_{1,18} = 0.006$, $p = 0.95$). Furthermore, subsequently mated and unmated males did not show significantly different responses during the playback trials ($F_{1,18} = 0.016$, $p = 0.90$).

Frequency bandwidth and the rate of repetitive elements of broadcast trills were positively correlated, although not significantly so, i.e. faster repeated trill elements tended to cover a broader frequency range than did trills of a slower rate ($r = 0.42$, $n = 21$, $p = 0.057$, Fig. 4). However, the stimuli trill rate was excluded in the final ANCOVA model as this variable did not significantly affect the responses of subsequently mated and unmated males ($F_{2,15} = 0.7$, $p = 0.5$).

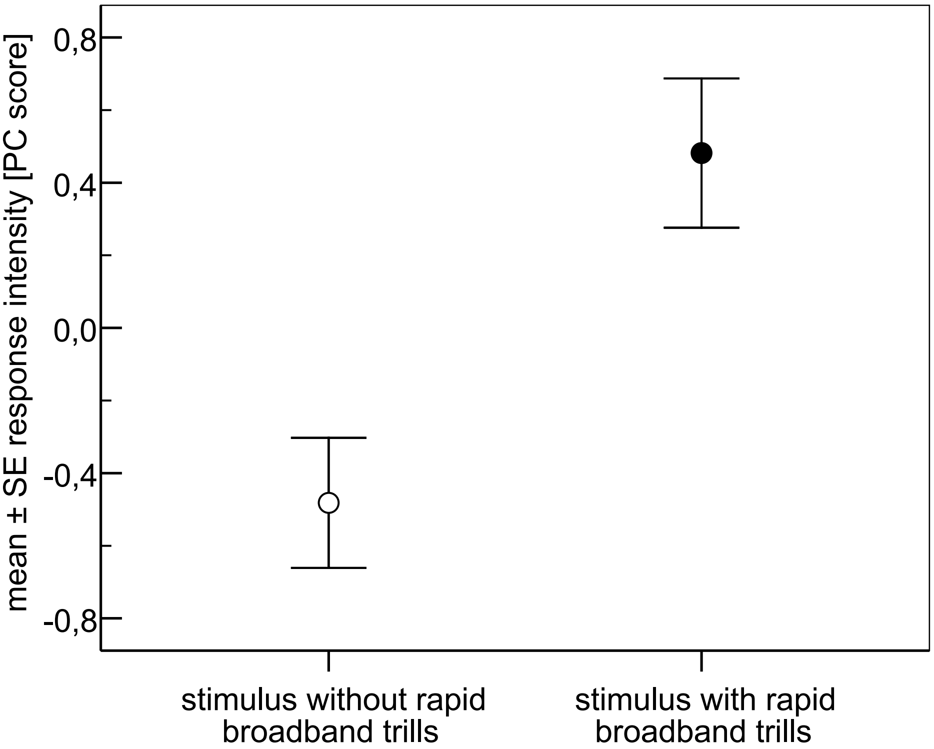

Figure 3

Mean ± SE response intensity (principal component scores as a composite measure of 5 variables) of 21 nightingales at the two experimental loudspeakers from which either exclusively songs without rapid broadband trills were presented (open circle) or songs that contained such trills (black circle).

Table 1

Mean ± SE responses of 21 nightingales during a two-loudspeaker playback experiment, separately shown for the two loudspeakers that differed in the proportion of rapid broadband trills of broadcast songs.

Response variable	Response at trill speaker	Response at no-trill speaker
Latency to closest approach	115 ± 28 sec	272 ± 45 sec
Latency to approach ≤ 4 m	283 ± 74 sec	618 ± 55 sec
Closest approach	4 ± 1 m	12 ± 2 m
Number of songs within 16 m	64 ± 12	35 ± 10
Number of songs within 4 m	30 ± 10	11 ± 5

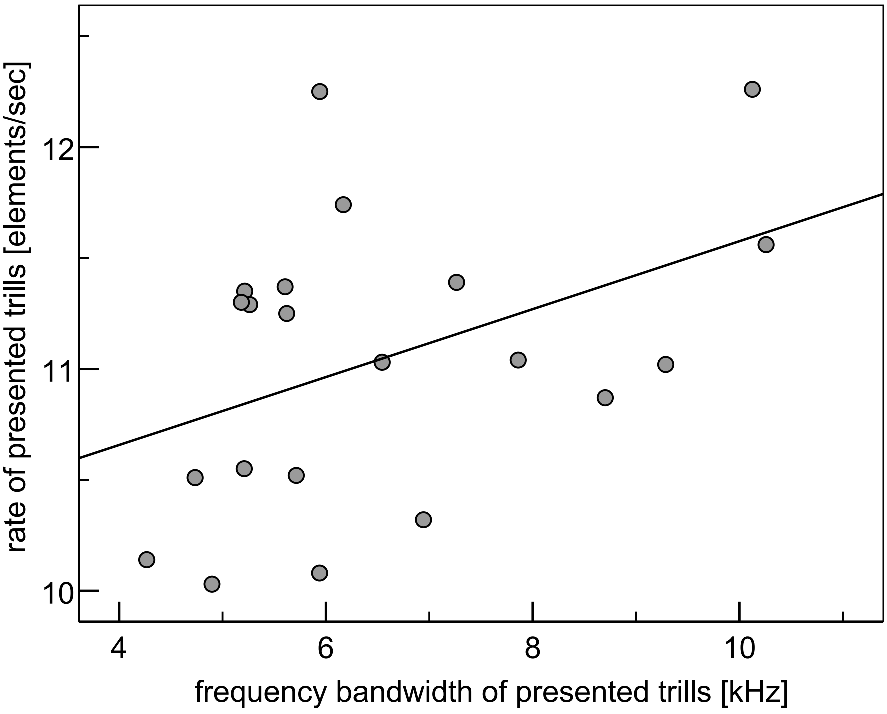

Figure 4

Relationship between mean frequency bandwidth and mean element repetition rate in 21 sets of 10 rapid broadband trills that were used for playback (r = 0.42, p = 0.057). The sets derived from recordings of nocturnal song of 21 different nightingales (see Methods for further detail).

Controlled for the stimuli trill bandwidth, the response intensity at the loudspeaker from which trills were presented was significantly different in subsequently mated and unmated males (ANCOVA, pairing success: $F_{1,17} = 9.1$, $p = 0.008$). Subjects showed different strategies in response to the variation in stimuli trill bandwidth according to their future mating success (ANCOVA, interaction of stimuli trills' bandwidth and pairing success: $F_{1,17} = 10.7$, $p = 0.005$). Males that remained unmated throughout the breeding season decreased their response intensity significantly with increasing frequency bandwidth of presented trills ($r = -0.67$, $n = 11$, $p = 0.024$, Fig. 5), whereas subsequently mated males tended to respond in the opposite direction, i.e. tended to increase their response intensity with increasing frequency bandwidth of presented trills ($r = 0.56$, $n = 10$, $p = 0.094$, Fig. 5).

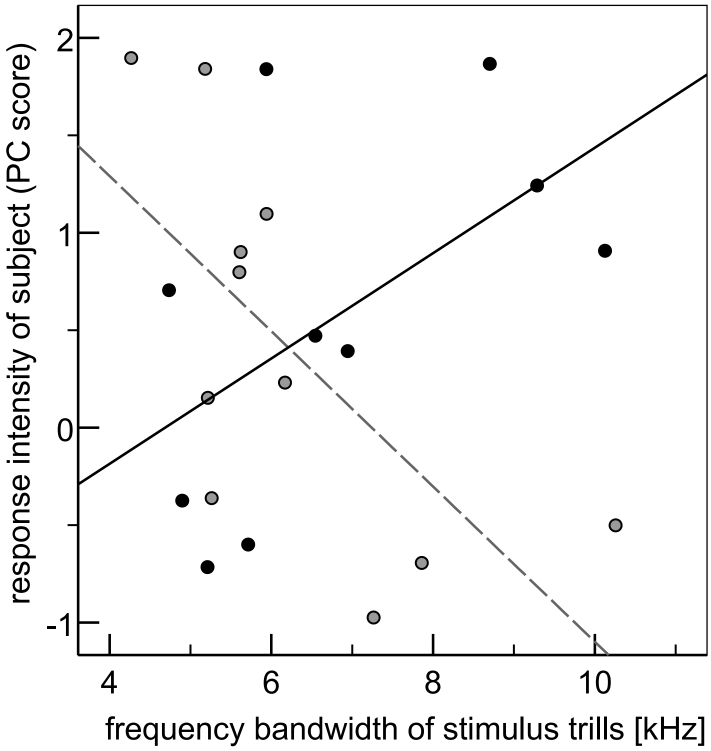

Figure 5

Response intensity (principal component scores) of subsequently mated (black circles and solid line) and unmated (grey circles and dashed line) male nightingales in relation to the frequency range of presented trills.

