

To tweet or not to tweet

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To tweet or not to tweet

The role of personality in the social networks of great tits

Lysanne Snijders

Thesis

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Natura nusquam magis est tota quam in minimis

Plinius

*Nature is to be found in her entirety nowhere more than in her
smallest creatures.*

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General introduction

General introduction

People differ in how social they are; some people are just more popular and have more friends than others. Already at the playground these differences are obvious. Similarly, many animals do not associate at random; some individuals have significantly more social relationships than others. Evidence that social relationships are important for people is well established (Pretty, 2003; Charuvastra & Cloitre, 2008; Helliwell & Huang, 2013), but social relationships can also be vital for nonhuman animals. Social bonds can help in fighting of competitors and predators (Grabowska-Zhang et al., 2012; Gilby et al., 2013; Goodwin & Podos, 2014), enhance reproductive success (Silk et al., 2003; Schülke et al., 2010; Grabowska-Zhang et al., 2011) and increase longevity (Silk et al., 2010). However, not all individuals weigh the costs and benefits of social interactions in the same way. How animals balance the costs and benefits of certain situations, for example the access to social information against the experience of social stress, is potentially modulated by personality differences.

Personality

In various animal species individuals differ consistently in their behaviour across time and context. These consistent differences are referred to as personality differences (Gosling, 2001; Réale et al., 2007; Bell et al., 2009; Wolf & Weissing, 2012). Personality differences explain variation in how individuals respond to stressful situations, i.e. their coping styles (Coppens et al., 2010). The fact that individuals are not completely flexible in their behavioural responses has puzzled biologists, since this consistency initially appeared to be maladaptive. Several functions and mechanisms have been proposed to explain the existence and maintenance of personality types. First, when individuals differ in their future fitness expectations, it might pay individuals with lower fitness expectations to take consistently more risks (Wolf et al., 2007; Biro & Stamps, 2008; Nicolaus et al., 2012). Second, the success of a certain personality type might depend on negative frequency dependent selection; whereby the rare type does best. This would result in a perpetual feedback loop alternating which personality type is most rare and thus most successful (Sih et al., 2004; Réale et al., 2007; Wolf & Weissing, 2010). Third, variation in personality types could be maintained by a variable environment. Varying predation levels and food availability can cause fluctuations in the cost-benefit trade-off for bold and explorative behaviour: one personality type could be successful in one year, but the contrasting type in the other year (Dall et al., 2004; Dingemanse et al., 2004). Fourth, sexual selection has been proposed to play a role in maintaining consistent differences in personality types; as

individual females could differ in their preference for male personality traits (Schuett et al., 2010). Finally, individual consistency can be advantageous in a social context, since it makes individuals predictable and so allows conspecifics to respond. This would be beneficial for behavioural coordination in social groups and the avoidance of costly outcomes in confrontations between competitors (Wolf et al., 2011).

If the social context indeed plays a key role in the maintenance of personality differences (Wolf et al., 2011), one might also expect social behaviour and social structuring to vary with personality (Wolf & Krause, 2014). This is important as the social environment is a key selection pressure in many animal populations, and in several species these personality differences indeed correlate with differences in social behaviour. For example, personality differences in barnacle geese (*Branta leucopsis*) predict the use of social information (Kurvers et al., 2010a; Kurvers et al., 2010b) and personality differences in great tits (*Parus major*) predict the response to social stress (Carere et al., 2001; Carere et al., 2003; van der Meer & van Oers, 2015). Different personality types could thus differ in the trade-off between benefits (social information) and costs (social stress) of social associations. Therefore it is likely that the number and strength of social associations will vary with personality type (Krause et al., 2010; Wolf & Krause, 2014).

Social networks

Social network analysis is a appealing approach for studying the role of specific individual characteristics, such as personality type, in the structuring of the social environment (Croft, James, & Krause, 2008). In a social network, individuals are represented as nodes that can be connected to

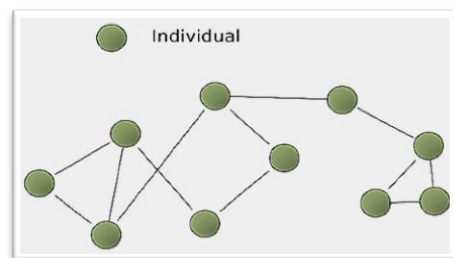


Figure 1. Example of a social network

each other via social associations or interactions, represented as edges (Figure 1). By using social network analysis, we can quantify if certain types of individuals within a social structure are better socially connected than others. The important role that social networks play in animal societies is increasingly recognized (Krause et al., 2007; Kurvers et al., 2014; Brent, 2015). Social network position can influence the likelihood of an individual finding new food (Aplin et al., 2012), acquiring novel foraging techniques (Allen et al., 2013), rising in social status (McDonald, 2007),

acquiring a mate (Oh & Badyaev, 2010), but also vulnerability to diseases and parasites (Weber et al., 2013). So, just like single social associations, certain networks positions have cost and benefits for individuals.

Recent studies have furthered our understanding of the role of personality in animal social networks, usually by focusing on swarming or flocking populations. Shy guppies (*Poecilia reticulata*) have more and stronger social associations than bold guppies (Croft et al., 2009). Similarly, shy three-spined sticklebacks (*Gasterosteus aculeatus*) have overall more social associations, but usually with the same individuals, while bold fish have more unique associations (Pike et al., 2008). Social network studies in great tit winter flocks reveal a similar pattern (Aplin et al., 2013). Yet, in captive barnacle geese there is no evidence for a relationship between personality and social network position (Kurvers et al., 2013). Although there is substantial variation between the above-mentioned study species, there seems to be a tendency for re-active personality types to have stronger social associations while pro-active personality types seem to have more unique, but weaker, social associations.

Social associations in swarming and flocking populations are, however, fundamentally different from those in territorial populations, in which individuals meet less frequently and the costs and benefits of spatial associations differ. Despite social associations also being highly relevant in territorial populations (Getty, 1987; Beletsky & Orians, 1989; Olendorf et al., 2004; Akçay et al., 2009; Grabowska-Zhang et al., 2011; Grabowska-Zhang et al., 2012; Goodwin & Podos, 2014) (Getty, 1987), very little is known about the social network dynamics in such populations.

Communication networks

Especially in territorial populations, conspecifics do not only socially connect via spatial proximity, but also by communicating. In communication networks conspecifics use long-range signal interactions to connect, often over large distances. Animals frequently use long-range signals (McGregor & Dabelsteen, 1996; McGregor, 2005; Peake et al., 2005) to facilitate (Maynard et al., 2014), maintain (Garland et al., 2011), but also discourage (Whitney & Krebs, 1975; Waser & Wiley, 1979; Naguib et al., 2001) spatial associations. Long-range communication networks and proximity networks are thus commonly inherently linked (Waser & Wiley, 1979). In territorial species, long-range signals are frequently used as a 'keep out' message (Krebs, 1977; Nowicki et al., 1998; Naguib et al., 2001). It would thus

be interesting to know if territorial individuals with fewer spatial associations are more active signallers.

Besides the influence of communication networks on proximity networks, they also provide valuable social information. Long-range signals can be used by conspecifics as a source of information: gaining information from others' signalling interactions via "eavesdropping" is an especially important component of communication networks (McGregor & Dabelsteen, 1996; McGregor, 2005). Eavesdropping in communication networks occurs in many species, ranging from fish to birds (McGregor & Dabelsteen, 1996; Naguib & Todt, 1997; Oliveira et al., 1998; Earley & Dugatkin, 2002; Mennill et al., 2002; Amy et al., 2010; Webster & Laland, 2013; Cvikel et al., 2015a). Eavesdroppers, males and females, can use the information retrieved from eavesdropping to adjust their current behaviour or their behaviour during subsequent encounters, for example by changing their mating decisions or their behavioural responses to rivals (Peake et al., 2001; Mennill et al., 2002; Amy et al., 2010).

Communication networks can thus provide valuable information, both on the quantity and quality of the social relationships within the social networks. Some social network studies have indeed integrated communication networks in their network analyses (Flack et al., 2006; Stopczynski et al., 2014). Quantifying the communication network within the social network study of Flack et al. (2006) gave relevant insights into the importance of 'policing' for the stability of the social structure. Understanding how proximity networks vary with communication networks is relevant for gaining more qualitative information on the types of social relationships and for the study of social networks in species that have little spatial associations but do use long-range signals.

The two general objectives of this thesis were therefore to test within a territorial population if (1) proximity networks can be predicted by personality and if (2) proximity networks vary with communication networks.

Study system

Study species

White cheeks, yellow breast, a black tie and a catchy tune; the characteristics of a great tit. These small songbirds generally live in deciduous forests, but can also commonly be found in gardens. They eat seeds and insects and are themselves a common prey species for sparrow hawks (*Accipiter nisus*). Great tits usually do not migrate and if they survive their first winter their mean life-expectancy is close to 2 years, although some individuals become much older. Especially in the first year of life mortality is very high, caused by strong predation in spring and summer and starvation in winter (Gosler, 1993). They are socially, but not sexually, monogamous and a male and female will together try to raise one or two broods

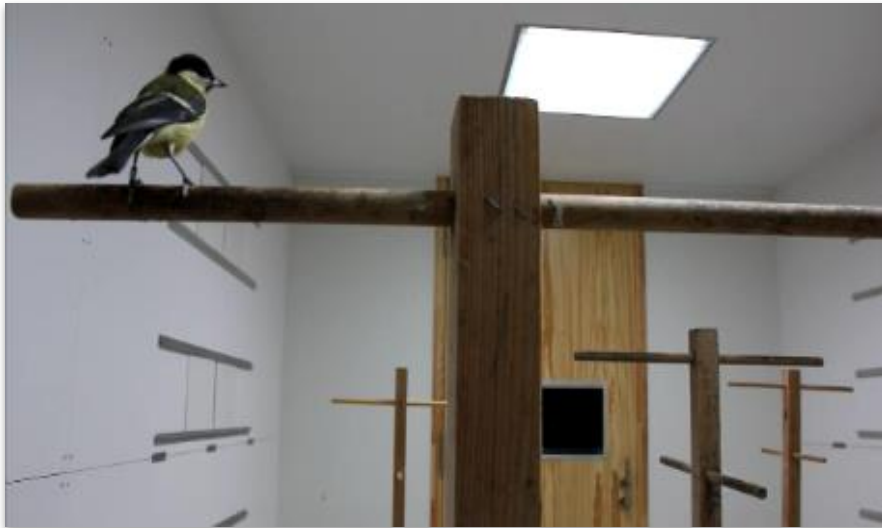


Figure 2. A juvenile great tit in the novel environment room

each year (Gosler, 1993). The great tit has been a key model species for the study of behaviour in the wild for decades, partly because great tits are cavity-breeders for which breeding success can be accurately monitored using data collected from nest boxes. This way, variation in behaviour can directly be linked to potential fitness effects and so provide adaptive explanations for variation in behaviour.

Since the nineties the great tit has also been an excellent model for the study of personality (Verbeek et al., 1994). For many years the great tit has provided important insights into the causes and consequences of personality differences

(Groothuis & Carere, 2005; van Oers & Naguib, 2013). Personality differences in great tits have been related to underlying factors such as genetic background (Dingemanse et al., 2002; Drent et al., 2003; van Oers et al., 2004; van Oers & Mueller, 2010; Mueller et al., 2013), physiological responses (Carere et al., 2003; Carere & van Oers, 2004; Baugh et al., 2013; van der Meer & van Oers, 2015), early life conditions (Naguib et al., 2011a) and future fitness expectations (Nicolaus et al., 2012). Moreover, personality differences associate with variation in aggression, dominance and competitive abilities (Verbeek et al., 1996; Verbeek et al., 1999; Carere et al., 2001; Dingemanse & de Goede, 2004; Carere et al., 2005; Cole & Quinn, 2012), learning abilities (Guillette et al., 2010; Titulaer et al., 2012), response to changes in the environment (van Overveld & Matthysen, 2010; Naguib et al., 2013; van Overveld & Matthysen, 2013), general social behaviour and communication (Carere et al., 2001; van Oers et al., 2005; Amy et al., 2010; Naguib et al., 2010; Aplin et al., 2014), anti-predation behaviour and foraging (Hollander et al., 2008; Quinn et al., 2011), movement ecology (Dingemanse et al., 2003; van Overveld et al., 2011) and ultimately components of fitness (Dingemanse et al., 2004; Both et al., 2005; van Oers et al., 2008; Patrick et al., 2011).

Territorial great tits are a intriguing model system for studying the role of personality in social networks, not only because much is known about the role of personality in great tit ecology, but also because they are a territorial songbird and thus do not only connect via spatial proximity but also via song (Krebs, 1971). Territorial singing in great tits is likely to act as an attractor for females as well as a keep-out signal for males (Slagsvold et al., 1994). Studying territorial great tits thus provided me with the possibility to examine whether proximity networks in a territorial system indeed vary with communication networks.

Study population

From 1955 onwards the Netherlands Institute of Ecology (NIOO-KNAW; IOO in 1955) has monitored the breeding behaviour of several great tit nest box populations in the Netherlands. I conducted my study in the long-term study population of great tits in the Westerheide forest, The Netherlands (52°01'00.0"N 5°50'20.0"E). Westerheide is a mixed pine-deciduous wood with about 200 nest boxes attached to trees distributed within a 1000 m x 1200 m area. Birds in this population are tested for exploration behaviour, an operational measure for personality, using the validated 'novel environment' test (Figure 2) developed by Verbeek et al. (1994). Throughout the year, with the exception of the breeding season, newly caught individuals (captured either by mist-netting during the day or

during a nest box check at night) undergo a novel environment test. The great tits are brought to the testing facilities of the Netherlands Institute of Ecology (NIOO-KNAW), where they are housed individually in cages (0.9 x 0.4 m and 0.5 m high), with a solid bottom and top, solid side and rear wall, a wire-mesh front and three perches. They have access to food and water *ad libitum* and undergo the novel environment test the following morning. During the novel environment test the birds are individually tested in a room (4.0 x 2.4 m and 2.3 m high) with five artificial trees. After they entered the experimental room, we record the total number of flights (movements between trees) and hops (movements within trees) within the first 2 min. These movements are subsequently used to calculate an overall exploration score ranging from low (slower explorers) to high (faster explorers) following Dingemanse et al. (2002). The birds are released near their nest boxes within 24 hours of the initial capture.

During the breeding season, nest boxes are checked twice a week to record laying date (of the first egg), clutch size and hatching date. Following an established procedure, breeding birds are identified during the chick rearing phase (when nestlings are 7-10 days old) by catching them inside their nest boxes using spring traps. We take biometry measures of the nestlings when they are 14 days old (with hatching date as day 0). Both body mass and tarsus length are measured for chicks and adults, and un-ringed birds are fitted with uniquely numbered aluminium leg rings.

Study methods

For this project I used a variety of methods to examine components of proximity and communication networks. To examine the proximity networks of wild territorial great tits (**Chapter 3 & 8**) I used the novel automatic tracking system: Encounternet (Mennill et al., 2012). This system is a very promising tool for the study of social networks (Krause et al., 2011; Rutz et al., 2012), since it can spatially track multiple individuals simultaneously. The other important characteristic of this system is that it can be used for studying small birds. To test if this technology has no long-term negative effects on great tits I analysed three years of tracking data for potential effects on survival, reproduction and provisioning behaviour (**Chapter 2**). A spatial association between two birds does, however, not provide information on who initiated the association. Thus, when a certain personality type has more spatial associations, it remains open if this personality type also initiated the majority of these associations. Therefore I designed a video-playback experiment

for captive personality-tested great tits in which an association could only take place if the subject bird initiated it (**Chapter 4**).

For studying the communication networks of the great tits I used three different methods: singing activity rounds, automatic song recordings and audio playback experiments. During the singing activity rounds, observers biked slowly through the forest, following a fixed route, and documented where and when a great tit was singing (Amrhein et al., 2002; Amrhein et al., 2008). To quantify the singing activity during the dawn chorus, these rounds started half an hour before sunrise (**Chapter 5 & 7**). During the breeding season, male great tits sing very actively at dawn, just before sunrise and maintain high levels of singing activity throughout the pre-laying, laying and incubation phase (Mace, 1986). I used automatic song recorders to collect more qualitative data on the dawn singing behaviour of the great tits (**Chapter 6 & 7**). Especially during the fertile period of their mate, great tit males sing close to the breeding nest box (Mace, 1986), which makes it possible to assign an identity to the singer afterwards. Finally, I conducted simulated territory intrusions, experiments during which we playback the song of an unfamiliar male great tit in the territory of the subject, to so document great tit spatial and singing behaviour in a confrontational context. These simulated territory intrusions also generated perturbations in the communication network (**Chapter 7 & 8**).

Outline of this thesis

The overall aim of this thesis was to identify the role of personality in the social networks of great tits, including proximity and communication networks. To study the proximity networks in this thesis I thus used a novel tracking system. In **Chapter 2** I investigate if this methodology had any negative effects on our study species and if so, when these effects were most pronounced. Using this tracking system in **Chapter 3** I examine if the proximity network positions of male territorial great tits relate to their personality. In **Chapter 4** I experimentally test if personality influences the individual preference to be close to a conspecific. To exclude the influence of the social partner I use a video-playback set-up with captive great tits. Whether personality also influences how actively wild great tits communicate is investigated in **Chapter 5**. In **Chapter 6** I study whether specific communication traits vary with the social structure of great tit territories. How personality, spatial proximity and communication interact during a territory intrusion I examine in **Chapter 7**. In **Chapter 8** all components: personality, communication and proximity networks come together when I test whether perturbations in the communication network could have personality-dependent consequences for the proximity

network. In the general discussion (**Chapter 9**) I review the link between personality and social networks in a broader context, discussing findings from a wide range of species. Additionally, I examine various ways that proximity networks and communication networks are linked. Finally, I shortly discuss the potential of animal social network analysis for conservation science. The conceptual overview of this thesis can be seen in Figure 3.