DISCUSSION

Our results demonstrate that male nightingales discriminated between rivals that differed in the percentage of songs containing rapid broadband trills and responded stronger to those rivals that sang a high proportion of such songs than to simulated opponents that sang only songs without trills. Generally, strong responses towards a simulated singing intruder, such as a quick approach (e.g. Mennill and Ratcliffe, 2004a), or a high song output (e.g. Hyman, 2003, Peake et al., 2002) are assumed to reflect male arousal and thereby also indicate the level of aggression of the intruder (reviewed in Naguib, 2005). Therefore, strong responses towards those simulated rivals that sang a high proportion of trills in our study provide evidence that songs with rapid broadband trills are interpreted as aggressive signals. This suggests that a territorial male's immediate response to being challenged is based on a general assessment of the rival. The presence or absence of conspicuous song traits such as specific song elements (this study), incomplete songs (Leitao and Riebel, 2003) or frequency jumps (ten Cate et al., 2002) can easily be detected and thus can be used as a first approximation of the level of threat.

Subsequently mated males showed different territorial defense strategies at the speaker that presented trills than bachelors. In particular, the response intensity in relation to the fine structure of presented trills can be seen as a predictor of the males' future mating success. Males that remained unpaired throughout the breeding season decreased their territorial defense behavior with increasing trill performance of the simulated opponent. In contrast, males that became paired later in the season increased the vigor with which they engaged in territorial defense with increasing trill performance of the simulated intruder. The response to inter-individual variation in structural characteristics of song has rarely been investigated in male birds (Illes et al., 2006; Leitao and Riebel, 2003; ten Cate et al., 2002), and different response strategies by males to such structural variants in relation to the males' quality have not been described previously. The body condition or the resource holding potential of a male appears to be a crucial factor determining a male's specific response strategy. Strong responses by high quality males only in response to high performance trills suggest that they risk to escalate a contest only when the opponent signals a high level of threat. Low quality males, in contrast, showed a high investment in territorial defense only in response to trills of lower performance. This may be an adaptive response strategy as it will not pay to signal the willingness to escalate a contest in response to an opponent of presumably higher resource holding potential or quality.

So far, the signal function of song performance has been investigated predominantly in an intersexual context as, for example, canary, *Serinus canaria*, (Draganoiu et al., 2002) and swamp sparrow, *Melospiza georgiana*, females have been shown to prefer high-performance trill songs (Ballentine et al., 2004). Song in these two species consists mainly of trilled note types or syllables that differ in the number of elements and repetition rate (Ballentine et al., 2004; Vallet and Kreutzer, 1995), so these song features may act as powerful drivers in sexual selection. In contrast, only about 10 to 20% of a nightingale's songs contain rapid broadband trills and the percentage of such songs is upregulated during agonistic interactions (Kunc et al., 2006; Schmidt et al., 2006). Therefore, physically challenging broadband trills can be seen as a signal indicating the motivation to engage in a close-range interaction. Additionally, using trills to signal aggression or motivation during a close-range interaction limits the probability of a long range transfer of this information to conspecifics not directly involved in that interaction due to effects of reverberation that increase with trill duration and repetition rate (Naguib, 2003; Wiley and Richards, 1982). Generally, males of various species have been shown to extract information on relative differences in motivation or social status between rivals by eavesdropping on their interaction (reviewed in Peake, 2005) and nightingales are attracted by interactions in which their neighbours are involved and intrude into their neighbour's territory the earlier the more intensely their neighbour had interacted with a stranger (Naguib et al., 2004).

In contrast to previous studies on constraints in trill production (Ballentine et al., 2004; Podos, 1996; Podos, 1997), we found a tendency that across stimuli frequency range and rate of trilled songs were positively correlated. This finding could be explained by our focus on those trills that were already at the upper boundary of the performance range. Podos (1997), however, analyzed a much wider range of repeated song elements including also slow and narrow trills in song recordings of 34 different Emberizidae species.

In conclusion, our study shows that nightingales discriminated between rivals based on the presence or absence of structural song components. An increase in the use of trills during male-male interactions as well as the signal value of high performance trills for males described here indicates that rapidly repeated frequency modulated song components are perceived and used as a signal of aggression in intrasexual contexts. Most interestingly, we showed that responses to being challenged prior to pairing differed between males according to their subsequent pairing success, which can be seen as an indicator of male quality. Our study provides new insights into the adaptive function of signaling strategies used in competition over resources by highlighting components of male quality as a source of variation in territorial defense behavior.

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Chapter IV

Communication networks in nightingales: how to assess a persisting rival that had challenged a neighbour?

Manuscript

Communication networks in nightingales: how to assess a persisting rival that had challenged a neighbour?

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In the social environment of territorial animals, information that is exchanged in a dyadic interaction may also be conveyed to conspecifics that are not directly involved in that interaction. While much attention has been paid to the signal value of specific signalling strategies in such communication networks, only little is known about the effects of possible individual response strategies of challenged individuals. Recent evidence in nightingales, *Luscinia megarhynchos*, suggests that a male's mating success, which can be seen as an indicator of male quality, correlates with the vigour of his territorial defence behaviour towards threatening opponents. Here, we investigated whether territorial male nightingales take information on a neighbour's future mating success that may be inherent in the neighbour's territorial defence strategy into account when eavesdropping on a neighbour's vocal interaction with an intruder. In a first step, we conducted a playback in the subjects' neighbouring territory simulating either an aggressively singing rival (by song overlapping) or a moderately singing rival (by song alternating). In a second step, we simulated the same rival to intrude into the subjects' own territory. Our results suggest that subjects adjusted their territorial defence behaviour to the level of aggressiveness the rival had interacted with before, i.e. residents eavesdropped on their neighbour's interaction. Furthermore, subjects' responses were influenced by the mating success of the challenged neighbour. Thus, in territorial species, resident males using their neighbour as 'early warning system' may additionally include the mating success of their neighbours in decisions on how to respond to rivals.

Communication is among the most conspicuous social behaviours performed by an animal and social relations between animals often are mediated by elaborate signals. In a social environment, communication occurs in a network of several signallers and receivers so that information that is exchanged between two individuals in a dyadic interaction may also be conveyed to and used by a third party (McGregor & Dabelsteen 1996). Owing to the long range of acoustic signals, the singing behaviour of territorial bird species has frequently been used as a model to investigate mechanisms underlying the information exchange in a communication network (McGregor & Dabelsteen 1996; McGregor 2005; Peake 2005). Neighbouring male songbirds often interact through countersinging to assess the competitive abilities of each other, and during such dyadic interactions males often respond to the counterpart's signals in a very specific way showing their motivation, social status or their competitive abilities (McGregor & Peake 2000; Todt & Naguib 2000; Naguib 2005; Searcy & Nowicki 2005). For example, males may directly refer to the rival by replying with the same song type (song type matching, Krebs et al. 1981) or increase the rate at which song types are switched They may also use a greater proportion of their repertoire or attempt to sing more songs relative to their opponent (Todt & Naguib 2000; Naguib 2005). Recent studies have highlighted the importance of the timing of songs relative to the opponent. These studies demonstrate that song overlapping in close-range interactions among males indicates the willingness to escalate a contest eliciting strong responses by the overlapped male, whereas song alternating is used in a less aggressive context (McGregor et al. 1992; Dabelsteen et al. 1997; Naguib 1999; Langemann et al. 2000; Mennill & Ratcliffe 2004b).