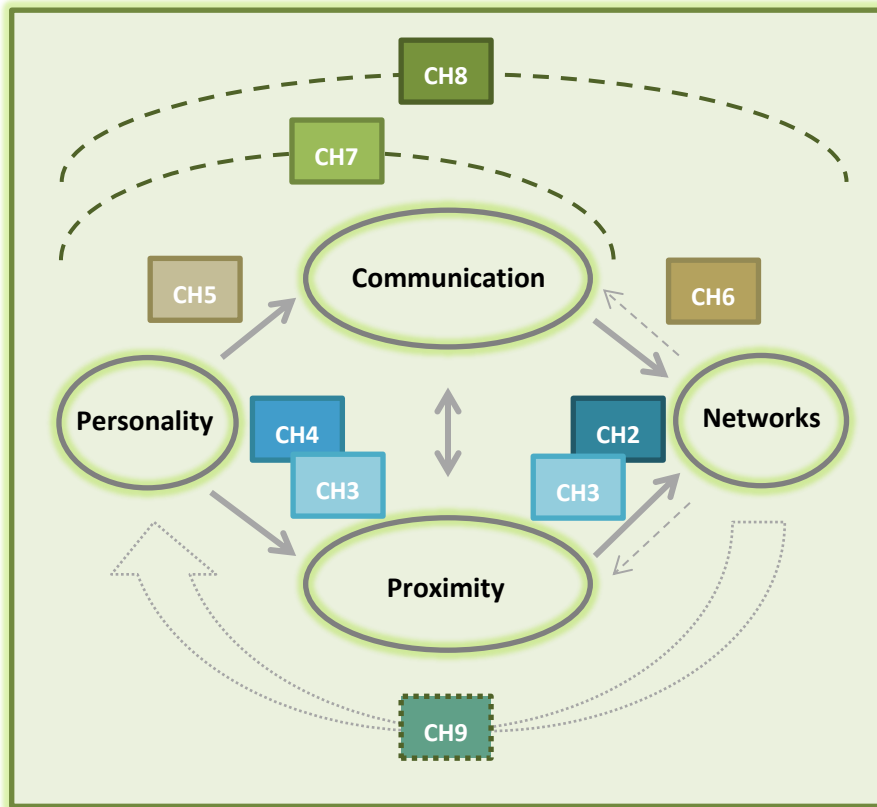


Figure 3. Conceptual overview of the PhD thesis

Societal relevance

Understanding the role of personality in animal social behaviour is important from both a fundamental and applied science perspective. There are billions of animals living in captive groups today (The Food and Agriculture Organization Corporate Statistical Database) and their holding conditions, group compositions, group sizes and densities are not the result of the animal's free choice. This can have severe consequences for the social interactions within the animal groups, resulting in physical injuries and even death (Savory, 1995). To understand and prevent these negative impacts on animal welfare it is thus important to know how individual animals vary in their natural social preferences (Rodenburg et al., 2004; Bolhuis et al., 2005).

Understanding of how individual animals are socially connected is also relevant for nature conservation. Social networks can predict disease transmission (Hamede et al., 2009; Bull et al., 2012; Hirsch et al., 2013; Rushmore et al., 2013), but also how resilient populations are to disturbances (Lusseau, 2003; Williams & Lusseau, 2006; Jacoby et al., 2010; Firth & Sheldon, 2015). Animal populations encounter many anthropogenic disturbances which could crucially disrupt the social connectivity and stability of these populations. Habitat fragmentation disrupts spatial connectivity, which can lead to a decrease or stop in gene flow (Frankham et al., 2002). Anthropogenic noise influences the effectiveness of communication (Halfwerk et al., 2011; Naguib et al., 2013; Gil et al., 2015), which can lead to severe problems for wide-ranging animals, such as marine mammals, that rely on communication to stay connected to family and potential mates. Lastly, the removal of specific individuals from the wild, for human consumption, entertainment or wildlife trafficking, has the potential to destabilize the whole population (Flack et al., 2006; Williams & Lusseau, 2006). Insights into social connectivity within wild animal populations is thus relevant for the effective conservation of animal populations.

Lastly, understanding animal behaviour, especially if it involves animals we can see in our own backyard, can make the general public very enthusiastic. When animal behaviour scientists share their experiences and findings with the general public they often generate more awareness, respect and appreciation for the natural world (e.g. BeleefdeLente.nl). Being aware of the individual animals that are moving around in everyday life and understanding what they do and why they do it, can inspire the very young to the very old.



2

Effects of radio transmitter attachment can be context-dependent

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Marc Naguib

In revision for Scientific Reports

Abstract

The widespread use of novel biotelemetry devices provides unprecedented insights in the behaviour and ecology of many animal species, accompanied by valuable implications for their conservation. However, adding a potential handicap might change the behaviour of the animal being studied. Quantifying the possible impacts of radio transmitter attachment on the animal is essential, but certain effects may become apparent only in particular stressful environments. Here we analysed the consequences of radio transmitter attachment in great tits (*Parus major*) over three environmentally dissimilar years. We found that the transmitters applied before breeding (ca. 5 and 7% of average body weight) in at least two years did not affect the probability of a bird breeding or catching a bird back several months later (apparent survival). Yet, in one year tagged birds were significantly less likely to breed compared to control birds (tags of 5% average body weight). Re-catching probability associated with tag weight in only one year (tags of 5% average body weight), but this was likely confounded by a general negative effect of lower body weights on re-catching probability that year. When tags were attached to parental birds during the chick provisioning period (7% of average body weight) it did not affect the likelihood of re-catching the birds in any of the three years. However, we found a significant effect of tagging birds, leading to immediate brood desertion, in the two out of three years. These were two overall poor years with also many nest desertions in general. Remarkably, in the other “better” year none of the tagged birds deserted. Birds that were tagged when they were provisioning larger broods, especially during relatively low average temperatures, were most likely to desert. Finally, we found no evidence for differences in provisioning behaviour between tagged and non-tagged parents in the year the birds did not desert. This suggests that the initial stress of the tag attachment itself rather than the weight led to the brood desertions. Our study shows that effects of transmitter attachment can be strongly context-dependent. We discuss transmitter attachment in relation to already present stressors and re-evaluate the use of the “5% rule” in biotelemetry studies.

Introduction

Biotelemetry and biologging studies can provide significant and concrete evidence-based advice for conservation measures (Sutherland et al., 2004; Wilson & McMahon, 2006; Cooke, 2008). Recent innovations in biotelemetry and biologging now provide us with unique and increasingly accurate techniques to remotely assess the behaviour and spatial ecology of many animal species (Rutz & Hays, 2009; Hays, 2015; Kays et al., 2015; Wilson et al., 2015a). These spatial data can reveal valuable information on the use of corridors (Jachowski et al., 2013; Pendoley et al., 2014) and specific habitats (Rhodes et al., 2006). For example, biotelemetry studies resulted in important knowledge for the management of key individual trees for roosting white-striped freetail bats (*Tadarida australis*) (Rhodes et al., 2006) and on the effectiveness of marine reserves for flatback marine turtles (*Natator depressus*) (Pendoley et al., 2014). Additionally, knowledge on proximity networks within populations can provide important insights in the risks of disease transmission (Bull et al., 2012; Weber et al., 2013). Also, the use of biological sensors, such as heart rate sensors (Laske et al., 2011) and accelerometers (Payne et al., 2015), can give important facts on changes in experienced stress and energy expenditure of wild animals in response to (human) disturbances (Wilson et al., 2015a).

The life history of species is one especially relevant aspect for which the use of transmitters and loggers can generate valuable insights for conservationists (Cooke et al., 2004; Wikelski & Cooke, 2006; Wilson & McMahon, 2006; Cooke, 2008). Accurate and detailed information on rate of reproduction, age of maturity and life-expectancy are often key in identifying species specific conservation challenges and in developing the appropriate conservation measures (Cooke, 2008). However, if a transmitter is experienced by the animal as a potential handicap, tag-attachment might influence energy investment, reproduction, or survival (Barron et al., 2010) and provide us with inaccurate information on animal behaviour (Wilson & McMahon, 2006; McMahon et al., 2007; Cooke, 2008; Jewell, 2013). For this reason, transmitters are advised to be as light as possible and recommended to not weigh more than 5% of the body weight (Kenward, 2001).

An animal's ability to fully compensate for negatively affected life history traits is likely constrained by physiological mechanisms (Ricklefs & Wikelski, 2002). However, sometimes such constraints in adaptability only become apparent when individuals are pushed to their limits by environmental circumstances (Saraux et al., 2011; Wilson et al., 2015b). This context-dependence provides an extra challenge

for the identification and understanding of potential tag-effects. For example, negative tag-induced effects on the foraging behaviour in Magellanic penguins (*Spheniscus magellanicus*) were clear only in years of low food abundance (Wilson et al., 2015b).

A meta-analysis on avian tag-effects by Barron et al. (2010) concluded that tagging had particularly strong consequences for the energy-expenditure of birds and the likelihood of breeding. Small passerines are usually even more sensitive to such sudden changes in energy-expenditure, with both body mass and the environment as important predictors of how much additional energy a bird can spend (McNab, 2015). Yet, the use of radio-tags in this often elusive group of animals is very useful; besides that passerines can be important conservation targets themselves (Cox et al., 2014), these wide-spread birds also frequently serve as important ecological model species and indicators (Schmidt et al., 2013). Excitingly, modern technology now allows us to automatically track the behaviour of small passerines (<20 g) (Mennill et al., 2012; Hallworth & Marra, 2015). But with this progress also the responsibility arises to carefully investigate and communicate the potential effects such devices might have on these small animals (Hill & Elphick, 2011).

In this study, we therefore investigate potential context-dependent effects of radio transmitters on a small passerine. We radio-tagged great tits (*Parus major*) both before breeding as well as during the chick provisioning period, an energetically demanding period, in three environmental variable years. We subsequently analysed effects of transmitter attachment on the likelihood of catching a bird back several months later (apparent survival), the likelihood of breeding, brood desertion and provisioning behaviour. Great tits are an ideal model species for quantifying such tag-effects since also the apparent survival, reproduction and behaviour of untagged birds can be accurately monitored and permit identification of tag-induced effects.

The data we present here were not gathered with the goal of investigating transmitter-effects, but were part of two behavioural studies. One study investigated the proximity networks of great tits before breeding and the other study investigated the social foraging behaviour of great tits during chick provisioning. Technical limitations in combination with the weight of our study species (approximately 18 g) did not allow us to keep the tag weight below the usually recommended 5% body weight for all three years. Birds were initially tagged with tags of ca. 5% of the body weight, but because of technical limitations

we also applied slightly heavier tags of ca. 7% body weight. However, this is less than the natural daily change in body weight in great tits (1.47 ± 0.25 grams, 7.6% body weight on average)(van Balen, 1967) and provided us with the unique opportunity to quantify potential effects of tagging for varying years as well as for slightly differing tag weights.

Attaching a transmitter to an animal will always cause a certain level of discomfort and this level of discomfort is likely to increase gradually with tag weight. The “5% tag weight rule”, applied as general guideline (Kenward, 2001), seems to find its basis in a study on manoeuvrability of insectivorous bats (Aldridge & Brigham, 1988), yet unequivocal scientific support that this is an appropriate threshold for all biotelemetry studies is sparse (Barron et al., 2010). Several studies reported no negative effects when using devices of more than 5% body weight (Pennycuik et al., 1989; Neudorf & Pitcher, 1997; Barron et al., 2010; Cvikel et al., 2015a). A recent study on foraging behaviour in insectivorous bats used transmitters of 12% body weight and found no deleterious effects (Cvikel et al., 2015a). In the original study (Aldridge & Brigham, 1988), the “5% rule” was given as a general recommendation and the authors themselves give 30% as an absolute upper limit when food is abundant, since pregnant female bats are known to gain up to 30% in body weight and still forage successfully (Aldridge & Brigham, 1988; Cvikel et al., 2015a). The meta-analysis of avian transmitter effects by Barron et al. (2010) did not find an increase in negative effects with tag weights increasing up to 10% body weight. A study analysing effects of long term carrying 5% extra mass, attached via backpacks to male great tits during chick provisioning, found short term effects of lower nestling mass and increased subject mass, but the study did not find effects on subject survival and the production of fledglings or recruits (Atema et al., Submitted).

Despite tremendous effort, for some studies it is technically not feasible to strictly adhere to the 5% recommendation (Neudorf & Pitcher, 1997; Barron et al., 2010; Cvikel et al., 2015a), often due to battery weight limitations. In the meta-analysis of Barron et al. (2010) 10% of the bird studies used tags above 5% body weight. Moreover, less than 11% of studies using radio-tags (less than 7% for conservation studies) reported tests of potential tag effects (Godfrey & Bryant, 2003). Sharing the results of biotelemetry studies may contribute to refining guidelines for acceptable practice in passerine biotelemetry studies (Dougill et al., 2000; Plous & Herzog, 2001; Godfrey & Bryant, 2003; Wilson & McMahon, 2006; Barron et al., 2010; Hill & Elphick, 2011).

Methods

Study population

This study was conducted in Westerheide, a long-term great tit nest box population near Arnhem, The Netherlands (52°01'00.0"N 5°50'20.0"E). Westerheide is a mixed wood forest of about 120 ha with approximately 120 nest boxes. As part of a standardized procedure we checked nest boxes throughout the year and un-ringed individuals were fitted with a uniquely numbered aluminium leg ring. Birds caught for the first time were taken to the laboratory for a maximum of 24 hours. Here they were subjected to a novel environment test to quantify exploration behaviour, an established personality trait in great tits (see Dingemanse et al. (2002) (Dingemanse et al., 2002) for details). During the breeding season we checked nest boxes twice a week and documented laying date, clutch size, start of incubation, number of hatchlings and number of fledglings (see van Oers et al. (2008) (van Oers et al., 2008) for details). To identify the breeding birds we caught the parents inside their nest boxes with spring traps on approximately the 7th (2012) to 10th day (2013-2014) after hatching of the chicks. Nestlings were ringed and measured (body mass and tarsus length) on the fourteenth day after hatching. Permission for this study was granted by the Dutch legal entity: KNAW Dier Experimenten Commissie (DEC) no. NIOO-10.05 to M.N and K.v.O and no. NIOO-12.02 to K.v.O and M.N.

Tagging procedure before breeding

We tagged great tits before the breeding season in three subsequent years (2012, 2013, and 2014) as part of a project studying social networks in great tits (**Chapter 3 & 8**). The radio transmitters were designed for use on small passerines and manufactured as light as possible (Supplementary Figure S1), while still allowing enough battery power for the use of the novel Encounternet technology (Mennill et al., 2012). The leg loops of all the transmitters used in this study were made of a stretchable and porous material to allow the birds to lose their tags, including the harness, usually within three months. We removed the transmitters of birds caught again during breeding (between two and three months after tagging). No tags were observed on birds after five months of tagging. The procedure of fitting the radio transmitter on a bird usually lasted from 30 seconds up to two minutes. The antenna had a total length of 16cm, which is below the suggested maximal length (Dougill et al., 2000). See **Chapter 3** for additional detailed information on the handling, trapping, tagging and housing procedures.

On March 7, 2012 after sunset we performed a routine roost check. Great tits we found in nest boxes were either placed back in their nest box or brought to the

laboratory, depending on the location of the nest box (inside or outside the designated automatic tracking area; both were equally intensively monitored) and on whether the bird had been personality-tested before. The following morning untested birds in the laboratory underwent the novel environment test for a maximum of ten minutes and a subset of the birds were fitted with a leg-looped backpack harness with a radio transmitter (*Mean weight* = 1.03 g; *Mean percentage body weight* = 5.6%, *Range* = 5.0-6.2%, *N* = 34). We released all birds in the afternoon near the nest boxes they were caught in.

On April 2, 2013 we performed a procedure similar to 2012 with the exception that we tagged the birds on the evening of the roost check in the field and returned them to their nest box the same evening. The tagging procedure took place later than in 2012 as a consequence of extremely cold temperatures in March that year (KNMI Deelen weather station: average temperature March 2013 = 1.6°C compared to March 2012 = 7.9°C and March 2014 = 8.1°C). Similar as before, we took a number of (untagged) birds to the lab for personality testing and a number of (untagged) birds were directly returned to the nest box. The great tits were fitted with similar radio transmitters as in 2012 (*Mean weight* = 0.99 g; *Mean percentage body weight* = 5.5%, *Range* = 4.8-6.5%, *N* = 36).

On March 10, 2014 the tagging procedure was similar to 2013. However, due to problems with the battery life of the radio transmitters the previous years we used a more robust (heavier) battery type, which resulted in a slightly heavier radio transmitter (*Mean weight* = 1.30 g; *Mean percentage body weight* = 7.2%, *Range* = 6.3-8.4%, *N* = 44). Some individual birds were tagged twice. Ten of the birds tagged in 2013 had also been tagged in 2012, and in 2014 twelve of the tagged birds had also been tagged in 2013.

Tagging procedure during chick provisioning

To study parental food provisioning we also radio-tagged great tit pairs with chicks (first broods) during the same three years. On May 11 and May 14 2012 we fitted a total of eleven pairs with transmitters on the seventh or eighth day (one pair on the ninth day) after hatching of the chicks. Because of previous issues with the battery life of the tags earlier in the season, we used a radio transmitter with a different battery compared to March 2012 (*Mean weight* 1.29 g; *Mean percentage of body weight* = 7.3%, *Range* = 6.8-7.8%, *N* = 22).

Between June 1st and June 10th 2013 we radio-tagged seven great tit pairs following a similar procedure to 2012, but we caught parents on the tenth day after hatching of the chicks (instead of after seven to eight days as in 2012). By delaying the catching day we hypothesized that parents would be less likely to desert the

nest because of the increased time and energy invested in the brood (*Mean* tag weight 1.29 g; *Mean* percentage body weight = 7.3%, *Range* = 6.6-7.7%, *N* = 14).

Based on the positive experience in 2013 we radio-tagged a total of four great tit pairs in 2014, also on the 10th day after hatching of the chicks, using the same tags as in 2013 (between May 9th and 13th 2014; *Mean* weight 1.29 g; *Mean* percentage body weight = 7.4%, *Range* = 7.1-7.9%). Eight parental provisioning subjects we tagged twice, four birds we had tagged earlier in the season (2012) and four birds a year before (2014). We tagged birds between 10.50 AM to 02.10 PM and each year we caught the pairs that did not desert their nest two to three days later to remove their radio transmitters. We qualified a nest as deserted when the total brood had died within two days after the tagging procedure.

Provisioning behaviour

In 2013, we used video recordings within the nest box of tagged and non-tagged couples to examine potential effects of tagging on chick provisioning behaviour. One female of the seven tagged great tit pairs in 2013 lost her tag before the recording and we therefore excluded the complete pair from the provisioning behaviour analysis. The cameras were inserted in nest box lids, which were used to replace the original lids. The camera lids were placed at least five days before the tagging procedure to give the parents time to habituate to the camera. The camera was connected to a battery and a video-recorder (Archos 405, 30 GB) between 7.00 AM and 8.00 AM the morning following the tagging procedure. All the recording equipment was placed in a black plastic box (20 x 30 x 20 cm) and hidden underneath leaves and branches on the opposite side of the nest box. The tagged nests comprised five nests with seven nestlings and one nest with six nestlings, and were video-recorded on the eleventh day after hatching. We recorded six additional nests following the same procedure, including catching parents on the tenth day after hatching. These procedural control nests, controlled for capture and restraint, included three nests with seven nestlings and three nests with six nestlings. All recordings were made between the 1st and 10th of June 2013. We used The Observer XT10.5 (Noldus, Wageningen, The Netherlands) to analyse the video material. We quantified the feeding rate (feedings/min) and the mean visit duration (sec) for both parents. One person observed all the videos. Of each nest, 60 minutes was analysed between 9.30 and 10.30 AM (one exception of 45 minutes due to recording problems).