This relative information on strength or weakness of potential rivals provided by the timing of songs during interactions is also extracted and subsequently used by eavesdropping males, as revealed by two-speaker playback experiments simulating a communication network (Naguib & Todt 1997; Naguib et al. 1999; Peake et al. 2001, 2002; Mennill & Ratcliffe 2004a; Naguib et al. 2004). Furthermore, females seem to alter their assessment of males based on the outcome of song contests. This has been shown nicely in great tits, *Parus major*, where females were found to be more willing to make excursions into neighbouring territories when the neighbour was simulated to 'win' a contest against the female's mate (by song overlapping), and also in black-capped chickadees, *Poecile atricapilla*, where females paired to high-ranking males engaged more in extra-pair copulations after their mate had 'lost' a song contest against a simulated song overlapping opponent (Mennill et al. 2002; Mennill et al. 2003).

These studies provided new insights into principles of information exchange in communication networks by focussing on the signal value of different pattern- or time-specific singing strategies a male may use in challenging an opponent. Much less attention has been paid to possible individual differences that may influence the response strategy of a male that is challenged by a rival. However, the response strategy a male adopts when territorial integrity is threatened is likely to be affected by the male's own resource holding potential, body condition, or quality. Black-capped chickadee males' responses to a simulated song overlapping opponent, for example, differ with their dominance status (Mennill & Ratcliffe 2004a) and in the nightingale, *Luscinia megarhynchos*, a male's mating success correlates with the vigour of his territorial defence behaviour towards threatening opponents (Kunc et al. 2006; Schmidt et al. 2006) Thus, dyadic vocal interactions may also be an important source of information on fundamental aspects of a resident's male quality that may be assessed through his territorial defence strategy. These findings raise the question whether this information on a male's social status or subsequent mating success is used by eavesdropping conspecifics in a similar way as information on short-term motivation and aggressiveness provided by time- or pattern-specific signalling strategies.

To address this question, we investigated whether territorial male nightingales adjust their responses towards an unfamiliar rival simulated by playback to prior experience that was to be obtained by eavesdropping on a vocal interaction of that rival with a familiar neighbour. We predicted that subjects responded differently according to the level of aggression the simulated rival had interacted with before, i.e. whether or not the simulated rival had overlapped the neighbour's songs. Assuming that a persisting rival that already had challenged a high quality neighbour is perceived as more threatening than is a rival that had challenged a neighbour of low quality, we also predicted that subjects adjusted their response towards the unfamiliar rival in relation to the neighbour's quality, measured by his mating success.

METHODS

Subjects and study site

We conducted playbacks on 36 neighbouring pairs of territorial male nightingales in the vicinity of the Petite Camargue Alsacienne (France) in an area of about 16 km^2 along the Upper Rhine Valley 10 km north of Basel, Switzerland (Amrhein & Zwygart 2004). In this agriculturally used area, 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 pairs of neighbouring territories we chose for playback were sufficiently isolated to avoid interference with responses from other territorial males. Therefore, subjects were individually recognizable by territory location.

Playbacks were conducted at the beginning of the breeding season in 2006 (between 18 April and 2 May). In a first interactive playback, we simulated an intrusion by an unfamiliar rival that sang either aggressively or moderately. Therefore, males used in the first playback received either an overlapping playback (n = 18, in which 71 ± 10% (mean ± SD) of subject's songs were overlapped), or a song alternating playback (n = 18, 4 ± 3% of subject's songs were overlapped). Thereafter, we simulated the same rival intruding into a neighbouring territory by conducting a non-interactive playback on males in adjacent territories with the same stimulus songs. For clarity reasons, we hereafter refer to males subjected to the second playback as 'subjects' because we were mainly interested in their responses and to males of the first playback as their 'neighbours'.

Sample size of the second non-interactive playback was 33 (three trials had to be excluded from analysis because the subject could not be detected), 16 playbacks were conducted 25.9 ± 3.4 (mean ± SD) min after a song overlapping playback, the other 17 were conducted 26.0 ± 3.0 min after a song alternating playback. Assuming that males eavesdrop on their neighbours' interactions, this design allowed us to investigate territorial response strategies of males exposed to an intrusion (the subjects in the second playback) with respect to the level of aggressiveness the intruder has interacted with before (with neighbours during the first playback) and also with respect to the quality of the neighbour, measured by his mating success.

As nightingales usually sing regularly at night only until a female has settled in their territory (Amrhein et al. 2002; Amrhein et al. 2004b), we monitored nocturnal singing activity of subjects and their neighbours each night over the breeding season from 3 April until 28 May to assess their mating status. Among the neighbours, six males were considered to be mated at the day of playback because they already had ceased nocturnal song, 18 males ceased nocturnal song later in the season, indicating that they became paired, and 12 males sang at night throughout the breeding season indicating that they remained unpaired (bachelors). Based on whether or not males became paired, we distinguished between males of high and low quality (n = 24 and n = 12, respectively) for analysis.

Playback design and stimuli

The stimuli songs were derived from nocturnal recordings of 36 different colour-banded nightingales made between 2002 and 2005 in the study population. Nocturnal song was recorded on Sony a TCD 5M tape recorder with a Sennheiser ME66/K6 microphone and digitised using Cool Edit 2000 (Syntrillium Software, Scottsdale, AZ, U.S.A, sample frequency: 44.1 kHz, resolution: 16 bits). From each song recording, we selected 22 different songs (Avisoft SASLab Pro 3.5, R. Specht, Berlin, Germany). We generated a new set of stimulus songs for each playback and used the songs of each source male only once. According to the naturally occurring proportions of different song categories in nocturnal song, each set of 22 songs consisted of three whistle songs, i.e. songs starting with a series of mostly unmodulated whistles

(Hultsch & Todt 1996; Kunc et al. 2005), and 19 nonwhistle songs. Three of these nonwhistle songs contained rapid broadband trills, a song trait that is used during agonistic interactions with rival males (Kunc et al. 2006). The 22 songs were randomly merged into one wave-file in Cool Edit and normalized to the peak amplitude. To enable the experimenter to play each song on demand during the first interactive playback on neighbours, we arranged the 22 single songs in one file of SyrinxPC 2.3s (J. Burt, http://www.syrinxpc.com), on a Toshiba Satellite notebook (S2210CDT). For the second non-interactive playback on subjects, we arranged the 22 songs in the same order in another file of SyrinxPC with silent intervals of 3 s between two consecutive songs, resulting in playback files of about 2 min duration.

Playback protocol

All playbacks were conducted between 0700 and 1100 hours CEST. We only used males that were singing before playback started. In all playbacks, the loudspeaker was placed inside the territory, within 10m to the male's previous nocturnal song post, at a height of about 2m. By positioning markers at distances of 4, 8 and 16m in the vicinity of the loudspeaker, the males' approach to the loudspeaker could be measured.

The notebook used for playback 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 song was adjusted prior to playback to 90 dB at 1 m distance, measured with a Brüel & Kjær precision SPL meter 2233 (C-weighting, fast response). This sound pressure level is within the range of naturally singing nightingales (Brumm 2004). Singing of a focal male and the distance to the speaker after each male's song were recorded on the left channel of a Sony TCD 5M stereo tape recorder with a Sennheiser ME66/K6 directional microphone. On the right channel we recorded the songs broadcast by the loudspeaker. This allowed us to assess the on- and offset of playback for analysis. The focal males' 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

During both playbacks, males occasionally interrupted their regular singing, leading to between-song intervals that differed markedly from the mean intersong intervals ± SD of 2.6 ± 0.6 sec in the first, and 2.9 ± 1.0 sec in the second playback. As the occurrence of such long silent intervals may confound the analysis of regular intersong intervals, we analysed these interruptions separately. By defining a singing interruption as a silent interval exceeding 10 sec, we followed previous studies which showed such interruptions to be characteristic responses to overlapping playback (Naguib 1999; Mennill & Ratcliffe 2004b; Schmidt et al. 2006).