To test potential differences in quality of nest box location between tagged parents and control parents we approximated the location quality as the mean number of fledged chicks per nest box by unique pairs over the last ten years

(2005-2014). There were no substantial differences in the average number of fledged chicks per nest box in previous years (Independent T-Test; $t_{10} = 0.44$, $P = 0.67$), between nest boxes of tagged and non-tagged birds.

Statistical analysis

Likelihood of re-catching (apparent survival)

As a proxy for survival we analysed whether an individual tagged before breeding was less likely to be observed (caught) again the subsequent breeding season or any time thereafter (at least 2 months later) compared to two procedural controls for capture and restraint: (1) handled in the field on the same night but left in the nest box and (2) brought to the laboratory on the same night and released next day close to their nest box (Supplementary Table S1). The second procedural control (brought to the lab) is the more appropriate control in 2012, when we also took the tagged birds to the lab. The first procedural control (handled in the field) is the more appropriate control for the tagged birds in 2013 and 2014, as birds were tagged in the field in these years. We analysed observations up to March 10, 2015, retrieved from standardized roost checks (December and March each year) and nest checks (April to June each year) and we used Fisher exact tests to compare the probabilities of re-catching a bird. Similarly for birds tagged during chick provisioning, we compared the probability of observing (catching) a bird after at least five months between tagged parents and untagged (but caught) parents (Supplementary Table S2). Additionally, for the birds tagged before breeding, we tested with a logistic regression whether the likelihood of observation for each year decreased with increasing tag weight (percentage body weight). Finally, we tested whether this potential effect was related to an overall effect of bodyweight (g) or the tagging procedure with a generalized linear model (GLM) with logit function, including bodyweight, treatment and their interaction as explanatory variables. Because we did not weigh the birds we merely handled during the roost check (procedural control 1) only birds we took to the lab (procedural control 2) could serve as a control for effects of tag weight.

Likelihood of breeding

To test whether birds tagged before breeding were less likely to breed that year we conducted a similar analysis as discussed above, but considering only data on birds caught during chick provisioning in the subsequent breeding season (Supplementary Table S3).

Brood desertion

With Fisher exact tests we analysed whether the likelihood of desertion increased due to the tagging procedure by comparing tagged parents to parents that we caught (but not tagged) on approximately the 7th (2012) to 10th day (2013-2014) after chick hatching of each year (Supplementary Table S4). We analysed which factors best explained whether or not tagged parents deserted their nest with a logistic regression pooling the data for all three years. Environmental variables were retrieved from the nearby (< 10 km) KNMI weather station in Deelen (Supplementary Table S5). We used average temperature on the day of tagging, rainfall (mm) on the day of tagging, age of the chicks, brood size, body condition of the male and female (the residual of weight over tarsus) and tag weight (percentage of body weight) of the male and the female as explanatory variables. Due to the relatively low sample size and the low variation in the explaining variables we could only fit a maximum of two variables into the model. Therefore we conducted a forward stepwise model selection, starting with a model containing a single variable with the best explanatory power (lowest Akaike information criterion (AIC) and highest significance). Subsequently we performed model comparisons and with likelihood ratio tests (*Chi-square*) to test if the starting model significantly improved when adding a potential explanatory variable. Finally, we likewise tested whether the final model was significantly better than a null-model.

Provisioning behaviour

Potential effects of tagging on chick provisioning behaviour were analysed by conducting two linear mixed model (LMM) analyses with either feeding rate or mean visit duration as dependent variables. We used treatment (tagged or control), sex and brood size (six or seven) as explanatory variables and nest box identity was used as a random factor. We tested differences in average chick body weight, chick tarsus length and territory quality between tagged nests and untagged nest with Independent T-Tests.

Normal distribution of the data and the residuals of the models were tested with the Shapiro-Wilk test. We used parametric tests when distributions were not significantly different from a normal distribution ($P > 0.05$). Post-hoc test were conducted for group comparison when $P < 0.1$. All statistical tests were performed in R Studio Version 0.98.1103 (RStudio, Inc.) or SPSS version 22 (IBM, Corp.).

Results

Likelihood of re-catching (apparent survival)

Birds tagged before breeding were as likely to be caught back as control birds (Table 1; Supplementary Table S1). This was also the case for birds tagged during chick provisioning (2012: $P = 0.82$, 2013: $P = 0.76$, 2014: $P = 0.72$; Supplementary Table S2). Birds carrying relatively lighter radio transmitters before breeding in 2012 and 2014 were not more likely to be re-caught (Logistic regression; 2012: $b = -1.21$, $SE = 1.62$, $P = 0.46$; 2014: $b = -0.97$, $SE = 0.87$, $P = 0.26$). However, tag mass as percentage of body weight had a significant negative effect on re-catching a bird that was tagged in 2013 (Logistic regression; $b = -2.96$, $SE = 1.39$, $P = 0.03$). Still, this effect could have been driven by the fact that in 2013, birds with a lower body weight had an overall lower probability of being caught back (Logistic regression; $b = -0.94$, $SE = 0.37$, $P = 0.01$). In support of this there was no significant interaction effect between treatment (tagged or control) and body weight (GLM; $b = -0.19$, $SE = 0.95$, $P = 0.84$).

Table 1. Fisher exact test p-values for comparing the proportion of tagged birds observed in the next breeding season or thereafter to procedural controls.

	Handled outdoors	Brought indoors	Overall
2012			
Brought <i>indoors</i> and tagged	$P = 0.77$	$P = 0.70$	$P = 0.67$
2013			
Handled <i>outdoors</i> and tagged	$P = 0.41$	$P = 0.51$	$P = 0.30$
2014			
Handled <i>outdoors</i> and tagged	$P = 1.00$	$P = 0.52$	$P = 0.75$

Likelihood of breeding

Birds tagged before breeding in 2012 and 2014 were as likely to raise nestlings as untagged birds (2012: $P = 0.38$; 2014: $P = 0.80$; Supplementary Table S3). Yet, birds tagged in 2013 were significantly less likely to raise nestlings (*birds handled outdoors and tagged* compared to *birds handled outdoors but not tagged*: $p = 0.03$; Supplementary Table S3). However, it is important to note that the proportion of tagged birds not raising chicks was equal to the proportion of birds not raising chicks after they had been taken indoors for one day (*birds handled outdoors and tagged*: 24/36, compared to *birds handled indoors but not tagged*: 8/12; Supplementary Table S3). The weight of the radio transmitter as percentage of bodyweight did not affect the likelihood of breeding within the same year (Logistic

regression; **2012**: $b = -1.24$, $SE = 1.56$, $P = 0.43$; **2013**: $b = -0.13$, $SE = 1.21$, $P = 0.91$, **2014**: $b = -0.47$, $SE = 0.77$, $P = 0.54$).

Brood desertion

The percentage of control birds deserting their nests tended to vary between the years (14%, 5% and 17% respectively; Fisher exact test; $P = 0.09$) and was significantly lower in 2013 compared to 2014 (Fisher exact test; $P = 0.04$) but not compared to 2012 (Fisher exact test; $P = 0.13$).

The percentage of birds that deserted their nest after a tagging procedure during chick provisioning varied significantly between the years (55%, 0% and 75% respectively; Fisher exact test; $P = 0.02$). In 2012 and 2014 tagged parents significantly more likely deserted their nest compared to parents that were caught but not tagged (Fisher exact test; 2012: $P = 0.003$, 2014: $P = 0.02$). In 2013 there was no effect of tagging on nest desertion (Fisher exact test; $P = 1.00$). A bootstrap analyses revealed that the negative results in 2012 and 2014 were not the consequence of chance effects due to small sample sizes. The percentage of nests deserted by tagged parents in 2012 (55% based on 11 tagged nests) fell outside the 99% confidence interval for desertion of nests by untagged parents (99% $CI = 0-45\%$ desertion, drawing 11 out of 124 control nests, 10000 bootstraps with replacement). Likewise, the percentage of nest deserted by tagged parents in 2014 (75% based on 4 tagged nests) fell outside the 95% confidence interval for desertion of nests by untagged parents (99% $CI = 0-50\%$ desertion, drawing 4 out of 81 control nests, 10000 bootstraps with replacement).

Overall, tagged parents with more nestlings more likely deserted their nest (Logistic regression; $b = 1.80$, $SE = 0.79$, $z = 2.26$, $P = 0.02$, $AIC = 21.82$) and the likelihood of brood desertion tended to increase with lower average temperatures (Logistic regression; $b = -0.41$, $SE = 0.24$, $z = -1.74$, $P = 0.08$, $AIC = 30.82$). Additionally, tagged females in lower body condition were more likely to desert the nest (Logistic regression; $b = -1.74$, $SE = 0.87$, $z = -2.01$, $P = 0.04$, $AIC = 28.24$). The combination of brood size and the average temperature ($AIC = 19.83$) was the best predictor of brood desertion (Model analysis of variance (ANOVA) with best starting model (brood size): *Deviance* = 3.99, $P = 0.046$; Figure 1). The daily amount of rain (mm), nestling age, male body condition and the tag weight as percentage body weight for both male and female were not significant predictors for brood desertion (Logistic regression; all $P > 0.1$). Average temperature and female body condition did not significantly associate with brood size (Spearman Rank Test; all $P > 0.1$). Thus over the three years, parents with a large brood and tagged during relatively low temperatures were most likely to desert their nests (Odds ratio for

brood size = 8.6; 95% CI = 1.25-58.56; Model ANOVA against null model; *Residual deviance* = 15.93, $P = 0.0003$).

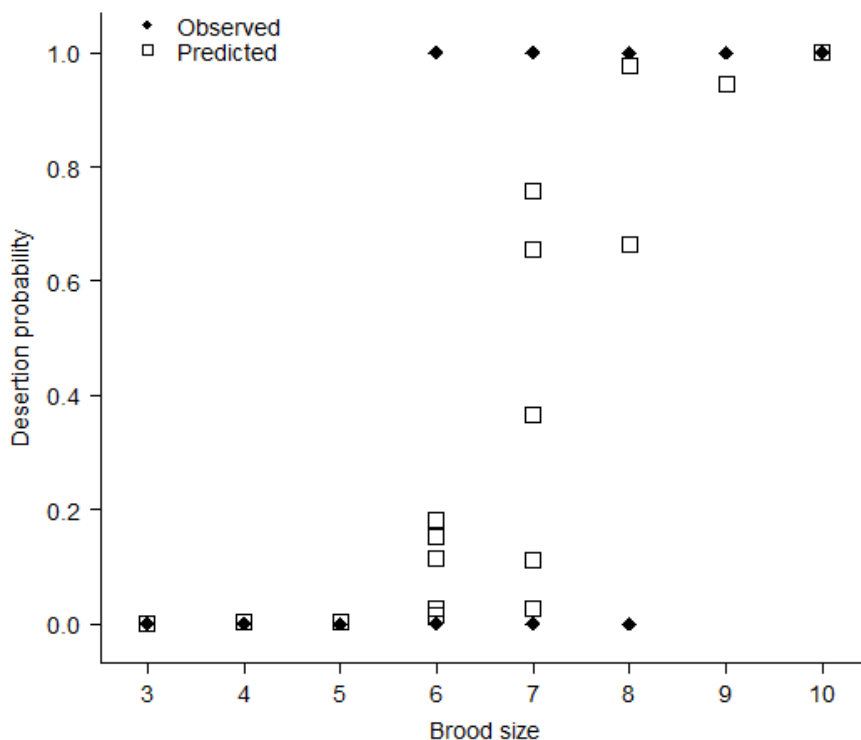


Figure 1. Probability of nest desertion in relation to brood size for observed values (closed diamonds) and predicted values by the final model (open squares).

Provisioning behaviour

Tagged parents with eleven-day old chicks in 2013 had similar feeding rates (Linear mixed model; $F_{1,10} = 0.83$, $P = 0.38$, *Mean* feeding rate = 0.46/min; $SD = 0.15$; Figure 2a) as non-tagged parents (*Mean* feeding rate = 0.40/min; $SD = 0.16$). Likewise, tagged parents, spent not more time in the nest box during visits (Linear mixed model; $F_{1,10} = 1.90$, $P = 0.198$, *Mean* visit duration = 14.98s; $SD = 9.30$; Figure 2b) compared to non-tagged parents (*Mean* visit duration = 11.04s; $SD = 5.46$). There was no effect of brood size (6 or 7 chicks) on both feeding rate ($F_{1,9} = 0.29$, $P = 0.60$) and mean visit duration ($F_{1,9} = 0.01$, $P = 0.91$). Male and females did not differ in feeding rate ($F_{1,11} = 0.002$, $P = 0.97$), but females did stay longer during visits ($F_{1,11} = 6.95$, $P = 0.023$; Figure 2b). There were no significant differences between tagged and non-tagged nests in the average weight of the chicks at fourteen days of age

(Independent T-Test; $t_{10} = 0.32$, $P = 0.76$) or the average tarsus length (Independent T-Test, equal variances not assumed; $t_{8.577} = 1.798$, $P = 0.11$).

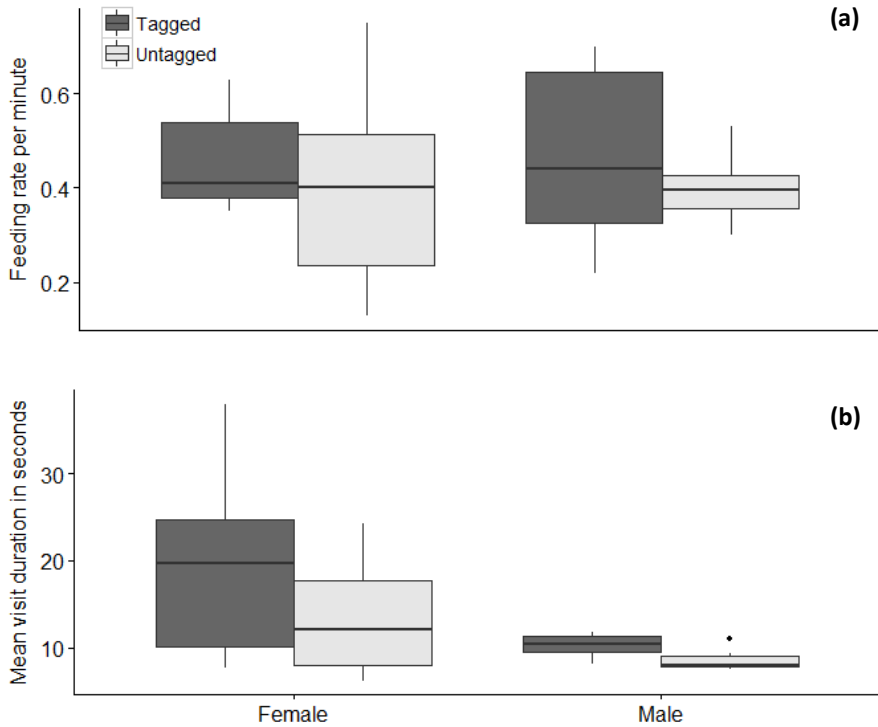


Figure 2. Difference between tagged (dark grey) and untagged (light grey) parents for male and female great tits in (a) feeding rate per minute and (b) mean visit duration in seconds.

Discussion

Our study revealed no negative effects of tagging on the apparent survival of our passerine species, regardless of tag weight. Yet, for one year, while using lighter tags (ca. 5% body weight), we documented a negative effect on the likelihood of breeding. Additionally, while using slightly heavier tags (ca. 7% body weight), we found an effect of tagging the birds during the nestling phase, with substantial desertion rates in the two poorer years out of the three study years. In contrast, in one (better) year none of the tagged parents deserted the nest, while the tag weights for parents were the same in each year. The brood size and the average temperature during tagging best predicted whether a nest would be deserted. In

the year that the tagged birds did not desert their nest, the parents did not differ in their feeding rate and mean visit time from untagged parents.

The tags used before breeding in the third year (2014) and for all years during breeding (2012-2014) were heavier (ca. 7% body weight) than the tags used in the first two years (2012-2013) before breeding (ca. 5% body weight). Yet all added tag weights were within the natural daily range of body weight changes for great tits (Van Balen, 1967) and within the range of tags applied in previous radio tracking studies on song birds (Neudorf & Pitcher, 1997; Barron et al., 2010). The only year we found a negative effect of tagging on the likelihood to breed was 2013, thus when we used the lighter tags (ca. 5% body weight). Spring temperatures around the time of tagging in 2013 were extremely low (> 6°C lower average temperature compared to 2012 and 2014), which might have influenced the initiation of breeding for tagged birds. Yet, the proportion of tagged individuals found breeding did not differ from birds that were taken away from their territories for one day, suggesting that birds overall were more stress sensitive to any disturbance in that year.

We found no effect on probability of re-catching (apparent survival) or likelihood of breeding in 2012 (5% tags) and in 2014 (7% tags). Additionally, we found no effects on apparent survival for the birds tagged during breeding (2012-2014, 7% tags). Our finding that tag attachment did not affect apparent survival for birds tagged with 5% tags and 7% tags agrees with a similar study that for three subsequent years attached 5% extra mass to male great tits and did not find effects on survival (Atema et al., Submitted). Only in one year we found a significant effect of tag weight as percentage body weight on apparent survival (2013, 5% tags), however this effect could not be disentangled from effects of body weight *per se*, since lighter birds in the relatively cold spring of 2013 had a lower probability in being caught back in general. Especially lighter birds might had to spend more energy on thermoregulation in the cold spring of 2013, consequently decreasing their survival chances. Interestingly, we did not detect an effect of the tag weight (as percentage of bodyweight) in 2014 even though those tags were heavier (*Range* = 6.3-8.4% body weight) compared to the tags used in April 2013 and March 2012 (*Range* = 4.8-6.5% body weight). Finally, we found no effects of tag weight on the likelihood to breed in any of the three years. This is in line with Barron et al. (2010), who did not find an increase in negative effects with heavier devices. Overall, these result suggest that for great tits tagged in spring the use of 7% body weight tags does not lead to more substantial tag effects on breeding initiation and apparent survival compared to 5% body weight tags.

Many studies on tag-effects focus on survival, which is logical, since effects on reproduction and specific behaviours are difficult to test when biotelemetry is used to obtain more insights in these behaviours in the first place (Roth et al., 2009; Mennill et al., 2012). Yet, here we show, in accordance to previous studies (Jewell, 2013), that there can still be negative effects on behaviour and reproduction even when no effect on survival is found (Wilson & McMahon, 2006). In all three years of our study there were no effects of tagging on the likelihood of catching parents back, yet tagging during nestling provisioning caused significant desertion in two of these three years. When quantifying potential tag-effects, efforts should thus be made to not only quantify effects on survival, but also on the behaviour of the animal.

The extremely varying results of the effects of tagging on reproduction are remarkable, since we used the same tag weights in all three years. Our bootstrap analysis shows that the varying desertion rates could not be explained by the smaller sample sizes for tagged birds. Yet, we cannot rule out that using tags exactly under 5% body weight would have resulted in an absence of significant desertion rates. For example, there was no significant effect on nestling survival within eight days after the 5% body weight backpacks were attached in the study of Atema et al. (Submitted). However, in that study only the male of a breeding pair was tagged, which will likely have led to lower desertion rates compared to tagging both parents. Effects on provisioning behaviour have also been found for birds tagged with transmitters below 5% of their body weight (Ackerman et al., 2004; Barron et al., 2013) and have been found absent for individuals tagged with transmitters above 7% body weight (Neudorf & Pitcher, 1997). These findings suggest that not the tag weight *per se*, but rather the procedure of tagging, such as the timing of tagging, is most relevant for predicting substantial tag effects (Dougill et al., 2000; Barron et al., 2010; Hill & Elphick, 2011). Next to this study there is evidence showing that also very light tags (< 3% body weight), and even flipperbands, can have significant consequences in one year but not in another (Bro et al., 1999; Saraux et al., 2011; van Wijk et al., 2015; Wilson et al., 2015b).

During the breeding season of 2013 none of the tagged parents deserted their nest. Selection of higher quality birds at the start of this cold year would be an appropriate explanation, since then only high quality individuals would be breeding, but we did not find strong evidence for this. For example, although there was a significant effect of female body condition on the likelihood of desertion, this was not the best explaining factor. Moreover, this effect became non-significant when the model included brood size. Unfortunately our sample size was too low to

be able to examine the effect of adding female condition to the final model which included both brood size and average temperature. Thus female body-condition could have played an additional role in affecting brood desertion.

The strong negative effect of brood size was surprising because we expected birds with many chicks to be less likely to desert, since their “investment loss” would be higher. However, birds with many chicks, especially in unexpected poor years, probably experience higher stress levels. Adding an additional load might have pushed them over their limits (Wilson et al., 2015b). The year 2014 turned out to be the most extreme breeding seasons over the last fifteen years in our study area (personal communication P.d.G., Supplementary Table S5), with 17% of the caught but untagged parents deserting (Supplementary Table S4). Also 2012 was a relatively bad year with 14% abandonment of caught but untagged birds, while in 2013 there was only 5% desertion by untagged birds (Supplementary Table S4). These percentages suggest that even untagged great tits in 2012 and 2014 were more likely to give up their broods. Given that the tagged parents deserted within two days already, it is very well possible that the overall disturbance of tagging, rather than the tag weight itself, let to their decision to desert. Seemingly in contrast to our significant desertion rates, Barron et al. (2010) did not uncover evidence that tagged birds invested less in caring for offspring for the benefit of self-preservation. However, their results could be biased if the analysis only included individuals that did not give up starting a nest in the first place, since the authors did find a significant effect of tagging on the probability of nesting.

Our analysis of provisioning behaviour also suffers from this selection issue, since it only includes parents that did not desert their brood. Still, it is noteworthy that we did not find any significant difference in provisioning effort between tagged and untagged parents. Tagged parents might be expected to show a lower provisioning effort due to increased energy demands resulting from the attached tags. Yet we did not find a decrease in provisioning effort or chick condition for tagged parents. In contrast, if the tagging procedure reduced the perceived chance of future offspring, tagged parents might be expected to actually increase their investment in current offspring (Trivers, 1972) and thus their provisioning effort. However, also this scenario appears not applicable, because the tagging procedure caused parents to give up provisioning all together in two out of three years and the tagging procedure did not affect apparent survival of the parents. There is still the possibility that the tagging procedure significantly affected provisioning effort in the other two years. Unfortunately, we do not have the video data available to test this. Moreover, although we did not observe a difference in provisioning behaviour

in 2013, there is still a chance that tagged parents had to invest more energy to keep up a sufficient feeding rate. Trade-offs in energy investment caused by external stressors are crucial to understand in order to estimate the impact of additional stressors such as transmitter attachment (Wikelski & Cooke, 2006; Cooke, 2008; Cooke et al., 2014).

Our study shows that transmitter-effects on the ecology and behaviour of a small passerine can be context-dependent, at least for tags that range between 5% to 7% body weight. We therefore argue that variable environmental stressors should be considered when evaluating potential negative consequences of tagging procedures, especially when species with high conservation priority are concerned (Godfrey & Bryant, 2003). To get the best out of the tremendous potential of biotelemetry and biologging (Cooke et al., 2004; Cooke, 2008; Hallworth & Marra, 2015; Wilson et al., 2015a), while reducing the potentially negative effects as much as possible, it thus is important to consider the context of tagging. Moreover, in the case that there are negative effects to report these, including information on the environmental circumstances and the tag weight. Finally, the general application of the “5% rule” needs to be supported by more scientific evidence and should be interpreted as a recommendation rather than as a fixed rule, taking into account varying environmental variables and the reproductive status of the study species.

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Supplement to Chapter 2

Table S1

Table S1. The number of birds (*percentage*) observed during nest box checks in subsequent breeding seasons or thereafter in relation to the experimental treatment.

Treatment	Observed	Not observed	Total
2012			
Handled outdoors	12 (86%)	2 (14%)	14
Brought indoors	18 (72%)	7 (28%)	25
Brought <i>indoors</i> and tagged	26 (76%)	8 (24%)	34
2013			
Handled outdoors	15 (68%)	7 (32%)	22
Brought indoors	5 (42%)	7 (58%)	12
Handled <i>outdoors</i> and tagged	20 (56%)	16 (44%)	36
2014			
Handled outdoors	35 (76%)	11 (24%)	46
Brought indoors	10 (67%)	5 (33%)	15
Handled <i>outdoors</i> and tagged	33 (75%)	11 (25%)	44

Table S2

Table S2. The number of unique parents (*percentage*) observed during nest box checks in subsequent breeding seasons or thereafter in relation to only being caught or being caught and tagged.

Treatment	Observed	Not observed	Total
2012			
Parents caught	101 (46%)	117 (54%)	218
Parents caught and tagged	11 (50%)	11 (50%)	22
2013			
Parents caught	70 (62%)	43 (38%)	113
Parents caught and tagged	8 (57%)	6 (43%)	14
2014			
Parents caught	76 (52%)	71 (48%)	147
Parents caught and tagged	5 (63%)	3 (37%)	8

Table S3

Table S3. The number of birds (*percentage*) observed raising chicks of at least seven days of age in the subsequent breeding seasons in relation to the experimental treatment.

Treatment	Observed	Not observed	Total
2012			
Handled outdoors	12 (86%)	2 (14%)	14
Brought indoors	16 (64%)	9 (36%)	25
Brought <i>indoors</i> and tagged	25 (74%)	9 (26%)	34
2013			
Handled outdoors	14 (64%)	8 (36%)	22
Brought indoors	4 (33%)	8 (67%)	12
Handled <i>outdoors</i> and tagged	12 (33%)	24 (67%)	36
2014			
Handled outdoors	25 (54%)	21 (46%)	46
Brought indoors	9 (60%)	6 (40%)	15
Handled <i>outdoors</i> and tagged	27 (61%)	17 (39%)	44

Table S4

Table S4. The number of couples deserting or not deserting (*percentage*) after the parents were only caught or caught and tagged (also includes second nesting attempts for untagged birds, but not tagged birds).

Treatment	No desertion	Desertion	Total
2012			
Parents caught	107 (86%)	17 (14%)	124
Parents caught and tagged	5 (45%)	6 (55%)	11
2013			
Parents caught	55 (95%)	3 (5%)	58
Parents caught and tagged	7 (100%)	0 (0%)	7
2014			
Parents caught	67 (83%)	14 (17%)	81
Parents caught and tagged	1 (25%)	3 (75%)	4

Table S5

Table S5. Daily environmental variables for the days of the transmitter attachment (source: KNMI Deelen (NL) weather station). Average temperature and rainfall were used as predictor values in the analysis for brood desertion.

Year	Average Temperature (°C)	Minimum Temperature (°C)	Sun hours	Rain hours	Rainfall (mm)
2012					
Mean	13.18	6.55	4.93	0.00	0.00
SD	1.40	1.12	5.18	0.00	0.00
N	11	11	11	11	11
2013					
Mean	14.04	5.93	11.43	0.00	0.00
SD	2.69	2.25	2.35	0.00	0.00
N	7	7	7	7	7
2014					
Mean	10.15	5.93	5.05	4.20	7.68
SD	1.1	2.47	3.51	6.23	10.99
N	4	4	4	4	4

Figure S1



Figure S1. A wild great tit carrying an Encounternet transmitter. The smaller pictures shows the radio-transmitters just before they are attached to the birds.



3

Social networking in territorial great tits: slow explorers have the least central social network positions

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Abstract

In various animal species individuals differ consistently in their behaviour, often referred to as personality. In several species these personality differences also correlate with differences in social behaviour. This is important as the social environment is a key selection pressure in many animal populations, mediated, for example, via competition or access to social information. Using social network analysis, recent studies have furthered our understanding of the role of personality in the social environment, usually by focusing on swarming or flocking populations. However, social associations in such populations are fundamentally different from those in territorial populations, where individuals meet less frequently and where the costs and benefits of spatial associations differ from those for swarming or flocking species. In this study we therefore tested whether social network position is related to individual differences in exploration behaviour, an established measure of an avian personality trait, using a wild, territorial, personality-typed great tit, *Parus major*, population. By means of novel, large-scale, automated tracking (Encounternet) we show, while controlling for average territory distance, that slower exploring males had less central social network positions. Yet, slower explorers overall did not travel shorter distances than faster explorers, indicating that a less central social network position was not merely a consequence of lower activity. Finally, males with less central social network positions did not have reduced breeding success compared to males with more central positions. Our results suggest that territorial individuals influence the structuring of their own social environment in relation to their personality. This is relevant, because the establishment of social relations and familiarity with possible competitors is predicted to be important in many territorial populations.

Introduction

The social environment in animal populations imposes a fundamental selection pressure on the fitness of individuals (Smith & Parker, 1976; Wolf et al., 1999). Thus, it is relevant to understand whether and how individuals influence the structure of this social environment. Socially relevant behaviours have been found to vary in relation to personality, defined as consistent individual differences in behaviour (Groothuis & Carere, 2005; Pike et al., 2008; Amy et al., 2010), and can, vice versa, vary depending on the social context (van Oers et al., 2005; Réale et al., 2007; Webster & Ward, 2011; Wolf & Krause, 2014). For example, in great tits, *Parus major*, a species in which boldness correlates positively with exploration behaviour (Groothuis & Carere, 2005), slow explorers are more strongly affected by social stress (Carere et al., 2003) and differ from fast explorers in the strength of their response to social confrontations (Carere et al., 2005; Amy et al., 2010). Additionally, slow exploring males are found to adjust risk-taking behaviour to the behaviour of a companion, whereas fast males do not (van Oers et al., 2005). Personality differences are thus likely to have an influence on the social structuring of a population (Krause et al., 2010; Wolf & Krause, 2014).

A relatively novel approach in animal behaviour for investigating the role of specific individual characteristics within the structuring of the social environment is social network analysis (Croft et al., 2008). In a social network individuals are represented as nodes that can be connected to each other via social associations or interactions, represented by edges. By using social network analysis, the position of an individual within the social structure can be quantified. Consequently, it can be analysed whether this social network position is related to a certain characteristic of an individual, such as its personality. A limited number of studies have examined how personality differences could affect the social network structuring in animal populations. In a study of guppies (*Poecilia reticulata*) social associations were assorted in relation to individual boldness (Croft et al., 2009). Also, in a study analysing winter feeder visits of non-territorial great tits, the most central network positions were occupied by the faster exploring individuals (Aplin et al., 2013). Findings on the relationship between personality and social network position are very relevant for understanding the costs and benefits of personality types, as social network studies have revealed that an individual's social network position can predict social rank (McDonald, 2007), discovery of new foraging patches (Aplin et al., 2012), acquisition of novel feeding strategies (Allen et al., 2013) and mating success (Oh & Badyaev, 2010).

These social network studies have greatly advanced our knowledge of social network dynamics but have primarily focused on swarming or flocking populations (Croft et al., 2009; Krause et al., 2009; Oh & Badyaev, 2010; Aplin et al., 2012; Aplin et al., 2013). However, social associations are highly relevant also in non-grouping contexts such as in territorial populations (Getty, 1987), yet very little is known about the social network structuring of such populations. Territorial individuals are often limited in having many spatial associations with conspecifics, yet these spatial associations with conspecifics can increase access to social information, such as on local resources, competition and threats (Amrhein et al., 2004a; Aplin et al., 2012; Krama et al., 2012), information that is similarly relevant for territory owners. Additionally, spatial associations at the start of the territory season may allow territory owners to familiarize themselves with each other. When individuals, later in the season, recognize each other as territory owners instead of as strangers, who could be trying to take over a territory, they are predicted not to escalate interactions among each other, as the payoff from winning would not balance the energetic costs and risks of injury (Smith & Parker, 1976). Indeed, territorial songbirds have the ability to remember and distinguish between familiar and unfamiliar neighbours (Godard, 1991; Akçay et al., 2009; Grabowska-Zhang et al., 2012) and neighbour familiarity has been found to correlate positively with reproductive success in several territorial songbirds (Beletsky & Orians, 1989; Grabowska-Zhang et al., 2011).

Thus, if certain personality types associate less with surrounding conspecifics, as a result of a general weaker tendency to approach other individuals (Carere et al., 2005), this could have consequences for finding local resources, because of decreased access to social information (Aplin et al., 2012; Allen et al., 2013) and for individual energy expenditure on territory defence, because of reduced familiarity with nearby territory owners (Getty, 1987; Temeles, 1994). Nevertheless, it is not well understood whether and how personality and social network position are correlated within territorial systems. Knowing whether such a relationship exists will provide insights into the social consequences of personality differences (Wilson et al., 2013) as well as into the social structuring of territorial populations (Wolf & Krause, 2014).

Hence, in this study we tested, at the start of the breeding and territory season, whether male great tits differ in spatial social network position depending on their exploration behaviour, an established operational measure for a personality trait (Groothuis & Carere, 2005; Réale et al., 2007). Exploration behaviour in great tits

not only relates to social behaviour (Carere et al., 2003; Carere et al., 2005; Amy et al., 2010), it also explains substantial variation in a wide range of other behaviours and life history traits, including general risk-taking behaviour (van Oers et al., 2004), cognitive performance (Titulaer et al., 2012), behavioural response to stressors (Naguib et al., 2013), extra-pair paternity (van Oers et al., 2008), reproductive success (Both et al., 2005) and survival (Dingemanse et al., 2004). Furthermore, slower explorers show a faster and higher endocrine stress response to standardized stressors (Baugh et al., 2013). Therefore, as slow explorers are known to take fewer risks (van Oers et al., 2004) and spatial associations in territorial populations are probably not without risk, we predicted slower exploring territorial males would have less central social network positions. This prediction is also in line with results from earlier studies, such as (Amy et al., 2010), in which fast exploring male great tits showed a closer approach to a simulated territory intruder and a more recent study measuring spatial associations at feeding stations of great tits in winter feeding flocks (Aplin et al., 2013), which showed that slower individuals had fewer unique contacts.

To determine spatial social network position, we used a novel automatized tracking system (Encounternet) at the initiation of the breeding season to track wild territorial male great tits, which had been tested earlier for exploration behaviour. By simultaneously tracking neighbouring birds we quantified close-range encounters between these individuals. Additionally, we quantified breeding success at the end of the breeding season to test whether strength of social network position is linked to components of fitness. A more central social network position could lead to increased access to social information about potential food sources and the whereabouts of aggressive competitors, which may be important for efficiently providing food to offspring, leading to a higher breeding success.

Methods

Study system

We conducted this study at our long-term study population of great tits at Westerheide near Arnhem, The Netherlands (52°01'00.0"N 5°50'20.0"E). Westerheide is a mixed pine–deciduous wood with approximately 200 nest boxes distributed within a 1000 m x 1200 m area (see (Dingemanse et al., 2002) for details). Using a routine procedure, we tested birds for exploration behaviour (Verbeek et al., 1994), an established operational measure for personality traits (Réale et al., 2007), using a novel environment test (Dingemanse et al., 2002; Groothuis & Carere, 2005).

Novel environment test

In a routine procedure, 61 great tits were taken from their roosting boxes, after sunset, in March 2012 and transported to the bird housing facilities at the Netherlands Institute of Ecology, where they were weighed and housed individually in cages (0.9 x 0.4 m and 0.5 m high), with a solid bottom and top, solid side and rear wall, a wire-mesh front and three perches. We provided food and water ad libitum. The following morning, exploratory behaviour of 22 untested birds was measured using the novel environment test following (Verbeek et al., 1994). Because birds are tested only when they are caught for the first time (as the test would no longer be testing the reaction towards a novel environment when retesting), only 22 of 61 birds were tested, since 39 birds had already been tested earlier. Birds were individually tested in a room (4.0 x 2.4 m and 2.3 m high) with five artificial trees. After they entered the experimental room, we recorded the total number of flights (movements between trees) and hops (movements within trees) within the first 2 min. The movements of the birds in the experimental room were subsequently used to calculate an overall exploration score ranging from low (slower explorers) to high (faster explorers; (Dingemanse et al., 2002).

As we do not have repeated exploration measures, our study design did not permit us to investigate in more detail the between- and within-individual variation in behavioural types, which has recently been suggested as an alternative procedure in the personality literature (Dingemanse & Dochtermann, 2013; Dall & Griffith, 2014). However, exploration behaviour in this population is established as a highly repeatable proxy for behavioural traits in wild great tits (Dingemanse et al., 2002). Moreover, a similar study using exploration score to explain territory defence (Jacobs et al., 2014) did not find a qualitative difference in the results when comparing the use of the first score with multiple scores of individual exploration behaviour. Therefore we have good evidence that our measurement of exploration behaviour reflects substantial between-individual variation.