We measured (a) the closest approach to the loudspeaker [m], (b) the latency to the closest approach [sec], (c) the latency to reach the 4 m radius around the loudspeaker [sec], (d) the percentage of songs sung within the 4 m radius around the loudspeaker, (e) the percentage of songs sung within the 16 m radius around the loudspeaker, (f) the number of songs, and (g) the percentage of songs containing rapid broadband trills. Moreover, we calculated (h) the total duration of singing interruptions [sec] and for the effective signalling time, i.e. after excluding the duration of interruptions, (i) the song rate [number of songs/min].

Responses by focal males were analysed identically in both playbacks by applying multivariate general linear models (SPSS 14.0, SPSS Inc., Chicago, IL, U.S.A) with 7 parameters (b-h) as dependent variables and two fixed factors: the playback treatment in the neighbour's territory (alternating or overlapping) and the quality of the neighbour, measured by his mating success, i.e. whether or not he was paired later in the season. We did not include the mating success of subjects in the analysis because the sample size was too low to include a third fixed factor. The influence of playback treatment on each song parameter and the relation between mating success of the neighbour and song parameters were then assessed with univariate general linear models. In both analyses, data on latencies and singing interruptions were log transformed and data on the percentage of songs given within the outer and inner radii around the loudspeaker were arcsine square root transformed to meet requirements for parametric statistics. As a normal distribution of residuals of the closest approach to the loudspeaker (a) was not obtained even after transformation, these data were analysed using U-tests. Results are given as mean ± SE; all reported tests are two-tailed.

RESULTS

From both multivariate general linear models, we removed the non-significant interaction terms of treatment and pairing success (playback on neighbours: $P = 0.47$, playback on subjects: $P = 0.99$).

Responses of 'neighbours'

The final model revealed that neighbours' responses were strongly influenced by playback treatment (MANOVA, $F_{8,26} = 6.21$, $P < 0.001$, Table 1a), and responses also differed between males according to their mating success, although on the threshold of significance level ($F_{8,26} = 2.32$, $P = 0.050$, Table 1b).

Table 1. Responses of 36 male nightingales (‘neighbours’ in the first playback) subjected to a simulated intrusion by an unfamiliar rival that either sang aggressively (by song overlapping) or moderately (by song alternating) in relation to (a) the playback treatment and (b) the males’ pairing success

(a)	mean ± SE responses related to playback treatment			
dependent variable	alternating	overlapping	$F_{1,33}$	P
overall effect			6.21[1]	< 0.001
latency to closest approach [sec]	152.4 ± 26.7	97.1 ± 36.7	7.06	0.012
latency to approach ≤ 4m [sec]	77.8 ± 15.2	44.1 ± 7.4	1.08	0.31
percentage of songs within 4m	73.1 ± 7.4	67.8 ± 7.7	0.34	0.57
percentage of songs within 16m	87.1 ± 5.0	99.3 ± 0.3	7.17	0.011
number of songs	113.4 ± 8.1	138.3 ± 7.0	8.63	0.006
percentage of songs with trills	18.7 ± 1.5	21.6 ± 1.3	2.40	0.13
duration of singing interruptions [sec]	200.1 ± 33.2	71.0 ± 27.9	24.04	< 0.001
song rate [songs/min]	11.7 ± 0.3	12.7 ± 0.3	6.44	0.016

[1] $F_{8,26}$ in the overall analysis

(b)	mean ± SE responses related to mating success			
dependent variable	unmated	mated	$F_{1,33}$	P
overall effect			2.32[1]	0.050
latency to closest approach [sec]	188.8 ± 61.4	92.7 ± 12.6	2.47	0.13
latency to approach ≤ 4m [sec]	75.6 ± 14.5	53.7 ± 10.9	1.93	0.17
percentage of songs within 4m	71.0 ± 11.0	70.1 ± 5.9	0.01	0.92
percentage of songs within 16m	90.0 ± 6.0	94.8 ± 2.8	0.33	0.57
number of songs	144.4 ± 8.2	116.6 ± 6.8	9.36	0.004
percentage of songs with trills	21.1 ±1.5	19.7 ± 1.3	0.72	0.40
duration of singing interruptions [sec]	74.3 ± 23.4	166.2 ± 32.6	4.53	0.041
song rate [songs/min]	12.6 ± 0.5	11.9 ± 0.2	3.39	0.075

[1] $F_{8,26}$ in the overall analysis

Although the closest approach to the loudspeaker did not differ significantly between the treatment groups (U-test, U = 133, P = 0.25), neighbours that received a song overlapping playback reached this closest approach sooner than males that received a song alternating playback (Table 1a). Males stayed significantly longer in the vicinity of the simulated intruder, i.e. within the 16m radius around the loudspeaker, when they were exposed to an overlapping playback but males of both treatment groups sang a similar percentage of songs close to the simulated intruder, i.e. within 4m to the loudspeaker. Neighbours gave about 20% more songs in response to being overlapped and interrupted their singing only about one third as long as did neighbours that received a song alternating playback, leading to a song rate that was higher in overlapped neighbours than in neighbours of the song alternating treatment group.

The closest approach to the loudspeaker did not differ significantly between males according to their mating success (U-test, U = 156.5, P = 0.65). Irrespective of playback treatment, males that succeeded in attracting a social mate responded to the simulated intruder less vigorously with respect to how many songs they sang and how long they interrupted their singing in comparison to bachelor males (Table 1b). Accordingly, mated males tended to sing at lower rates than did bachelors.

Responses of 'subjects'

Overall, the final model suggest that subjects' responses were strongly influenced by the preceding playback treatment the neighbour had received (MANOVA, $F_{8,23}$ = 3.52, P = 0.008, Table 2a), and responses also differed between subjects according to their mating success ($F_{8,23}$ = 2.67, P = 0.031, Table 2b).

Although subjects whose neighbours had received a song overlapping treatment entered the inner circle around the simulated intruder much sooner than did subjects whose neighbours had received a song alternating playback, we did not find a significant influence of the neighbour's treatment neither in this nor in any of the other seven response variables (Table 2b). Furthermore, playback treatment also did not significantly affect the closest approach of subjects to the simulated intruder (U-test, U = 134, P = 0.94).

With respect to the quality of the neighbour, subjects differed in the percentage of songs containing rapid broadband trills, although not quite significantly so. Subjects whose neighbours became paired sang about 35% more songs with trills than subjects whose neighbours remained unpaired (Table 2b). Neighbour's mating success did not significantly affect any of the other seven responses measures of subjects and also subjects' closest approach to the speaker was not significantly related to the neighbours' mating success (U-test, U = 90, P = 0.47).

Table 2. Responses of 33 male nightingales (‘subjects’ in the second playback) exposed to a simulated intrusion by an unfamiliar rival in relation to (a) in relation to the treatment of the preceding first playback on their neighbour, and (b) in relation the pairing success of the neighbour that was challenged by the intruder.

(a)	mean ± SE responses related to neighbour’s treatment			
dependent variable	alternating	overlapping	$F_{1,30}$	*P*
overall effect			3.52[1]	0.008
latency to closest approach [sec]	93.8 ± 18.0	120.4 ± 35.2	0.008	0.93
latency to approach ≤ 4m [sec]	218.8 ± 70.4	135.4 ± 57.9	1.76	0.20
percentage of songs within 4m	54.7 ± 9.4	57.4 ± 8.9	0.04	0.84
percentage of songs within 16m	88.5 ± 6.2	88.6 ± 5.0	0.03	0.89
number of songs	108.2 ± 10.1	93.0 ± 9.2	1.32	0.26
percentage of songs with trills	18.8 ± 1.7	22.5 ± 1.8	1.53	0.23
duration of singing interruptions [sec]	208.4 ± 46.1	251.4 ± 46.9	0.01	0.92
song rate [songs/min]	1 2.5 ± 0.40	12.0 ± 0.5	0.31	0.58