Reproductive success

During the breeding season, using a standardized procedure (van Oers et al., 2008), we checked nest boxes twice a week to identify the breeding birds and quantify their breeding success. The main recorded breeding measures were laying date, clutch size, start of incubation, number of hatchlings and number of fledglings. Adults were caught inside their nest boxes using spring traps (metal doors that close the nest hole when the bird enters the nest box) on approximately the 7th day after hatching of the chicks. We caught nestlings on the 14th day after

hatching. We fitted unringed individuals with uniquely numbered aluminium leg rings and measured body mass and tarsus length.

Spatial network tracking

We equipped 34 of 61 caught birds with an Encounternet tag of approximately 1 g, using a leg-looped backpack harness (see Supplementary material, Figure S1). This selection was based both on the limited number of available tags and on the location of the caught birds, i.e. birds caught in the outer corners and at lower density areas of the study site were not included. The Encounternet tags are active radio transmitters, set to transmit every 5 s, and were especially designed to be as light as possible for use on small passerines. Within 24 h after catching, we released birds next to their roosting nest box. During a subsequent roosting check a few days later we tagged directly at their roosting nest box an additional five birds and retagged four birds, whose tag had failed to work. In total 29 males and 10 females were tagged. We selected 13 males for analysis based on (1) holding nearby territories, (2) having more than 100 localizations and (3) having simultaneous observations with all other males within the analysis. During two complete roost checks in March we identified 27 unique males within an area comprising all nest boxes within a 242 m radius (two times the maximum documented un-manipulated mean territory size; (Both & Visser, 2000) of the subject roosting boxes, which thus represented approximately 48% (13/27) of the nearby male population. Tracking data were analysed only from the day following the day of release (9–13 March 2012) to control for birds' behaviour potentially being affected immediately after tagging.

Encounternet (Encounternet LLC, Portland, OR, U.S.A.) consists of roaming nodes (tags fitted to the birds), base nodes (receivers) distributed across the study site and master nodes used to download data from the receivers. We used up to 93 receivers, which were placed in strategic locations throughout the study site. With this novel tracking system the locations of birds are mapped simultaneously and automatically, thus expanding data collection considerably beyond traditional radio tracking in which single individuals are followed manually (Naguib et al., 2001). Tags transmitted a digital ID code every 5 s which was logged by receivers within signal range, along with a time stamp and a received signal strength indicator (RSSI). We GPS-mapped the locations of the receivers (Garmin GPSmap 62st). Using Pymaster software version 22 (Encounternet LLC, Portland, OR, U.S.A.) we downloaded and organized all received signals per individual tag. Subsequently, the RSSI value was used to calculate the distance between the tag and the receivers based on a fixed distance–RSSI curve (Mennill et al., 2012). Tags can be detected up

to approximately 90 m from a base node, with the distance–RSSI curve getting less steep around 50 m. We triangulated the locations of each bird by combining all the RSSI values of at least three receivers within the same half-minute, using MATLAB (The MathWorks, Natick, MA, U.S.A.).

The 13 subject males were on average detected for 2007 (971–4698) half-minutes and triangulated 722 (134–2809) times. Detection numbers vary per bird, depending on the difference in tag life span and birds moving out of the detection range of a base node. There are fewer triangulations than detections as birds were not always in the range of at least three base stations. A simultaneous observation is defined as two individuals both having a triangulated location during the same minute. In calibration tests, in which we placed two tags within 5 m of each other, the system calculated them to be 7.5 ± 6.5 m apart. We therefore decided 10 m apart to be a good cut-off point (approximation) for an encounter as it would capture most of the real encounters between individuals (birds being aware of each other), while taking into account the inaccuracy of the localization. An encounter was thus a priori (before analysis) defined as two triangulated individuals being within 10 m of each other. The association strength between two individuals was defined by the number of times two individuals were within 10 m of each other divided by the total number of times they were observed simultaneously. Individuals in our analysis had on average 472 (173–1129) simultaneous observations. A unique combination of two males was observed on average 39 times simultaneously and 68 (87%) of the 78 $((12 \times 13)/2)$ possible pair combinations were observed more than 10 times simultaneously. Thus with all possible pair combinations we mean to say that the 13 males could each meet 12 of the other males. We divided this number by 2 as in a spatial association we cannot distinguish between male A meeting male B and male B meeting male A.

Statistical analysis

Spatial network tracking

Using an unprecedentedly large data set of individual locations, a total of 9380 localizations, 3066 simultaneous observations and 70 encounters within 10 m (Supplementary Figure S1), we calculated and visualized the social network properties with the software programs UCINET (Borgatti et al., 2002) and SOCPROG (Whitehead, 2009). The individual network properties analysed were Strength (the sum of association strengths per individual), Eigenvector centrality (this measure of connectivity takes into account how well an individual is connected to other well-connected individuals) and Degree (the number of unique connections per individual). We used the linear permutation model in the *lmPerm* package

(Wheeler, 2010) version 1.1-2 in R 2.15.2 (R Core Team, Vienna, Austria) for statistical analysis of dependent data. For all other analyses, unless mentioned otherwise, we used SPSS 19.0 (IBM Corp., Armonk, NY, U.S.A.). Because values for significance in permutation models are based on approximation, significance levels are shown using '<' or '>'. The 'Prob' method of ImPerm was employed, which approximates the permutation distribution by randomly exchanging pairs of dependent values and iterations are stopped when the estimated standard error of the estimated proportion p is less than $px0.1$ (Wheeler, 2010). Every 10 cycles a complete random permutation was performed.

All the linear permutation models for the effect of exploration score on the individual network properties were controlled for the average nest box distance of the individual (the average of the distances between the subject roosting box to the other 12 individuals) as a continuous variable, age of the individual as a categorical factor (younger or older than 1 year) and condition of the individual calculated as the residual of the function of weight against tarsus (continuous). Because birds in the centre of the study area would have a higher chance of encountering other individuals at random than birds on the edge of the study area we controlled for this using the average territory distance. Males with territories more towards the edge of the study area also had the largest average territory distance (independent t test: $t_{11} = -4.245$, $P = 0.001$). Thus to correct for spatial dependence in a territorial species at all times and because there was a nonsignificant trend for Degree to be larger with a smaller average territory distance, we kept average territory distance (an approximation of spatial dependence) as a control in all the final models. We used backward model selection for age and condition and removed them when there was less than a trend effect ($P > 0.1$), starting with the least significant term.

Results were checked for possible systematic biases, that is, biases introduced by the systematic data collection itself which could consequently be driving the results we found. We tested with approximate Spearman correlation tests (9999 permutations) in the R Coin package (Hothorn et al., 2008) whether exploration score, Strength, Eigenvector centrality and Degree were correlated with average territory distance (only for exploration score to check for possible collinearity between exploration score and average territory distance), number of localizations, detectability (locations/h) and number of simultaneous observations. There was only a trend for an effect ($Z = 1.8335$, $P < 0.08$) for number of simultaneous observations on Degree; however, controlling for this in the linear model did not significantly change the outcome and it was therefore left out of the final model. Additionally, birds with territories more towards the edge of the study

area were not characterized by a significantly higher or lower exploration score (independent t test: $t_{11} = -0.142$, $P = 0.89$).

To check whether individual social connectivity was not just driven by individual activity, we calculated the median speed (m/s) for each individual by calculating the distances moved between two consecutive (half a minute apart) locations (31–193 values per individual). The median of speed was used to account for non-normality. To account for the variation in sample size we randomly drew 10 000 times 31 samples from each individual (without replacement) and calculated the median speed for all of them (thus 10000 times per individual). Subsequently, we performed a Spearman correlation test between exploration and these median speeds 10000 times.

Reproductive success

Linear models were used to analyse the relationship between the number of fledged offspring and the breeding male's Eigenvector centrality (excluding one failed nest). Age of the birds (younger or older than 1 year) did not have a significant effect and was left out of the final model. Including the nest in which none of the chicks fledged did not significantly change the outcome.

Ethical note

Within 1.5 h after being caught the birds were transported by car from the field to the institute (maximum 20 min). The exact duration between catch and transport ranged from approximately 10 min to 1.5 h between individuals, depending on when they were removed from their roosting box. During this time, and during transport to the institute, birds were individually housed in standard darkened wooden carriers containing 10 compartments (10 x 10 x 10 cm). There were no apparent effects of the transport on health (no visual injuries or mortality). When moved from carrier to indoor cage, and vice versa, birds were caught by hand. Before being housed in individual standard indoor cages (see Novel Environment Test), tarsus and weight were measured (maximum duration of handling was 5 min). In the laboratory, birds were provided with mealworms, fat balls, apple, sunflower seeds and water ad libitum. The exploration test is part of an established routine procedure and was conducted between 0800 and 1200 hours. During this procedure birds commonly do not lose weight outside their natural range (Dingemanse et al., 2002). Individual cages are connected to the novel environment room (see Novel Environment Test) via a 20-20 cm sliding door and the individual cage was darkened, so a bird could enter the room without being handled. When the test was terminated, after at most 10 min, the room was darkened (birds stay

perched then) and the bird was caught by hand and released back to its cage within 1 min. None of the birds died in captivity.

After being tested for exploratory behaviour and tagging (see Spatial Network Tracking), which lasted a maximum of 5 min, birds were released back in the field close to their nest box within 24 h. After these procedures, birds usually stay on their territory and breed, as evidenced by yearly breeding records and also by radio tracking (Amy et al., 2010). Tags had a weight of, on average, 5.6% of body weight (maximum 6.2%). We are aware that 5% of body weight is often recommended as an upper limit and we agree that transmitters should be as least disruptive and stressful as possible. However, a meta-analysis on avian transmitter effects (Barron et al., 2010) did not find greater transmitter effects when proportionally heavier devices were used. Additionally, to maximally reduce the possibility of a negative effect of the transmitter on the birds, the Encounternet tags were especially designed to be as light as possible for use on small passerines, while still allowing for the use of this novel technology (simultaneous observation of multiple individuals). Of the 34 birds caught and tagged on 7 March 2012, 25 individuals were later found breeding (73%), which was not significantly different from the 12 of the 14 birds we checked that same night but that we left in their nest box and were later found breeding (86%) (see **Chapter 2**). Additionally, tagged parents did not have fewer fledged chicks when comparing parent birds that only differed in receiving an Encounternet tag on 7 March (*Median* = 6 chicks) to birds that did not (median = 5 chicks). These data indicate that during the time between tagging and provisioning the chicks (approximately 2 months) birds with tags did not have a higher risk of mortality, for example by increased vulnerability to predation, or impaired parental abilities. Tagged birds were found breeding in the same nest box in which they were caught or in a nest box within 200 m. Only one male bred more than 200 m away from the place of catching. Birds lose their Encounternet tags, including the harness, usually within 3 months, because the harness wears out. Breeding birds that had not lost their tag were trapped as parents in their nest box during chick feeding using spring traps, as part of a routine procedure to establish the identity of the breeding birds. These birds had their tag removed and were released within a few minutes. Birds were not trapped inside their nest box for longer than 15 min. It is unknown how long birds took to go back to their broods after trapping, as we left the area to avoid disturbing the birds; however, the trapping, for parent identification, was done following a standard protocol and our data did not suggest that trapping had any profound effect on chick mortality or brood desertion. Permission for this study was granted by the

Dutch legal entity: KNAW Dier Experimenten Commissie (DEC) no. NIOO-10.05 to M.N. and K.v.O. and no. NIOO-12.02 to K.v.O and M.N.

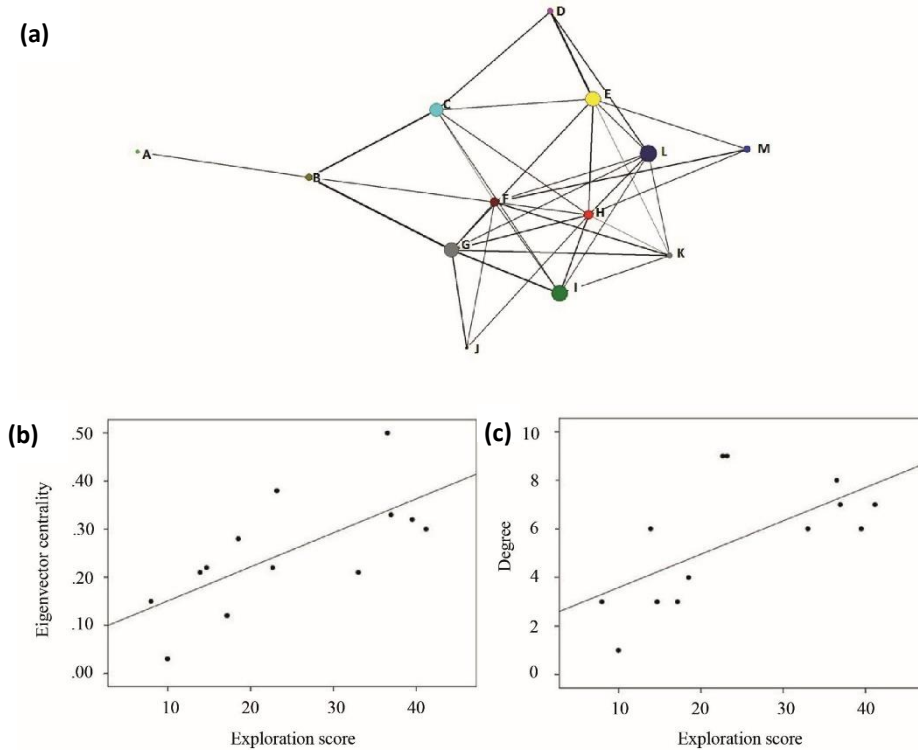


Figure 1. (a) Visualization of the social network with NetDraw (Borgatti, 2002) using a spring embedded layout based on distances, node repulsion and equal edge length. The size of the nodes reflects the relative exploration score of individuals (different letters) and the thickness of the lines represents the relative association strength between each pair. (b) A bird's Eigenvector centrality, a network position measure including direct and indirect connectedness, according to its exploration behaviour. (c) Degree, the number of direct connections per individual, according to the bird's exploration score. Lines are regression lines.

Results

Spatial network position

Strength, a measure of how much time a male spends close to the other males relative to the time he spent alone, was lower for slower exploring males (Table 1). As a consequence, slower explorers also occupied the least central positions in the network (Figure. 1a), reflected by a lower Eigenvector centrality (Figure. 1b, Table 1). Interestingly for a territorial species, some of the males did encounter up to

nine of the 12 other tagged males, with a trend for slower males to have the fewest unique contacts, also called Degree (Figure. 1c, Table 1). Males that had the fewest unique contacts were characterized by the lowest Strength (linear permutation model: *Estimate* = 0.048, *Iterations* = 5000, *N* = 13, *P* < 0.005) and Eigenvector centrality linear permutation model: *Estimate* = 0.036, *Iterations* = 5000, *N* = 13, *P* < 0.005). In all final models we included the average territory distance for each male to all other 12 males as a control variable, but only the number of unique contacts (Degree) tended to decrease when the average territory distance between males became larger (Table 1).

Table 1. Permutation model statistics for social network position

Social network position		Estimate	Iterations	P	N
Strength	Exploration score	0.008	3996	< 0.03	13
	Average territory distance (km)	-0.703	103	> 0.40	13
	Condition	-0.005	51	> 0.90	13
	Age	0.048	141	> 0.20	13
Eigenvector centrality	Exploration score	0.007	5000	< 0.02	13
	Average territory distance (km)	-0.440	77	> 0.50	13
	Condition	0.000	51	> 0.90	13
	Age	-0.070	323	> 0.20	13
Degree	Exploration score	0.101	1801	< 0.06	13
	Average territory distance (km)	-29.335	1340	< 0.08	13
	Condition	-0.126	51	> 0.70	13
	Age	0.102	51	> 0.90	13

Values are based on the last model still including the variable, using backward model selection. Variables in bold were kept in the final model.

Movement speed

Slower explorers were not slower during tracking, and thus when they were in their natural environment, as 98.3% of the 10 000 correlation tests of exploration score and median speed, based on randomly drawn samples of equal sample sizes for each individual, were not significant (Figure. 2). This indicates that the lower social connectivity of slower explorers was not a side-effect of lower spatial activity.

Reproductive success

There was no relationship between the number of fledged offspring (*Range* 2–6) and the Eigenvector centrality of breeding males (linear model: $F_{1,7} = 0.76$, $N = 9$, $P = 0.41$).

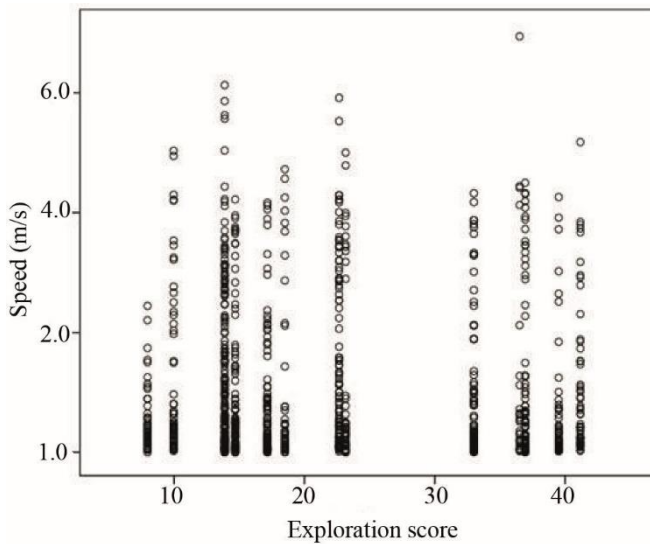


Figure 2. Median speed (m/s) according to individual exploration score. Each visualized single speed measurement represents the distance moved between two consecutive, half a minute apart, locations (31–193 values per individual).

Discussion

The automated tracking of male territorial great tits revealed that slower exploring males showed an overall weaker social connectivity, in terms of less central spatial social network positions, than faster exploring males. Moreover, our findings suggest that higher connectivity in the wild is an active process and does not result from a generally higher activity of faster explorers, as the median distance travelled did not correlate with individual exploration behaviour. Finally, we found no evidence that a weaker social network position was correlated with lower breeding success.