[1] $F_{8,23}$ in the overall analysis

(b)	mean ± SE responses related to neighbour’s mat. success			
dependent variable	unmated	mated	$F_{1,30}$	*P*
overall effect			2.67[1]	0.031
latency to closest approach [sec]	70.8 ± 16.2	120.1 ± 25.5	1.00	0.33
latency to approach ≤ 4m [sec]	136.4 ± 74.4	193.9 ±57.0	0.08	0.77
percentage of songs within 4m	50.9 ± 11.6	57.9 ± 7.8	0.12	0.73
percentage of songs within 16m	88.8 ± 7.5	88.4 ± 4.7	0.03	0.85
number of songs	98.6 ± 14.6	101.7 ± 7.9	0.17	0.67
percentage of songs with trills	16.4 ± 1.8	22.2 ± 1.5	3.87	0.058
duration of singing interruptions [sec]	276.1 ± 65.5	211.7 ± 37.7	1.55	0.22
song rate [songs/min]	12.9 ± 0.6	12.0 ± 0.3	1.46	0.24

[1] $F_{8,23}$ in the overall analysis

DISCUSSION

The response behaviour of neighbours during the interactive playback was significantly influenced by playback treatment. Neighbours that received a song overlapping playback approached the simulated intruder much sooner, interrupted their singing less and sang at higher rates than did males that received a song alternating playback. In line with previous studies (Dabelsteen et al. 1997; Todt & Naguib 2000; Osiejuk et al. 2004), these strong responses to the song overlapping opponent demonstrate that song overlapping serves as a directed agonistic signal in close-range interactions indicating aggression and the motivation to escalate a contest. In our study, males interrupted their singing during song alternating playback for significantly longer than during song overlapping playback. This results contrasts with previous studies on nocturnal song of nightingales where the opposite effect of playback treatment on the duration of singing interruptions was found (Naguib & Kipper 2006; Schmidt et al. 2006). These contrasting results suggest that nightingales use different territorial defence strategies at night when reactions to rivals usually are restricted to countersinging and during the day when territorial responses also involve approach behaviour and visual inspection of the opponent. Moreover, in our study, males had to respond to a territorial intrusion which is a more direct threat than is a rival simulated to sing from outside the male's territory as simulated by Naguib & Kipper (2006) and Schmidt et al. (2006).

Overall, subjects responses to the simulated intruder during the second playback were influenced by the playback treatment the neighbour had received before. This differential response, however, was not significantly reflected in any of the measured response variables suggesting that a combination of subtle changes in the timing and intensity of approach behaviour or singing patterns may accumulate to a statistically detectable different territorial reaction. The most striking albeit non-significant differential response according to the treatment of the preceding playback in the neighbouring territory was the latency to enter the central zone around the loudspeaker, i.e. the latency to approach the simulated intruder up to at least 4m. Although we did not find a significant difference in the variables of territorial behaviour related to the playback treatment the neighbour had received before, the overall analysis suggests that subjects eavesdropped on the nature of the interaction in their neighbours' territory and adjusted their territorial defence behaviour to the level of aggression the opponent had interacted with beforehand. Thus, our results obtained in naturally occurring networks (see also Naguib et al. 2004) confirm current knowledge on the importance of eavesdropping in communication networks (Peake et al. 2001, 2002; Mennill & Ratcliffe 2004a)

Similarly, subjects responses overall were significantly influenced by the mating success of the challenged neighbour, which can be seen as an indicator of male quality. Subjects whose challenged neighbour was a high quality male tended to respond to the persisting intruder by increasing the percentage of songs containing rapid broadband trills, which are assumed to be a signal of aggression in male-male interactions (Schmidt et al. 2006). These results suggest that resident males use the quality of a neighbour to indirectly assess the threat an unfamiliar rival poses to territorial integrity. To our knowledge, such an indirect rival assessment has not been described previously for songbirds.

In territorial systems, residents usually respond less aggressively towards familiar opponents, such as neighbours, than to strangers, as territorial neighbours pose less of a threat than do strangers seeking to establish a territory (Falls 1982; Stoddard et al. 1991; Weary et al. 1992; Godard & Wiley 1995; Stoddard 1996; Schmidt et al. 2006). This phenomenon, commonly referred to as the dear enemy effect (Ydenberg et al. 1988; Temeles 1994), reduces the costs of territorial defence against neighbours (Godard 1991; Stoddard 1996). Once established a stable relationship to a neighbour in a communication network, males then may use their neighbours as border guards or 'early warning system' when territorial integrity is threatened (Eason & Stamps 1993; Hyman 2003; Naguib et al. 2004). Thus, a resident male may benefit by the presence of a neighbour because he can focus on territorial defence against strangers.

Taking into account the mating success of their neighbours in decisions on how to respond to persisting rivals, may be beneficial for residents because this information may be used in addition to the singing strategy of an unfamiliar rival (Peake et al. 2001, 2002; Mennill & Ratcliffe 2004a) in order to assess the level of threat posed by that rival. As males that are successful in attracting a female may be more aggressive or may have a higher resource holding potential (Kunc et al. 2006; Schmidt et al. 2006), persisting strangers that had challenged such males beforehand may be more of a threat to territory tenure than strangers that had challenged a less aggressive neighbour. Therefore, our results suggests that territorial defence should be viewed from a broad perspective that takes into account dyadic vocal interactions as an important source of information on fundamental aspects of a resident's male quality that may be assessed through his territorial defence strategy. Combining direct information on a stranger's motivation that is reflected in his singing strategy with indirect information that is reflected in the challenged neighbour's response strategy, may enable a male to respond appropriately to newly arriving or non-territorial strangers that may prospect during the day to assess singing residents or territory occupancy (Stutchbury 1991; Amrhein et al. 2004a; Bruinzeel & van de Pol 2004). As such strangers may challenge a resident repeatedly early in the breeding season, a resident male may benefit by establishing a territory in the neighbourhood of a high quality male because this enables a male to use the neighbour as ‘yardstick’ for his own response.

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Synthesis
Future Prospects
Acknowledgments

Synthesis

Exposing territorial nightingales to simulated rivals that differed in the amount of song overlap (**Chapter I**) or in the occurrence of a specific song structure, i.e. the rapid broadband trills (**Chapter III**), my studies have shown that territorial defence strategies convey information on the challenged males' subsequent mating success. Males that remained unpaired throughout the breeding season interrupted their singing significantly more often in response to an aggressively singing rival (i.e. to an overlapping rival, **Chapter I**) than in response to a moderately singing, i.e. alternately singing rival. In contrast, subsequently mated males showed a similar number of singing interruptions, regardless of whether or not their songs had been overlapped by playback. In **Chapter III** I have shown that subsequently mated and unmated males also differ in their territorial defence when challenged by a rival that was simulated to sing a high proportion of rapid broadband trills. In particular, the response intensity in relation to the fine structure of presented trills can bee seen as a predictor of the males' future mating success. Males that remained unpaired throughout the breeding season decreased their territorial defence behaviour with increasing trill performance of the simulated opponent. In contrast, males that became paired later in the season increased the vigour with which they engaged in territorial defence with increasing trill performance of the simulated intruder. Thus, the subsequent pairing success of a male appears to be a crucial factor determining his specific response strategy.

Throughout my work, I have claimed the pairing success of a male to be an indicator of his quality. One may argue that the quality of a male should be measured on a larger scale, for instance by his lifetime reproductive success which does not necessarily have to be linked to the pairing success in one year. A male that remains unpaired in a given season may have had a successfully breeding female the preceding year and he may also become paired in the following year. However, as nightingales form only seasonal pair bonds, i.e. usually have different pair mates in consecutive seasons (personal observation, unpubl. data), a female in a given year has to choose a male based on the 'status quo' of his quality or that of his territory. Even though I can only speculate about the factors that are important in female choice, such as the quality of a male's territory, his ability to feed the offspring, or his competitive abilities, the fact that some males are preferred over others implies that they are for some reason more attractive to females or of higher quality. Furthermore, a male nightingale's capability to attract a social mate can be seen as the main factor limiting a male's reproductive success in a given season because only 8% of nestlings are sired by an extra-pair male (Amrhein 2004). Therefore, I think it is reasonable to treat males which successfully attract a social female as males of higher quality than those males that remain unpaired throughout a breeding season.

In order to establish a territory in a suitable habitat, individuals may use information on environmental factors that may affect breeding success, such as the presence of predators (Marzluff 1988; Muller et al. 1997; Eggers et al. 2006; Fontaine & Martin 2006), the food availability or the type and density of vegetation (Davies & Houston 1984; Tobias 1997b). According to public information hypothesis (Valone & Giraldeau 1993; Doligez et al. 2003), animals further may monitor the presence or density of conspecifics to estimate habitat quality (Stamps 1988; Reed & Dobson 1993; Muller et al. 1997), or they may directly monitor the reproductive success of conspecifics to assess local habitat quality, for instance by evaluating the density of juveniles (Danchin et al. 1998; Doligez et al. 1999; Reed et al. 1999; Piper et al. 2006). However, in many species this information may be available only at the end of the breeding season and, thus, can be used for breeding habitat selection only in the following year (Boulinier et al. 1996; Danchin et al. 1998). Therefore, the use of public information may be associated with a time delay for reproduction which might reduce the fitness of an individual (Doligez et al. 2003). Assessing the subsequent pairing success of a conspecific, for example by his specific reactions to territorial challenges early in the breeding season prior to mating, as shown in **Chapters I** and **III**, may enable a male to evaluate indirectly the quality of a habitat. I thus can hypothesize, based on my findings, that males seeking a suitable habitat to establish a territory may use the territorial defence strategy of an opponent as a predictor of his mating success which thereby indirectly reflects the quality of a habitat. Thus, my findings demonstrate the adaptive function of signalling strategies used in competition over resources by highlighting components of male quality as a source of variation in territorial defence behaviour.

Besides these differential strategies in territorial defence in relation to the subsequent pairing success, the results presented in **Chapters I-IV** also show that nightingales discriminate between rivals according to the way the rivals use their song during vocal interactions. My findings in **Chapters I, II** and **IV** support the hypothesis that song overlapping serves as a directed agonistic signal in close-range male-male vocal contests (Collins 2004; Naguib 2005). There is increasing evidence for this hypothesis provided by numerous studies on the nightingale (Hultsch & Todt 1981; Naguib 1999; Kunc et al. 2006; Naguib & Kipper 2006; Kunc et al. 2007) and other bird species, e.g. on great tits (Dabelsteen et al.

1996) , black-capped chickadees (*Poecile atricapilla,* Mennill & Ratcliffe 2004b), yellowhammers (*Emberizia citrinella*, Osiejuk et al. 2004), or European robins *Erithacus rubecula* (Brindley 1991; Dabelsteen et al. 1997) suggesting that song overlapping generally is perceived as a signal indicating the aggressiveness of the overlapper or his motivation to escalate a contest.

Chapter III shows that nightingales discriminate between rivals based on the presence or absence of structural song components. To my knowledge, this has rarely been investigated in male birds (ten Cate et al. 2002; Leitao & Riebel 2003; Illes et al. 2006). These findings expand on a previous study by Kunc et al. (2006) which revealed that nightingales sing an increased proportion of songs containing rapid broadband trills when they are confronted with a countersinging rival compared to their spontaneous song. Moreover, during vocal interactions with a persisting rival, males increase the amount of songs containing trills in response to a rival that had challenged a neighbour of presumably high quality as measured by the neighbour's mating success (**Chapter IV**). Taking these results together, rapidly repeated frequency modulated song components, such as the rapid broadband trills in nightingales, apparently are perceived as a threatening signal in intrasexual contexts.

So far, the signal function of trills has been investigated mostly in an intersexual context. A repetitive note production in form of a trill appears to require a precise coordination of vocal tract movement and airflow (Hartley & Suthers 1990; Westneat et al. 1993; Podos 1996; Hoese et al. 2000) resulting in a tradeoff between how fast a bird can repeat the trill units and the frequency each unit can span (Podos 1996, 1997; Podos & Nowicki 2005). The performance of physically challenging trills, therefore, may reflect male quality and females have been shown to prefer males that perform trills closer to the production limit (Vallet & Kreutzer 1995; Draganoiu et al. 2002; Ballentine et al. 2004).

In **Chapter II**, I have shown that the vigour with which males engage in territorial defence during the day depends on information from prior signalling experience. Males that had received a song overlapping playback the preceding night approached the simulated intruder more quickly and closer and sang more songs near the loudspeaker than did males that had received a song alternating playback. This adjustment of territorial defence strategies depending on information from prior signalling experience suggests that integrating information with time plays an important role in territorial defence. In general, disputes over territory boundaries are likely to occur frequently early in the breeding season when newly arriving or non-territorial males may prospect during the day to assess singing residents or territory occupancy (Stutchbury 1991; Amrhein et al. 2004a; Bruinzeel & van de Pol 2004). In such situations, in which repeated challenges by non-territorial rivals can be expected, territorial males may benefit by acting upon prior experience (Switzer et al. 2001). So far, only a few studies have addressed long lasting effects of territorial intrusions on territory proclamation or territory defence strategies, for instance in winter wrens (*Troglodytes troglodytes*, Amrhein & Erne 2006) and banded wrens (*Thryothorus pleurostictus*, Hall et al. 2006). My study presented in **Chapter II** expands on these findings as it shows that nightingales adjust territorial defence based on previous singing interactions with non-intruding rivals, i.e. based exclusively on auditory information. My results, therefore, also emphasize long-range singing interactions to be an important source of information in a communication network, specifically at early stages of the breeding cycle during territory establishment and mate attraction.

In **Chapter IV**, I investigated, based on the findings presented in **Chapter I** and **III**, whether resident males eavesdrop on a dyadic interaction between their neighbour and a simulated stranger. I hypothesized that a resident male could use the territorial defence behaviour of his neighbour against a stranger as a 'yardstick' for his own response. In line with previous studies (Peake et al. 2001, 2002; Mennill & Ratcliffe 2004a), I found that males adjusted their responses to an unfamiliar rival to prior experience on the rival's aggressiveness. Resident males responded stronger to persisting rivals that had interacted aggressively with a neighbour beforehand (by song overlapping) than to rivals that had interacted moderately with a neighbour (by song alternating). Furthermore, I found that subjects' response performances to the unfamiliar rival overall were influenced by the quality of the neighbour the rival had challenged beforehand, although I did not find significant changes in any of the measured response variables. This suggests that a combination of subtle changes in the timing and intensity of approach behaviour or singing patterns may accumulate to a statistically detectable different territorial reaction, an issue that requires further testing.

In territorial systems, the relationship between familiar neighbours is assumed to be stabilized by the 'dear enemy effect' (Falls 1982; Ydenberg et al. 1988; Temeles 1994; Stoddard 1996) which predicts that residents usually respond less aggressively towards familiar neighbours than to strangers, as territorial neighbours pose less of a threat to territory tenure than do strangers seeking to establish a territory. Numerous playback experiments have shown that territorial male songbirds discriminate between neighbours and strangers (Stoddard et al. 1991; Weary et al. 1992; Godard & Wiley 1995; Stoddard 1996), and neighbours may be memorized over a prolonged period of time (Godard 1991). Due to the dear enemy effect, costs of territorial defence against neighbours are reduced which enables a resident male to focus on defending his territory against strangers. Thus, males may benefit by establishing a territory next to an already existing territory. Once established a stable relationship to a neighbour in a communication network, males then may use their neighbours as border guards or 'early warning system' when territorial integrity is threatened (Eason & Stamps 1993; Hyman 2003; Naguib et al. 2004). My results presented in **Chapter IV** expand on these studies as they suggest that residents also include the mating success of their neighbours in decisions on how to respond to rivals.

After having discussed the signal value different singing strategies may have in male-male communication, I would like to consider their possible implications for intersexual communication. Given that vocal interactions provide information on interindividual differences among males (**Chapters I, III** and **IV**), I can hypothesize that even at the very beginning of the breeding season, i.e. prior to mating, females could eavesdrop on singing interactions of males and prefer the more vigorously performing male as social mate. Indeed, females have been shown to eavesdrop on male-male interactions and to base their choice of a social mate or extra-pair copulations on the performance of their mates in vocal contests (Otter et al. 1998; Otter et al. 1999; Mennill et al. 2002; Mennill et al. 2003; Leboucher & Pallot 2004). Thus, females may impose an indirect selection pressure on males to vocally interact with each other, when only those males that perform vigorously or aggressively in intra-sexual conflicts are preferred in female choice (Wiley & Poston 1996; Naguib 2005).