Our finding that territorial males differed in social network position in relation to their exploration behaviour has important implications. Slower explorers spent

relatively the least time in the vicinity of other males (lower Strength) and this was strongly related to the number of unique contacts (lower Degree) of individuals. When individuals spend less time near other conspecifics this will presumably lead to less familiarity among conspecifics. This is relevant, because familiarity, mediated by repeated encounters among individuals, can lead to a general decrease in the level of aggressive interactions (Ward & Hart, 2003; Geffroy et al., 2014). Familiarity between individuals, which exhibit consistent behavioural differences (personality), is predicted to promote social responsiveness, as it makes individuals predictable and so allows individuals to match their behaviour to that of their interaction partner (McNamara et al., 2004; Wolf et al., 2011). This can mediate the use of tit-for-tat strategies (Akçay et al., 2009), reducing unnecessary aggressive interactions and allowing for cooperative alliances (Elfström, 1997; Booksmythe et al., 2010; Grabowska-Zhang et al., 2012; Goodwin & Podos, 2014). For example, neighbouring great tits that shared a territory boundary the previous year were more likely to join each other in nest defence than neighbours that had not shared a boundary (Grabowska-Zhang et al., 2012). However, individuals that are already generally less aggressive, such as slower explorers (Verbeek et al., 1996; Carere et al., 2005), might also have fewer benefits from increasing familiarity with conspecifics, as they are anyway less likely to escalate territorial defence interactions. Indeed, slower exploring male great tits showed a weaker approach during territory defence when confronted with a simulated intruder (Amy et al., 2010). Additionally, a similar trend has also been found in other species, such as rainbowfish (*Melanotaenia duboulayi*) in which lower ranked males, which were less aggressive, were also less social (Colléter & Brown, 2011).

Still, social connectivity also relates to how much an individual is exposed to public information about potential food resources and feeding techniques (Wolf et al.), competitors (Gil & Gahr, 2002; Amrhein et al., 2004a) and mates (Oh & Badyaev, 2010; Mennill et al., 2012), all components that directly affect individual fitness. For example, in songbirds and whales spatial associations led to new feeding opportunities (Aplin et al., 2012; Allen et al., 2013) and for some songbirds also to better mating opportunities (Oh & Badyaev, 2010). Nevertheless, we did not find an effect of proximity network centrality on breeding success, which could be the result of several factors. First, it is certainly possible that our sample size was too small to detect a correlation between proximity network position and breeding success. A larger sample size would be necessary to rule out this hypothesis. Second, the lack of an effect on breeding success could be related to personality-dependent social responsiveness and use of social information: for example, slow exploring male great tits were shown to be more socially responsive, by adjusted

their risk-taking behaviour in response to a companion's behaviour (van Oers et al., 2005) and in barnacle geese (*Branta leucopsis*) shyer individuals were more inclined to use social information than bolder individuals (Kurvers et al., 2010b). Finally, it might be that, for slower explorers, the costs of a more central social network position outweigh the benefits of increased access to social information. It can also be risky to be close to a conspecific, as it might result in a fight (Rowell, 1974; Carere et al., 2005), increased social stress (Rowell, 1974; Carere et al., 2003) and more exposure to parasites (Croft et al., 2011) and predators (Grabowska-Zhang et al., 2012; Krama et al., 2012). If the costs of a stronger social network position indeed vary with personality it would explain both why slower explorers, which are more risk-averse (Réale et al., 2007) and more vulnerable to social stress (Carere et al., 2003), showed weaker social connectivity, but also why we did not find a correlation between social network position and breeding success.

Songbirds can also connect to each other via signal interactions, often referred to as communication networks (McGregor, 2005). Great tits have been found to differ in relation to exploration behaviour in using stronger spatial or signal responses when confronted with conspecifics (Verbeek et al., 1996; Carere et al., 2005; Amy et al., 2010). Slower exploring males confronted with a male conspecific showed a longer latency and frequency of attacks (Verbeek et al., 1996; Carere et al., 2005), spent less time close to a simulated intruder (Amy et al., 2010) and showed a longer latency to approach when confronted with a female (Carere et al., 2005). However, slow males did show a higher frequency of agonistic displays during the male to male confrontation (Carere et al., 2005) and sang with a higher song rate during the simulated intrusion in (Amy et al., 2010). Connectivity in terms of spatial associations might thus not equal social connectivity.

Our findings expand on a previous study on wintering (non-territorial) great tits visiting artificial feeding stations (Aplin et al., 2013), also showing that slower birds have a weaker social connectivity. Social encounters during the territorial period, however, could be expected to differ from winter encounters since there is less effect of flock composition and use of shared resource locations. Encounters by territorial birds are more likely to reflect direct social interactions among neighbouring birds in competition for space and reproduction. This is relevant, because when certain personality types prefer to avoid social confrontations (Wolf et al., 2011), as the results of this study suggest, this could lead to different personality types thriving better under different population densities (Sih et al., 2012), especially in a territorial system.

Moreover, a personality type is also likely to be partly shaped by the specific social environment of an individual (Bergmüller & Taborsky, 2010; Wolf & Krause, 2014). Experimental studies would be necessary to confirm the causes and consequences of the correlation between personality and social network position. Still, irrespective of this, we reveal here that individuals in a territorial songbird system do not merely encounter each other at random.

Taken together, our results suggest that territorial individuals influence, and consequently differ in, the structuring of their social environment in relation to their personality. This is important, because the establishment of social relations and familiarity with possible competitors is predicted to be significant in many territorial populations.

Acknowledgements

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Supplement to Chapter 3

Figure S1

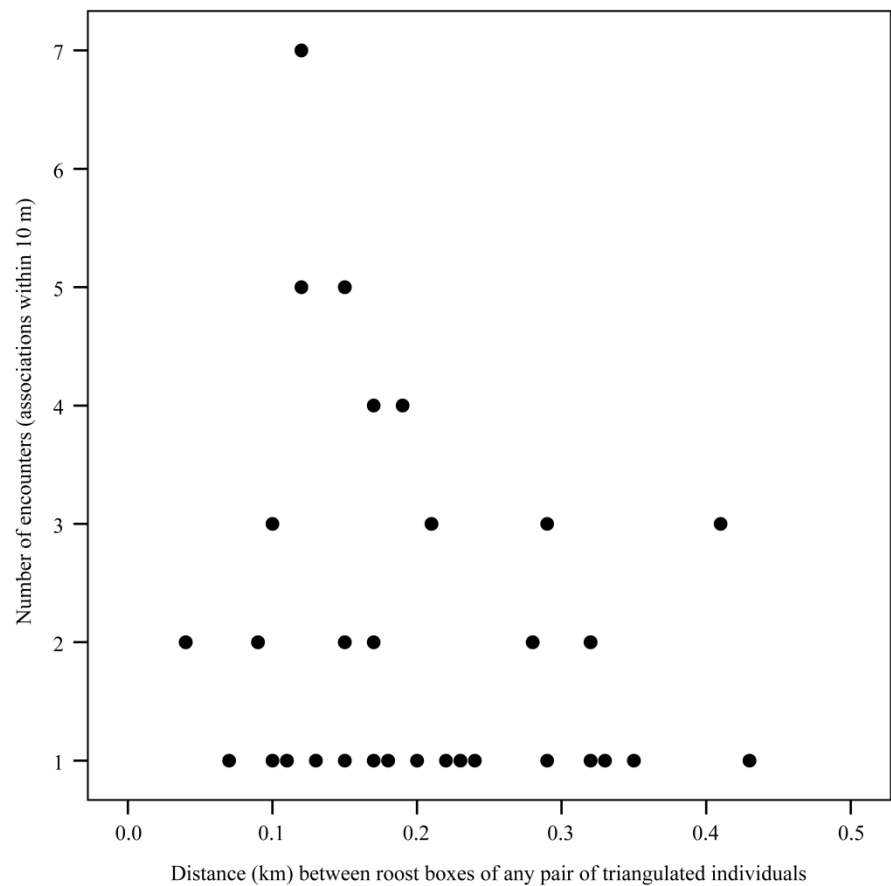


Figure S1. The number of encounters between any possible pair combination of the 13 tracked great tit males in relation to the distance (km) between their roosting boxes.



4

Dominance rank and boldness predict social preference in great tits

Lysanne Snijders, Marc Naguib, Kees van Oers

Submitted

Abstract

Social connectivity is key for several ecological processes, and can have important fitness consequences. Social network positions often correlate with other behavioural traits, but whether such correlations are caused by underlying differences in social preference remains unclear. To identify effects of individual traits on social preference it is essential to experimentally exclude the influence of the social partner. Using standardized high definition video playback on captive great tits (*Parus major*) we effectively demonstrate the influence of individual traits on the motivation to be near a conspecific. We show that lower-ranked and shyer individuals exhibit the strongest social preference. The effect of rank remained after experimentally changing dominance ranks by altering group compositions, suggesting the use of a plastic social strategy in relation to rank. Our findings indicate that social individuals can influence their position in their social network by strategically changing their social behaviour. Our results provide insight into how social relations and thus social structures form and change, processes that are key for understanding the long-term consequences of social network positions.

Introduction

In many animal populations social associations are an essential part of an individual's ecology. Social connectivity modulates exposure to social information about where to forage and settle (Stamps, 1988; Kurvers et al., 2010b; Aplin et al., 2012; Templeton et al., 2012), but may at the same time increase social stress and aggression (Rowell, 1974; Verbeek et al., 1996; Carere et al., 2003), indicating that an individual's social network position can have important fitness consequences (McDonald, 2007; Oh & Badyaev, 2010; Formica et al., 2012). When the costs and benefits of such social associations differ between individuals, it is likely that the preference for social association, the motivation to be near conspecifics, will also vary.

Previous studies have revealed intriguing correlations between the social associations and the dominance rank as well as the behavioural characteristics of individuals (Rushmore et al., 2013; **Chapter 3**). Lower-ranked individuals can increase their likelihood of rising in rank by social association (McDonald, 2007; Gilby et al., 2013), but they usually also have a higher chance of encountering aggression. Higher ranked chimpanzees (*Pan troglodytes*) had more unique social contacts (Rushmore et al., 2013), similar to more 'exploratory' great tits (*Parus major*) (Aplin et al., 2013; **Chapter 3**) and 'bolder' three-spined sticklebacks (*Gasterosteus aculeatus*) (Pike et al., 2008). Variation in social preference in relation to dominance rank, boldness and exploratory behaviour could underlie these observed differences in social network position.

The role of social preference in animal social behaviour still has many open questions (Webster & Ward, 2011; Wolf & Krause, 2014). One of the challenges of studying individual social preferences is that the formation of social associations can often be the consequence of the behaviour of both the focal individual and their social partner. For example, stronger social connectivity of high-ranking chimpanzees is more likely to be caused by lower-ranking individuals seeking out (grooming) associations with higher-ranked individuals (Rushmore et al., 2013) than by higher-ranking individuals having a higher overall preference for social associations. Insight into the individual factors driving variation in social preference therefore represents the next step towards understanding the formation and stability of social structures (Flack et al., 2006; Shizuka et al., 2014).

To tease apart the effect of variation in social preference from the response of a social partner, it is necessary to perform experimental manipulations that exclude

the influence of the social partner. Hence, in this study we conducted a standardized high definition video playback experiment to study social preference, in great tits, an important model species for studying social connectivity (Aplin et al., 2013; Aplin et al., 2014; **Chapter 3**). Video playback is now more realistic than ever before as a consequence of modern developments in recording devices and monitors, such as high definition and LCD technology which circumvent previous challenges to conducting video playback with species with high flicker fusion frequencies, like birds (Oliveira et al., 2000; Bird & Emery, 2008). Successful video playback studies have been conducted to study the social behavior of a variety of species, such as gloomy octopusses (*Octopus tetricus*), nutmeg manakins (*Lonchura punctulata*) and rooks (*Corvus frugilegus*) (Bird & Emery, 2008; Rieucan & Giraldeau, 2009b, a; Pronk et al., 2010). We expected lower-ranked birds to show less social preference for the unfamiliar same-sex stimulus bird, especially in a food context which is often highly competitive (Dingemanse & de Goede, 2004). We additionally expected bolder and more exploratory individuals to show a stronger social preference (Pike et al., 2008; Aplin et al., 2013; **Chapter 3**).

Methods

Experimental subjects

We used captive hand-reared first year great tits hatched in the spring of 2014, both as video playback stimuli ($N = 38$) and experimental subjects ($N = 36$). These birds were chicks of captive parents, but were raised by wild birds during the first ten days after hatching and subsequently hand reared under standard conditions (van Oers et al., 2005). Each bird was fitted with a unique aluminium leg ring and three colour leg rings for individual identification. Birds (12 females, 24 males) were sexed using molecular markers (Griffiths et al., 1998). For each bird the tarsus (0.01 cm) was measured before the experiment and weight was monitored before, during and after the experiment (0.1 g) using a digital balance. Birds experienced natural daylight and temperature conditions (daily average: 3 - 10 °C) in semi-open aviaries (2 x 4 x 2.5 m). The indoor rooms with individual cages (0.9 x 0.4 x 0.5 m) were kept on a light regime similar to the natural day-night cycle, and temperature maintained between 15 and 17 °C. Birds had auditory contact with each other at all times. All food and water was provided ad libitum, with the exception of mealworms, which were only provided in the aviaries and during experimental trials. See van Oers et al. (2005) for more details on housing conditions. Work was carried out under permit no. 14.12 granted to KVO and MN by the Dutch legal entity: KNAW Dier Experimenten Commissie (DEC).

Experimental design

All 38 birds (36 subjects) were recorded as stimulus videos 3 weeks before the start of the experiment. Before the experiment birds were always housed in individual cages and so had not been in physical contact with each other since the hand-rearing period (three to four months earlier). At the start of the experiment 36 birds were randomly assigned to single-sex groups of six birds (two female and four male groups) and subsequently housed in six aviaries. We conducted dominance observations from six to nine days after the birds were grouped together, as dominance ranks in great tit groups stay relatively stable from the sixth day after group formation (Verbeek et al., 1999). After a minimum of twelve days in a group, birds were individually housed again for the onset of the video playback trials. After we conducted four video playback trials with each individual from all six groups (Supplementary Figure S2), the birds were reassigned to six new groups based on their dominance ranks as measured in their original groups. In these new groups we placed birds with similar dominance ranks together. The previous protocol was then repeated, after which one final video playback trial was conducted with each bird. See Supplementary Figure S1 for the exact timeline of the complete experimental setup.

Dominance

We assessed dominance ranks by observing interactions between birds in the aviaries from behind a one-way window. Groups were directly observed six times for half an hour directly and for two to three times half an hour by video. We conducted direct observations for each group on four consecutive mornings (07:30-13:00) and two afternoons (13:00-16:45), while video recordings were made only on mornings. During observations we documented displacement, waiting, defined as an individual waiting for another bird to finish before feeding, and aggressive behaviour between two individuals (chasing and attacking). For each interaction we noted the identity of the actor and the identity of the receiver. During the first round of dominance observations 98-165 interactions were recorded per group, while the second round (after group composition was changed) resulted in 166-236 interactions. We assigned dominance ranks based on the number of birds with whom an individual had lost the majority of its interactions. Group members could have the same dominance rank, as more than half of the groups did not have a significant linear hierarchy based on the linearity index h' (De Vries, 1995). When it was unclear exactly how many birds an individual had lost the majority of its interactions with due to unknown or tied relationships, we assigned the average of

the minimum and maximum possible rank (six out of 36 during the first round; five out of 36 during the second round).

The six single-sex groups during the second round were composed by grouping the three highest-ranked birds of one group during the first round with the three highest-ranked birds of another group. This was likewise done, in a randomly paired fashion, for the three lowest-ranked birds of each group. We used this specific procedure to force previously dominant individuals to adopt more submissive roles and vice versa.

Boldness

Individual boldness was assessed for all except one bird, using two standardized novel object tests as described in detail by (Carere & van Oers, 2004), approximately three months before the start of the experiment (at 50 days of age). Briefly, a novel object was presented in the home-cage on the furthest right of the three perches. Tests lasted 2 minutes and behaviour was observed from behind a curtain. The boldness score was calculated following (Drent et al., 2003), but was adjusted to incorporate activity of the subject. Individuals not reaching the perch with the novel object within 2 min were given a score of 0-5 depending on the number of movements within these 2 min. Scores from 6–17 were given to animals that visited the perch, taking into account the latency to reach the perch and how close they approached the novel object. This score was then transformed to a score from 0 to 1, with 0 meaning that a bird did not move at all in the test and 1 indicating the bird touched the novel object repeatedly within one minute. This measure was highly repeatable between the two novel object tests ($r = 0.44$, $SE = 0.13$, $N = 37$); the average score was used in our analysis.

Exploratory behaviour

The exploratory behaviour of a bird (with exception of two individuals with temporary flight problems) was assessed with a standardized novel environment test following Drent et al. (2003) approximately 3.5 months before the start of this experiment (at 30-40 days of age). Exploratory behaviour is a repeatable and partially heritable behavioural trait in great tits (van Oers & Mueller, 2010) and explains variation in various social behaviours, such as aggression (Verbeek et al., 1996), territory defence (Amy et al., 2010; **Chapter 7**), approach of conspecifics (Groothuis & Carere, 2005), social foraging (Aplin et al., 2014) and social network position (Aplin et al., 2013; **Chapter 3**). The exploration score was calculated as the number of movements in the first 2 minutes (Dingemanse et al., 2002; **Chapter 3**).

Stimulus videos

Stimulus videos were constructed by recording a great tit from a fixed distance (40 cm) for eight to ten minutes in a white cage (0.67 x 0.37 x 0.38 m) including two perches and a Plexiglas front. Videos were recorded with a Full HD Panasonic HC-V550 (AVCHD, W 1920 x H 1080, 25 fps). In total 29 of the 38 recorded videos were used in the experiment, including only videos of birds that were life size on full screen. We removed the sound of the videos to avoid potential influences of calls and songs, and excluded the first two minutes of the original video. Using Adobe Premiere Pro (Adobe Systems, San Jose, US) we selected an approximately two-minute time-frame (Min: 1:46, Max: 2:05), in which the bird occupied the same location and body position at the start and end (See Supplementary Figure S3). This procedure allowed us to make realistic uninterrupted loops of the videos. Movement control videos were constructed in Adobe Premiere Pro by blurring a rectangle concealing the great tit in the stimulus video with Gaussian blurr (75.0) and Mosaic (250) frame by frame (See Supplementary Figure S4).

To test for the potential influence of stimulus bird activity, the stimulus videos were tracked with Ethovision XT (Noldus, static substraction, sample rate: 0.0040 / sec). We checked the tracks manually afterwards for incorrect detections and adjusted them if necessary. We used the total distance moved by center point (cm) as a proxy for stimulus activity.

Video playback protocol

All individual cages connected to the same experimental room (4.0 x 2.4 x 2.5 m) through sliding doors. The experimental room (Figure 1) contained two wooden cages (0.85 x 0.40 x 1.0 m) with wire mesh on the front and attached on a rolling base (Reparaz et al., 2014), which we positioned against the back wall. Each of the two cages contained a black LCD computer monitor (HP Compaq LA2306x, 60 Hz) allowing the birds a visual of the stimulus videos up to 20 cm. Additionally the room contained three artificial 'trees' (as described by Dingemanse et al. (2002)) with one tree in the middle of the room providing a view of both screens simultaneously and one tree in front of each screen that only allowed the bird to view one screen. We made observations through a one-way window situated at the opposite site of the room. Each stimulus video started before a subject entered the room and lasted fifteen minutes starting from the time a bird had entered the experimental room. Birds freely moved into the room by themselves. We turned off the lights after fifteen minutes (birds stay perched then) and the subject was put back into its individual cage.