To conclude, experiments in my thesis provide new insights into the adaptive function of signalling strategies that are used in the competition over resources in territorial animals. Using the nightingale as a model species, my studies demonstrate that territorial defence should be viewed from a broad perspective taking individual differences between competing animals and the adjustment to past territorial challenges in a communication network into account to understand the evolution of strategies in territory settlement and maintenance.

Future Prospects

The timing of song delivery and the choice of song types are functional components of vocal interactions, where overlapping on the one hand and songs containing rapid broadband trills on the other hand are threatening signals that have separate consequences for the behaviour and the song performance of a challenged male. The next step would be to investigate how males combine these two functional components in rival assessment. Studies addressing this question could show how animals use different categories of information obtained in several interactions in the assessment of a rival.

If the subsequent pairing success at least in part reflects local habitat quality, and if neighbours may serve as border guards then we can expect that males arriving from migration establish a territory in the vicinity of a territory already occupied by a high quality male although spatial distribution of patches of suitable habitat may be equal across an area. Thus, the question arises whether models based on information exchange in vocal interactions can predict settlement patterns in a population. By regularly monitoring and mapping the diurnal and nocturnal singing activity of nightingales in the vicinity of the Petite Camargue Alsacienne during the breeding seasons 2004-2006, I obtained arrival dates of up to 120 males, data on their pairing success and on territory boundaries (see Appendix for detailed information and maps). Based on a combination of these data and the results of my thesis, I would like to investigate whether the settlement patterns within a population of territorial animals can be explained with individually based models taking into account information gathering on local habitat quality (**Chapters I** and **III**) and on the aggressiveness or quality of conspecifics (**Chapters I**, **III**, and **IV**), information from prior signaling experience (**Chapter II**) and the possibility to obtain this information indirectly by eavesdropping on interactions of conspecifics in a communication network (**Chapter IV**). Furthermore, territory quality, which surely is an important factor influencing an animal’s decision where to settle, can be estimated by the number of years the territory had been occupied. I am convinced that such models will broaden our understanding of the significance of acoustic communication in territorial species as they might reveal the influence of information exchange in communication networks on strategies in territorial settlement and territory tenure.

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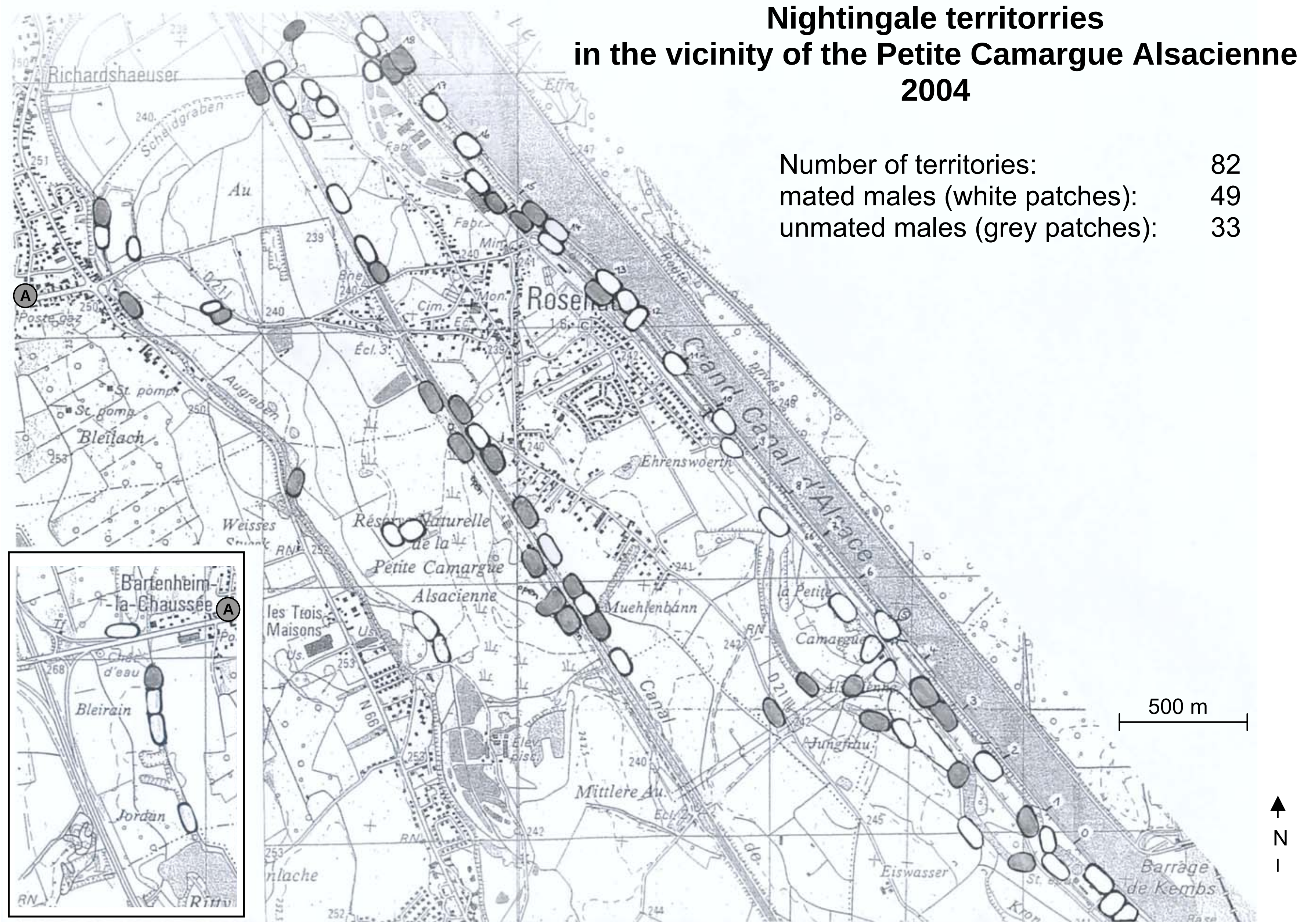
Appendix

Census of Singing Activity

So far, extensive research has been conducted on the study population of nightingales in the Petite Camargue Alsacienne (PCA) in France, about 10 km north of Basel, Switzerland (e.g. Amrhein 2004; Kunc 2004). In my first year as PhD student, I used the established and standardized methods of monitoring diurnal and nocturnal singing activity (Amrhein et al. 2002; Amrhein 2004; Amrhein et al. 2004b; Amrhein et al. 2007) to estimate the number of territories and the mating success of males in a larger area in the vicinity of the PCA. I censused an area of about 16 km^2 mainly around two canals (the Grand Canal d'Alsace and the Canal de Huningue) between the villages Village Neuf, Rosenau and Bartenheim-la-Chaussée. This area is part of the Upper Rhine Valley near Basel with more than 200 territorial nightingales (Amrhein & Zwygart 2004). In this agriculturally used area, nightingales usually settle in patches of dense bushes or woods along rivers and footpaths. Territories often border grasslands or fields, resulting in clear territory boundaries. In that area I also conducted all the playback experiments presented in this thesis.

I established a new fixed route to be followed by bike twice a day throughout the breeding seasons 2005 and 2006. In 2004, these rounds were conducted every other day. The direction of the rounds was changed from day to day. One round of inspection started 75 min before sunrise (the exact time is provided by http://www.sunrisesunset.com for the city of Basel) to assess singing activity during the dawn chorus. Song posts during the dawn chorus were used to estimate territory boundaries and to estimate the number of territorial males that were present in the area. The other round started at midnight to survey nocturnal singing activity. Based on the presence or absence of nocturnal song, the mating status of males could be assessed as usually nightingales cease nocturnal song after pairing (Amrhein et al. 2002; Amrhein et al. 2004b). Thus, males that regularly sang at night over the entire breeding season were considered to be unmated males. In contrast, males that stopped singing at night but still were singing during the day were considered to be mated.



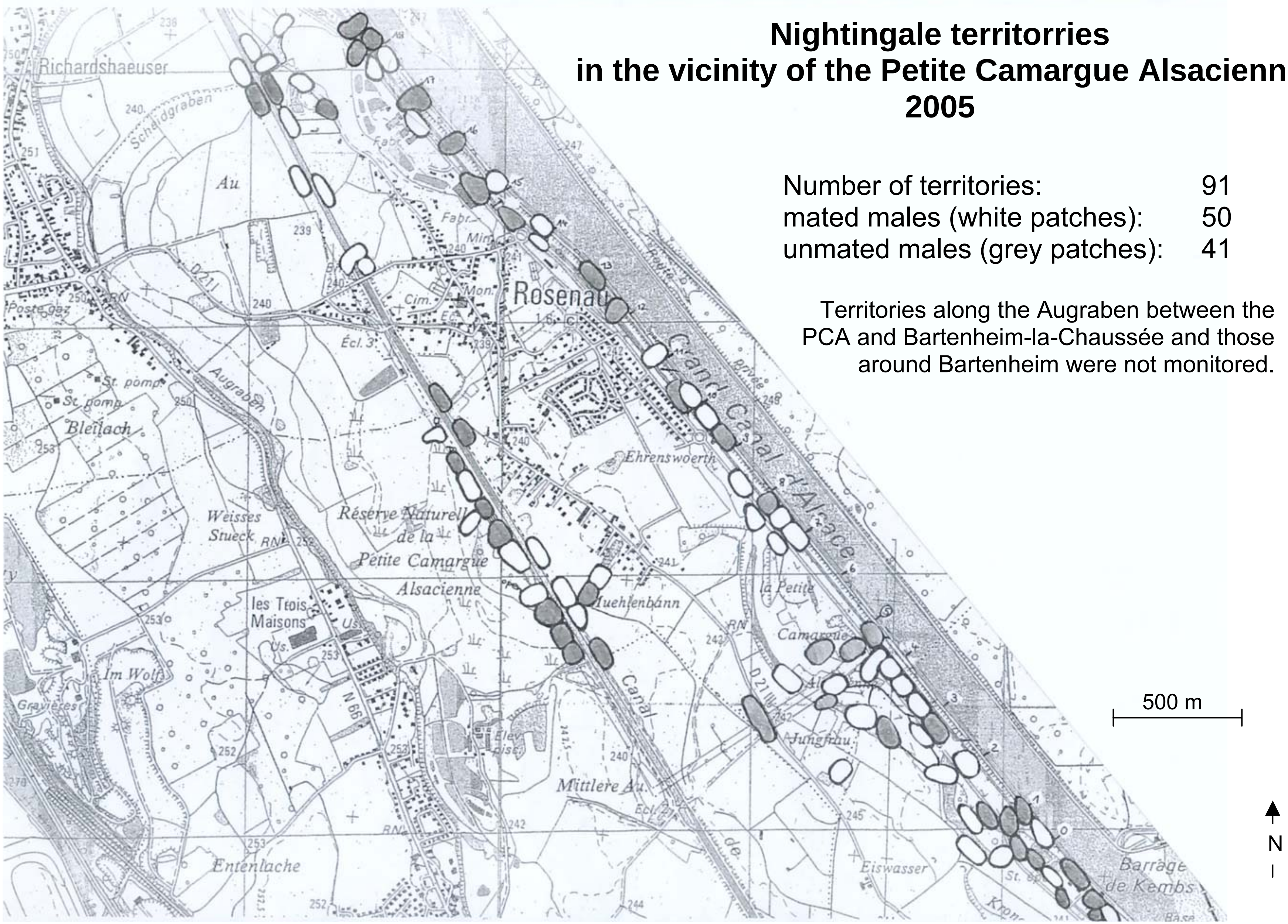
Nightingale territorries in the vicinity of the Petite Camargue Alsacienne 2004

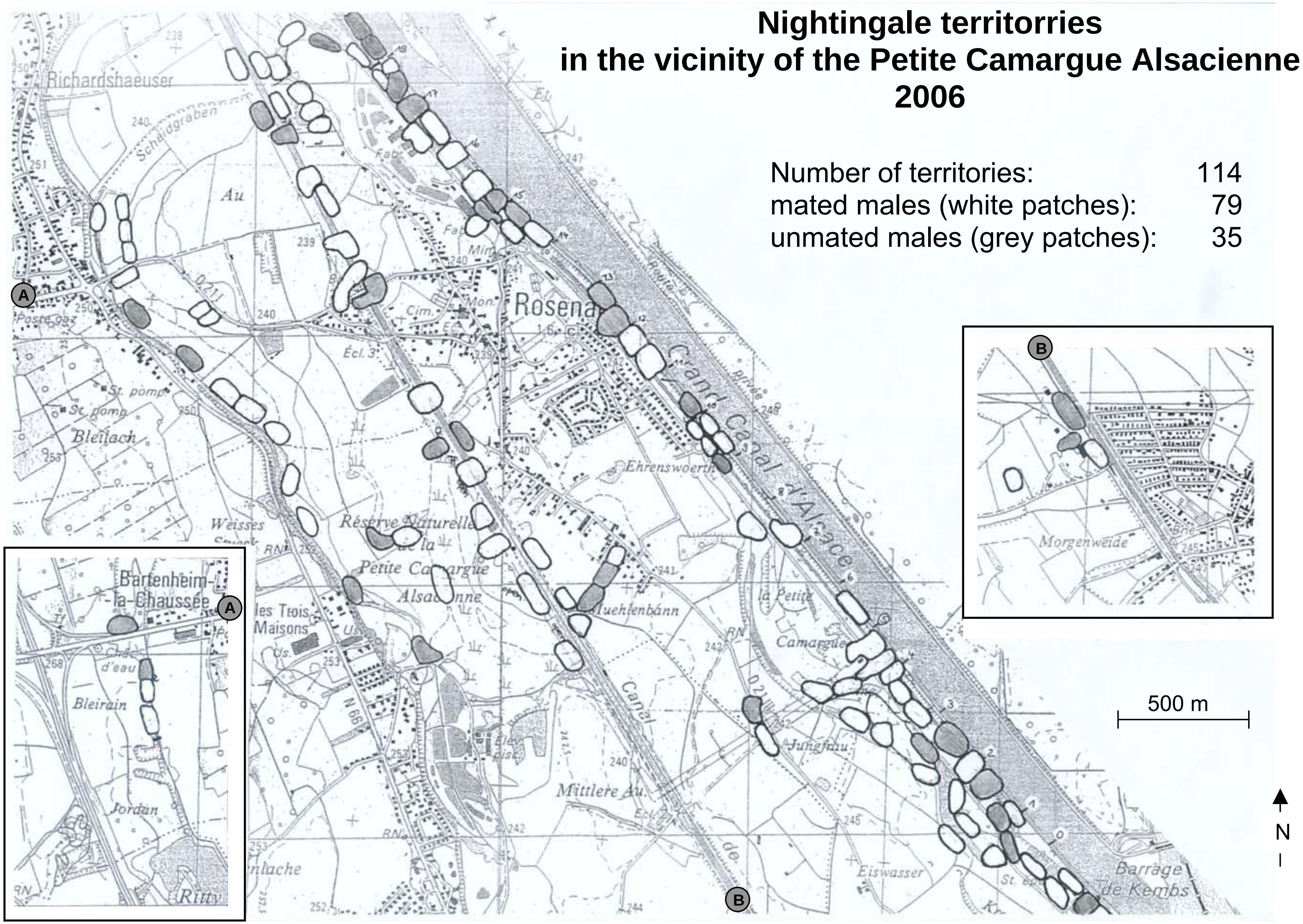
Number of territories:	82
mated males (white patches):	49
unmated males (grey patches):	33

Nightingale territorries in the vicinity of the Petite Camargue Alsacienne
2005

Number of territories:	91
mated males (white patches):	50
unmated males (grey patches):	41

Territories along the Augraben between the PCA and Bartenheim-la-Chaussée and those around Bartenheim were not monitored.





Nightingale territorries in the vicinity of the Petite Camargue Alsacienne

2006

Number of territories: 114
mated males (white patches): 79
unmated males (grey patches): 35