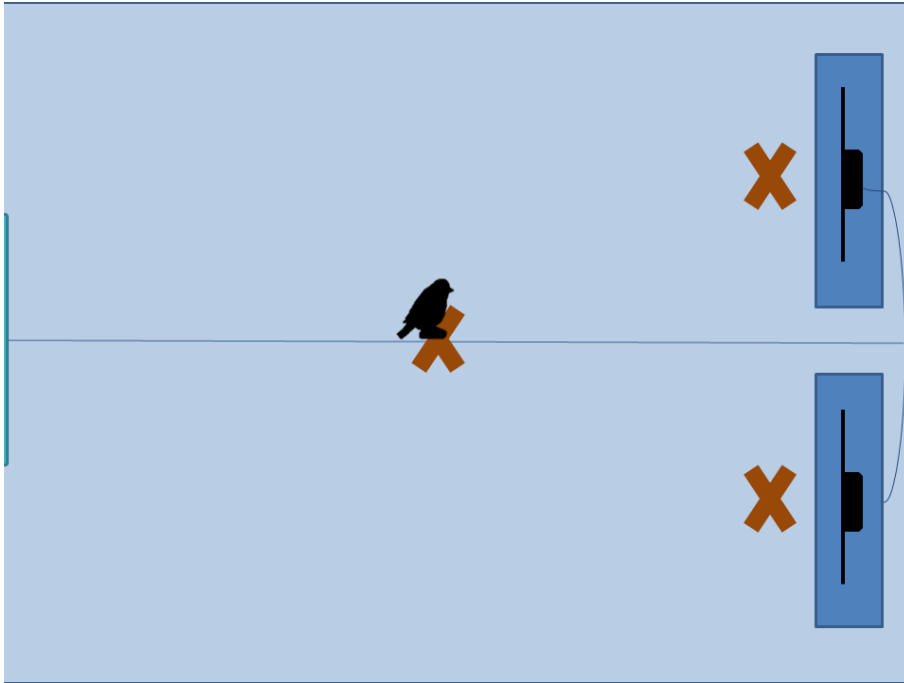


Figure 1. Schematic overview of the experimental room in which the video playbacks were conducted. A wide-angle camera was located on the left hand side in the door of the experimental room above the one-way window. The observer was located behind the one-way window. A bird would enter from their home cage either along the upper side or lower side. Three artificial trees were present, one in front of each of the screens and one in the middle of the room. The bird could see both screens simultaneously when perched on the tree in the middle, but only one screen when perched on one of the other trees.

During the first round, each individual received four video playback trials. Per unique video stimulus bird (two for each subject) each subject received both an experimental trial and a movement control (video playback validation) trial in a random order. The experimental trial included a screen showing a bird in a cage and a screen showing the same cage empty (Figure 1 and Supplementary Figure S2). The movement control trial was similar to the experimental trial except that the video showing the empty cage video was replaced by a great tit stimulus video, in which the bird in the video was “blurred” using video-editing software. Subjects only received stimulus videos of birds which were unfamiliar to them (no previous or current group mates) and that were of the same sex. Movement control trials

always showed the identical video stimulus bird (both blurred and original) as the subject would receive or had received during the accompanying experimental trial.

We randomized and balanced the screens (left or right) from which a video was shown. Trials per individual bird were one day apart and the order between birds was kept constant. During the first two trials (first video stimulus bird) food was present in the form of four small cups attached to the trees in front of the screens. Each cup contained one mealworm. During the following two trials (second video stimulus bird) no food cups were present. We initially provided the mealworms to motivate the birds to come closer to the screens. However, to test if the presence of food was not biasing the observed social preference we removed the food during the following two trials. During the second round (after group compositions changed), subjects received one experimental trial with a third unique video stimulus bird (food was present). The experimental design thus included three experimental trials (social preference tests) and two control trials (movement controls) for each of the 36 subjects (Table 1).

Data analysis

All 180 trials were recorded (with exception of three movement control trials due to video recording problems) using a broad angle camera from a fixed position (Observer, Noldus, Wageningen, The Netherlands). We used the program EthoVision XT version 9 (Noldus, Wageningen, The Netherlands) to manually score the total duration and the frequency of visits on (1) the middle tree, (2) in front of the left cage, (3) in front of the right cage or (4) in the rest of the experimental room. Sample sizes differ as a consequence of three failed data video recordings (movement control trials: $N = 3$) or of birds that for a total of thirteen times did not making a decision within fifteen minutes (movement control trials: $N = 7$; experimental trials: first round $N = 4$, second round $N = 2$). We calculated general interest in the videos as the total time spent in front of one of the videos divided by the total experiment duration (15 min). Our key metric, social preference strength, was calculated as the proportion of time spent in front of the great tit video divided by the total time spent in front of the great tit video and the other video (empty cage or blurred great tit video). This measure has been used as a reliable indicator in mate-choice experiments (Schielzeth et al., 2008; Reparaz et al., 2014) and has been successfully used in captive great tits to quantify personality differences in social exploration in response to social defeat (Carere et al., 2001).

Table 1. Overview of the experimental design which included three experimental trials (social preference test) and two control trials (movement control) for each of the 36 subjects.

First video stimulus Two trials for each subject	Second video stimulus Two trials for each subject	Third video stimulus One trial for each subject
Experimental trial & Control trial	Experimental trial & Control trial	Experimental trial
OR Control trial & Experimental trial	OR Control trial & Experimental trial	

Analysis of movement control trials (methods validation)

To analyse whether great tits distinguished the great tit video stimuli from mere movement, we tested if average social preference strength was significantly higher than random ($Mean = 0.5$). The subjects spent a significantly higher proportion of time near the un-manipulated great tit video than would be expected if birds were randomly dividing their time over the great tit video and the identical blurred version of the video (2 out of 4 trials $P < 0.05$). Interestingly, birds only spent more time than expected at random near the social stimulus when they had received the control trial before the experimental trial (Table 1), thus when they had not seen the specific stimulus video bird before (One sample T-Test Social preference strength against $Mean = 0.5$; First stimulus video: 1st encounter; $Mean = 0.64$, $SD = 0.17$, $t_{(14)} = 3.11$, $P = 0.008$; 2nd encounter; $Mean = 0.55$, $SD = 0.17$, $t_{(16)} = 1.36$, $P = 0.19$; Second stimulus video: 1st encounter; $Mean = 0.62$, $SD = 0.20$, $t_{(15)} = 2.42$, $P = 0.03$; 2nd encounter; $Mean = 0.52$, $SD = 0.32$, $t_{(15)} = 0.30$, $P = 0.77$; Figure 2). Interest (the total time the great tits spent in front of the videos) was overall high with 71% ($SD = 20\%$) when the first unique video stimulus was presented and 64% ($SD = 24\%$)

when the second unique stimulus video was presented. That the birds showed a significant preference during the first and again in the third trial (when they received a novel video stimulus bird) but not during the second and the fourth trial (when they had already encountered the video stimulus bird before during the experimental trial), strongly suggest that neither habituation nor differences in clarity or contrast between the social stimulus videos and the movement control videos can explain these results. In summary, the subjects distinguished the great tit video stimuli from mere movement, responding with significant social preference when a new video stimulus bird was shown.

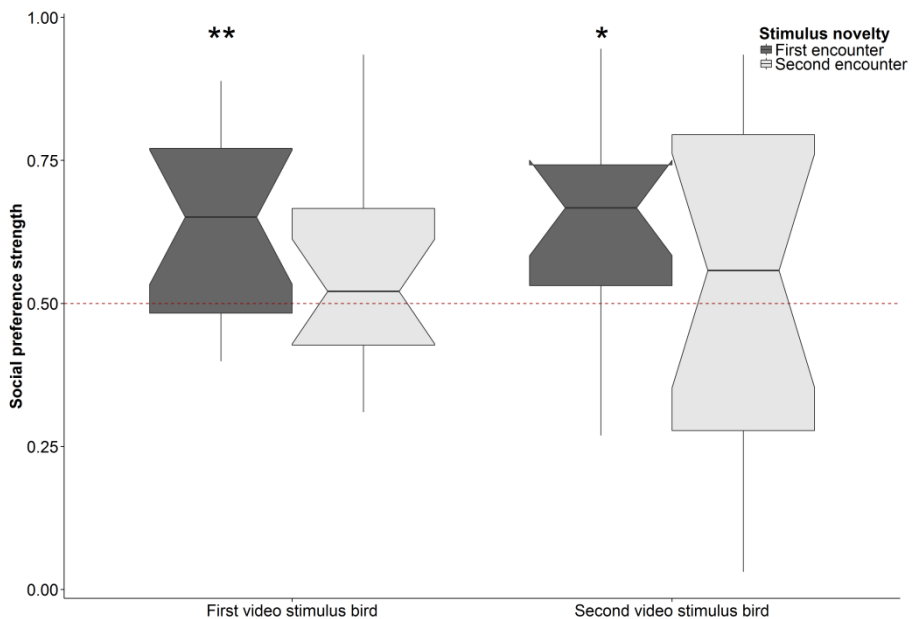


Figure 2. The average proportion of time birds spent in front of the original great tit video stimulus relative to the time spent in front of the “blurred”, but otherwise identical, great tit video was higher than expected by chance (0.5). However, this distinction from mere movement was only shown when a bird was confronted with a certain great tit video stimulus for the first time (dark grey: stimulus novelty = first encounter, light grey: stimulus novelty = second encounter). This was true for the first as well as for the second video stimulus bird presented. Notches not overlapping the chance line (0.5) visualize a significant difference from chance.

Statistical analysis of social preference

Next to *social preference strength*, *social choice* was calculated by dividing the total number of times the subjects moved to the great tit video from the middle tree by the total number of times the subject moved to either of the two videos. For analysis of the experimental trials (control trial were excluded from this analysis), we extracted the principal component (PC-Social preference) of *social preference strength* and *social choice* (arcsine transformed for normality) for each context (Loadings on component > 0.9, *Eigen value* > 1.6; Percentage variance explained > 81%). Both measures were strongly correlated (Weighted Regression of *social choice* against *social preference strength*; $\beta > 0.59$, $P < 0.001$ for movement control trials; $\beta > 0.64$, $P < 0.0001$ for experimental trials).

We conducted a mixed model analysis (REML) with residuals weighted for the number of active choices made (from the tree in the middle) per individual per trial. We considered the observed social preference of individuals that made more choices to be more reliable than of individuals that only made a small number of choices, since with less choices there is a higher risk of extreme chance effects. The starting model analysing the first round of video playback experiments included PC-Social preference (dependent), boldness (covariate), exploratory behaviour (covariate), dominance rank (covariate), stimulus novelty (first or second time encounter with the specific great tit stimulus; factor), food present (factor) and individual nested in aviary group (random factor). Additionally, based on our results with the movement control trials, the model included the two-way interactions between dominance rank and stimulus novelty, boldness and stimulus novelty and exploratory behaviour and stimulus novelty. In our dataset boldness was not correlated with exploratory behaviour (Spearman correlation test; $r_s = -0.22$, $P = 0.21$, $N = 34$) or dominance rank (Spearman correlation test; $r_s = -0.01$, $P = 0.94$, $N = 35$). Also, exploratory behaviour was not correlated with dominance rank (Spearman correlation test; $r_s = 0.00$, $P = 0.98$, $N = 34$). A back-wise model selection procedure was conducted by removing the least significant terms from the model step-wise ($0.1 < P < 1.0$), starting with the highest-level interactions.

To test for potential effects of sex and body condition on social preference, physical characteristics were added as control variables to the final model. We calculated body condition as the residual of weight over tarsus for each sex separately. Neither sex ($F_{1,21.434} = 0.21$, $P = 0.65$) or body condition ($F_{1,20.024} = 2.23$, $P = 0.15$) had a significant effect. Moreover, activity of the video stimulus bird did not influence the PC-Social preference (Spearman correlation test; First video stimulus bird: $r_s = 0.20$, $P = 0.25$, $N = 34$; Second video stimulus bird: $r_s = 0.02$, $P = 0.92$, $N = 33$). Similarly, there was no effect of stimulus activity when solely analyzing the first

and third trial ($r_s < 0.24$, $P > 0.35$). Stimulus activity as a control variable in the final model also did not have a significant effect ($F_{1,42.091} = 1.11$, $P = 0.30$).

Social preference after dominance rank manipulation

To test whether the newly acquired dominance rank had an effect on social preference we again used a mixed model analysis (REML) with the residuals weighted by the number of choices made per individual per trial. The starting model included PC-Social preference (dependent), dominance rank (covariate), boldness (covariate) and aviary group (random factor). The old dominance rank value was added to the final model as a control variable. One bird made twice as many choices as the bird with the second highest number of choices. Reducing the number of choices (weight) of this individual to the value of the second highest bird resulted in a trend effect of new dominance rank in the same direction as in the original model (see Results).

All statistical analyses were conducted in IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp, Armonk, NY). We used non-parametric tests if the data was not normally distributed according to the Shapiro-Wilk test.

Results

Dominance rank, boldness and exploratory behaviour in relation to social preference

The average time a great tit spent in front of the videos was over 60% of the total time, and this did not decrease with total number of trials received (60.8%, 69.7%, 66.3% and 67.6% respectively).

Lower-ranking individuals showed a stronger social preference (PC-Social preference) compared to higher-ranked birds (Table 2; Figure 3). Shyer birds showed the strongest social preference when they encountered a unique video stimulus for the first time, while bolder birds showed a relatively stronger social preference when they encountered a unique video stimulus bird for the second time (Table 2; Figure 4). When solely considering novel video stimuli birds, shy birds still tended to show a higher social preference (Mixed model: $F_{1,21.583} = 3.38$, $P = 0.08$), while there was no effect of boldness when only considering video stimuli birds that were presented for the second time (Mixed model: $F_{1,15.420} = 1.24$, $P = 0.28$). Finally, exploratory behaviour did not predict social preference either as main effect (Mixed model: $F_{1,28.983} = 0.01$, $P = 0.94$) or in interaction with stimulus novelty (Mixed model: $F_{1,49.955} = 0.85$, $P = 0.36$).

Table 2. Final mixed model statistics for PC-Social preference in relation to behavioural characteristics and experimental design following a stepwise backward selection procedure.

Independent	Test statistic	P-value
Stimulus novelty	$F_{1, 59.469} = 1.58$	0.21
Rank	$F_{1, 26.801} = 5.58$	0.03
Boldness	$F_{1, 36.009} = 1.21$	0.28
Boldness * Stimulus novelty	$F_{1, 58.879} = 4.27$	0.04

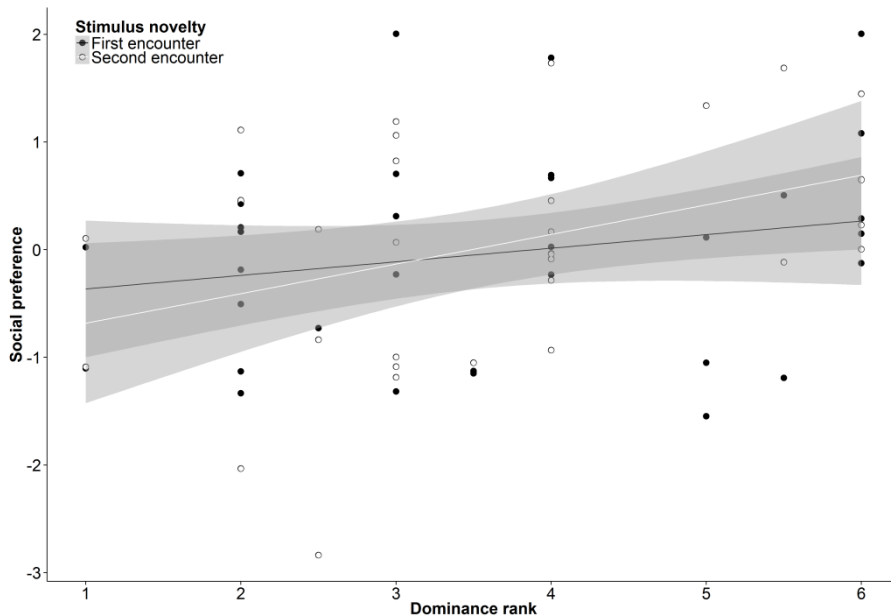


Figure 3. Birds with lower dominance ranks showed the strongest social preference (PC-Social preference). The first time a bird was confronted with a certain great tit video stimulus (stimulus novelty = first encounter) is represented in black while the second time (stimulus novelty = second encounter) is represented in white. Grey areas represent 95% CI.

Influence of dominance rank on social preference after manipulation

The time spent in front of the videos did not decline during the final experimental trial, after the experimental change in dominance rank, and was even significantly higher (77%) when compared to the first experimental trial (Paired T-Test: $t_{(32)} = -2.83$, $P = 0.01$).

Again, even after the change in ranks (Supplementary Figure S5), the birds with the lowest dominance rank showed the strongest social preference ($F_{1, 26.994} =$

4.54, $P = 0.04$, $AIC = 101.2$), while there was no significant correlation between old dominance rank and new dominance rank (Pearson correlation; $r = 0.21$, $P = 0.22$, $N = 36$). Adding the old rank as a control variable did not have a significant effect ($F_{1,10.304} = 0.42$, $P = 0.53$, $AIC = 103.2$), indicating a causal effect of dominance rank on social preference. This is further supported by social preference strength tending to increase with a decrease in dominance rank (Weighted Regression, weighted by minimum number of choices, $\beta = -0.34$, $P = 0.07$).

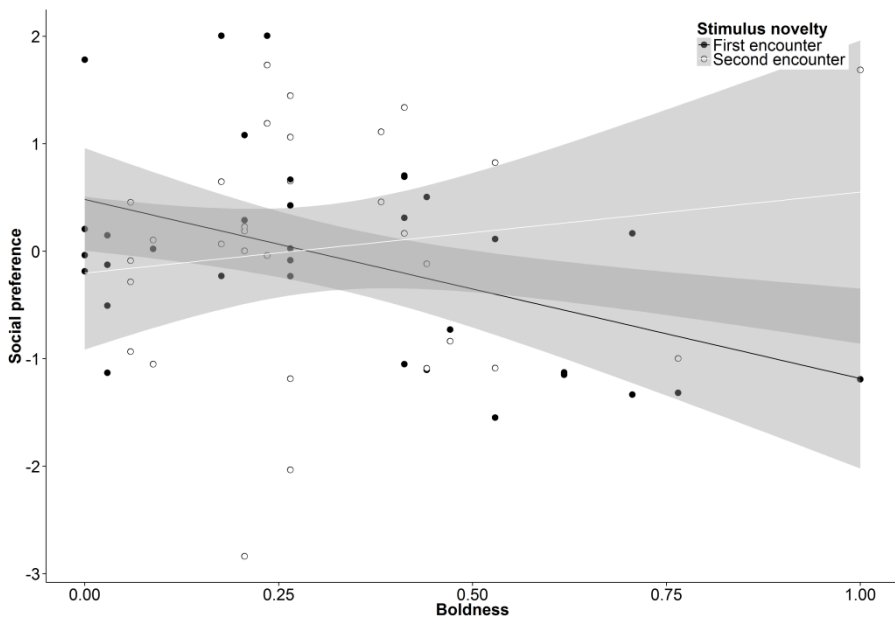


Figure 4. Shyer birds showed a stronger social preference (PC-Social preference) compared to bolder birds, when confronted with a specific great tit video stimulus for the first time (stimulus novelty = first encounter), while bolder birds showed a relatively stronger social preference than shyer birds when they were confronted with a specific great tit stimulus for the second time (stimulus novelty = second encounter). Grey areas represent 95% CI.

Discussion

Using High Definition video playback, we uncovered effects of individual behavioural characteristics on social preference. More specifically, we here reveal a negative effect of dominance rank and boldness on the preference for being in proximity to a simulated conspecific. Even after our manipulation of dominance rank, low ranking individuals showed the highest social preference. Responses of

the subjects were unlikely to be driven by mere curiosity for movement on the screen, as we showed that the subjects could distinguish between the video stimulus and mere movement. Moreover, the birds significantly increased their observed social preference during the control trials when a new video stimulus birds was encountered and twice decreased their social preference when they had already seen a specific video stimulus bird before. Finally, we did not find evidence of any relationship between stimulus activity and observed social preference. Our results agree with a previous study that revealed a significant relationship in male great tits between the proportion of time spent near a real same-sex conspecific and personality (Carere et al., 2001). Several previous studies have revealed that video-playback experiments can be effective tools to study social behavior in birds (Partan et al., 2005; Bird & Emery, 2008; Rieucou & Giraldeau, 2009a, b; Boogert et al., 2013; Zoratto et al., 2014).

We reveal that lower-ranked birds showed a significant higher preference for social association, when offered the choice between a video of an unfamiliar great tit and a video of an empty cage. This result is somewhat surprising as we expected subordinate birds to be more evasive of unfamiliar conspecifics, especially in a foraging context. Indeed, in crimson-spotted rainbowfish (*Melanotaenia duboulayi*), lower-ranked individuals were interpreted to be less social during real-life confrontations (Colléter & Brown, 2011). Yet, as it is the subordinate's behaviour that often determines the outcome of an interaction ("you cannot chase someone who doesn't flee") lower ranked individuals have been suggested to be more socially responsive (Rowell, 1974).

The evidence we found for the negative causal relationship between rank and social preference suggests that the increased social association could be an active strategy employed by lower ranked individuals. Possibly, lower ranked individuals actively sought out a social association as soon as they realized that the stimulus bird was not a threat to them. Seeking social association could increase social status or dominance rank as shown for long-tailed manakins (*Chiroxiphia linearis*) (McDonald, 2007) and eastern chimpanzees (*Pan troglodytes schweinfurthii*) (Gilby et al., 2013). In nature, it could also lead to more social information on feeding locations (Aplin et al., 2012) and thereby increase survival chances, since subordinate birds are known to suffer higher mortality from starvation (Gosler, 1996). Finally, it could be a strategy to increase overall vigilance. It would benefit lower-ranked individuals, who have less time to spend on vigilance (Krams, 1998), to have the additional vigilance of a social partner in addition to the increased safety in numbers. For example, shared vigilance during foraging has

recently been revealed as a likely driver of same-sex pair formation in rabbitfish (*Siganus* spp.) (Brandl & Bellwood, 2015). The existence of such behavioural strategies in great tits is not uncommon; for example competitive ability in great tits has been suggested to be more the consequence of a behavioural strategy than of individual quality (Cole & Quinn, 2012). Another (not mutually exclusive) possibility is that higher-ranked birds experience a stronger loser effect after losing confrontations (Rutte et al., 2006), and so adopted more evasive behaviour as soon as they noticed the video stimulus bird was not fleeing from them. This emphasizes the difficulty of drawing causal conclusions from un-manipulated social associations that are the consequence of both the focal individual and the social partner and stresses the necessity to perform fully-controlled social stimulus experiments.

We show that shy birds spent relatively more time near a novel stimulus bird, indicating a higher social preference when confronted with an unfamiliar conspecific, while bolder birds only became a bit more interested the second time they saw a particular stimulus bird. A study looking at social exploration in male great tits, measured as the time spent close to the cage of a unfamiliar male conspecific, revealed that slower explorers (exploration score is a combination of the novel environment score and the novel object score in this study) spent more time on social exploration (Carere et al., 2001). Combined with this study, these findings suggest that great tits with re-active personality types have a higher motivation to spent time near unfamiliar conspecifics, at least in a non-threatening context. Indeed, individuals have been revealed to differ in relation to their boldness in how sensitive they are to the social environment (Carere et al., 2001; Kurvers et al., 2010b; Webster & Ward, 2011; Jolles et al., 2015; van der Meer & van Oers, 2015). Especially shy reactive individuals are predicted to be more sensitive to their environment (Jolles et al., 2014; Guillelte et al., 2015), but see (Marchetti & Drent, 2000), thus if shy birds discovered that the stimulus bird did not provide any social information (Kurvers et al., 2010b; Aplin et al., 2012), it possibly decreased the usefulness of a social association and led to a decrease in social preference when birds were confronted with a certain stimulus bird for the second time. At the same time, it is possible that bolder birds became more interested in this “persistent” stimulus bird (Amy et al., 2010; **Chapter 7**) the second time they encountered it. Context-dependence of behavioural characteristic effects is a commonly encountered phenomenon in behavioural studies (Dingemanse & de Goede, 2004; van Oers et al., 2005; Webster & Ward, 2011; Jolles et al., 2014). Since individual differences are expected to matter significantly for social functioning and social structure (Wolf & Krause, 2014), it is

important to take context dependence into account when drawing conclusions about personality and social behaviour.

Previous studies in the wild found a positive effect of exploratory behaviour on the time spent close to conspecifics (Aplin et al., 2013; **Chapter 3**), while we did not find an effect in this study. Fast explorers are more risk-prone (van Oers et al., 2004) and are often the more aggressive and stronger responders in social conflicts (Verbeek et al., 1996; Amy et al., 2010). Because we created a much less hostile environment by presenting social stimuli via (none-threatening) videos, this might explain why we did not find effects of exploratory behaviour on social preference.

In conclusion, by using effective manipulation of social stimuli via High Definition video playback, we have experimentally demonstrated the causal effect of dominance rank on the strength of social association in a key model species for the study of social dynamics (Verbeek et al., 1996; Aplin et al., 2013; Aplin et al., 2015b; **Chapter 3**). It is important to understand if and how individuals can strategically adapt to socially imposed traits, such as dominance rank, which can have large fitness consequences (Gosler, 1996; McDonald, 2007; Colléter & Brown, 2011; Gilby et al., 2013). Moreover, insight into how social relations and thus social structures form, are maintained, and change, is crucial for understanding the long-term consequences of social network positions (McDonald, 2007; Oh & Badyaev, 2010; Formica et al., 2012; Shizuka et al., 2014; Culina et al., 2015). We therefore aim to stimulate the future use of such novel technologies to advance our understanding of the mechanisms of social network structuring in ecology and evolution.

Acknowledgements

We are grateful to Marylou Aaldering, Coretta Jongeling and Ruben de Wit for animal caretaking.

Supplement to Chapter 4

Figure S1

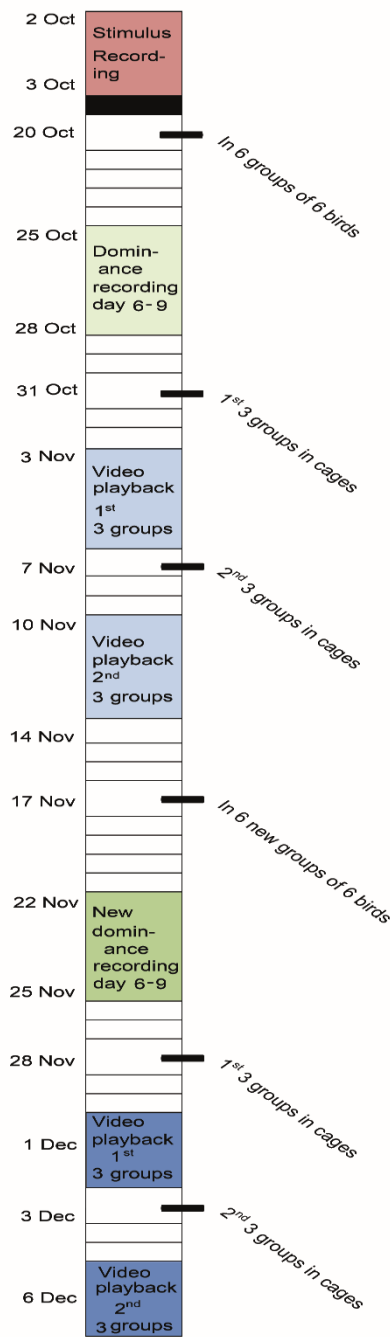


Figure S1. Stimulus video recording took place 3 weeks before the start of the experiment. Subjects had not been in physical contact with each other since the hand-rearing period. We assigned 36 birds to single-sex groups of six birds (two female and four male groups) and housed them in six aviaries. We conducted dominance observations from the 6th until the 9th day birds were grouped together, as dominance ranks in great tits stay relatively stable from that moment onwards (Verbeek et al., 1999). After a minimum of twelve days in the group, birds were individually housed in cages connected to the experimental room. Because of space limitations, we conducted the first four video playback trials (one trial per day) with individuals from only three groups. Subsequently, individuals from the first three groups were moved to new individually cages and individuals from the other three groups were moved into the cages adjacent to the experimental room. After we conducted all four playback trials also with these three groups, all birds ($N = 36$) were reassigned to six new groups based on their previous dominance ranks. We placed birds with similar dominance ranks together. After a new round of dominance recordings, one final video playback trial was conducted with each bird, again in two batches of three groups following the same protocol as before.

Figure S2

Figure S2. Pictures of the experimental set-up. A male great tit in the experimental trial can choose sitting close to a screen showing a unfamiliar male conspecific or a screen showing an empty cage.

Figure S3

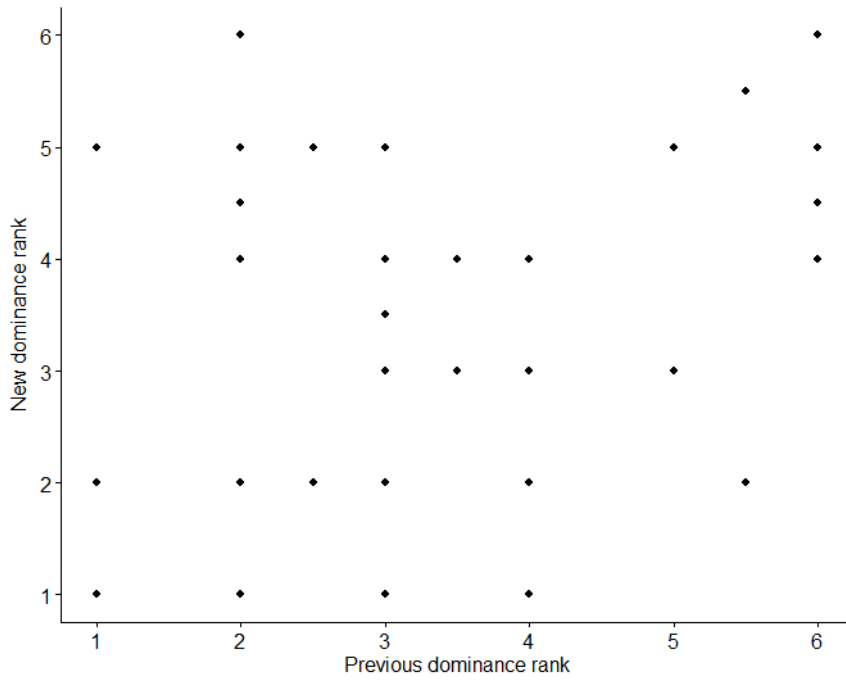


Figure S3. A picture example of a social stimulus video.

Figure S4



Figure S4. A picture example of a control stimulus video (the manipulated “blurred” version of the video shown in Figure S3).

Figure S5**Figure S5.** New rank after manipulation in relation to old rank



5

To sing or not to sing: seasonal changes in singing vary with personality in wild great tits

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Behavioral Ecology (2016) In press

Abstract

Expression of sexually selected signals in many species varies over time of day and season. A key model system to study this variation in signal expression is birdsong. Yet, despite good ecological understanding of why song varies across time of day and season, much of the individual variation remains unexplained. While some of the inter-individual variation in singing depends on the quality or motivation of an individual, it can also vary with other male characteristics. Because singing has been shown to vary with personality traits in specific contexts, personality is thus an important candidate to explain part of the variation in seasonal and daily singing. Using a personality-typed field population of great tits (*Parus major*), we here show that singing activity peaked at dawn during the fertile period of the females and that the association between male personality and singing activity depended on the reproductive stage of his mate; faster explorers significantly increased in singing activity during main periods of fertility and maternal investment (egg laying and incubation). Moreover, males with higher singing activity tended to raise more fledglings. Increased singing by faster explorers during key periods of female reproductive investment suggests that faster explorers are more responsive to changes in female reproductive stage, contrasting the general view that faster explorers are less responsive to environmental and social changes. Most importantly, these findings highlight that multiple factors including personality need to be integrated when assessing causes of variation of highly variable sexually selected signal traits.

Introduction

Individuals vary in sexually selected signals and numerous studies have shown that part of such variation can be assigned to variation in signaller quality (Andersson, 1994; Andersson & Simmons, 2006). Yet, there is increasing evidence that selection acts on sexually selected signals not merely along the line of a quality continuum but that also other factors are relevant in the evolution of mating signals. For instance, condition- and experience-dependent signal preferences are expected to lead to substantial variation in mating decisions (Jennions & Petrie, 1997; Cotton et al., 2006; Holveck & Riebel, 2010; Dunn et al., 2013; Honarmand et al., 2015), as do uncertainties in decision making (Wiley, 2015).

A key system in understanding the causes and consequences of such multiple factors affecting variation in sexually selected signals is birdsong. Birdsong is a well-studied and an important signalling behaviour, used by males to attract females and to defend territories against rivals (Catchpole & Slater, 2008; Naguib & Riebel, 2014). Most male songbirds in the temperate zones show a clear peak of singing in spring, i.e. the breeding season, when they become territorial and when females are fertile. Within the breeding season, males further vary song output with the actual reproductive state of their mate: for instance, *Acrocephalus* warblers reduce singing after a female settles in their territory (Catchpole, 1973) and nightingales (*Luscinia megarhynchos*) cease nocturnal song upon mating (Amrhein et al., 2002; Amrhein et al., 2004b), while great tits (*Parus major*) have been shown to peak in singing around female fertility (Mace, 1987; Amrhein et al., 2008). Such striking variation in seasonal song output suggests that the selection pressures on song output are different at different seasons.

Moreover, most males also show a striking diurnal variation in song output, with a peak of singing around dawn (Staicer et al., 1996). In temperate zones, this is most noticeable in spring, when males defend breeding territories and attract mates. Dawn song has been shown to be particularly important in female attraction, extra-pair matings, and territory defence (Kacelnik & Krebs, 1983; Slagsvold et al., 1994; Poesel et al., 2001; Kunc et al., 2005; Dalziel & Cockburn, 2008; Murphy et al., 2008; Suter et al., 2009). There are several factors which may have selected for this peak in dawn song, including female behaviour, sound transmission characteristics, territory invasions, and low foraging success at dawn (reviewed in: (Kacelnik & Krebs, 1983; Kunc et al., 2005)).

Yet, despite a good social and ecological understanding of why singing varies across time of day and season, much of the individual variation within a species remains

unexplained. Recent studies have revealed that personality traits, such as exploration behaviour, explain variation in singing in specific contexts (Garamszegi et al., 2008; Amy et al., 2010; Naguib et al., 2010; **Chapter 7**), yet whether personality traits also explain daily and seasonal intra-individual variation in singing is still an open question. Previous studies focusing on effects of personality on birdsong focused on one particular time of day or period and thus could not integrate daily and seasonal variation. For instance, an aviary study revealed that faster explorers have higher song rates before egg laying at dawn and throughout the day (Naguib et al., 2010), while an analysis of dawn song rate before egg laying and personality in the year of the study presented here did not reveal a relation between personality and dawn song rate in wild great tits (**Chapter 7**). Yet, experimental field studies before egg laying showed that faster explorers sing at a lower song rate but with longer songs (Amy et al., 2010), generally show a stronger vocal response (**Chapter 7**), and during egg laying are more likely to switch song types (Jacobs et al., 2014), than do slower explorers in response to a simulated intruder. Because personality is such an important candidate to explain behavioural variation in specific contexts, it may well explain also so far unidentified individual variation of both seasonal and daily singing. Indeed personality might be particularly relevant in explaining changes in singing that co-vary with such environmental changes as reactive individuals (slow explorers) have been shown to be more responsive than proactive individuals (faster explorers) to changes in the environment (Benus et al., 1987; Verbeek et al., 1994; Titulaer et al., 2012).

Great tits are among the best-studied species with respect to the variation and function of male song (McGregor et al., 1981; McGregor & Krebs, 1982; Lambrechts & Dhondt, 1990; Slagsvold et al., 1994; Rivera-Gutierrez et al., 2010; **Chapter 7**), and are among the key species in studies on the function of diurnal and seasonal variation of song (Slagsvold, 1977; Mace, 1986; Slagsvold et al., 1994; Amrhein et al., 2008). Moreover, great tits also have been extensively studied with respect to the ecology and evolution of personality traits (van Oers & Naguib, 2013). In great tits, repeatable variation in exploration behaviour in a novel environment, a validated measure of a personality trait, has been shown to explain variation in territorial behaviour (Amy et al., 2010; Jacobs et al., 2014; **Chapter 7**) and male song rate in captivity (Naguib et al., 2010).

In order to determine the relation between personality and temporal variation in singing, we here quantified singing activity, measured as the decision to sing or not to sing (Amrhein et al., 2002; Amrhein et al., 2004b; Amrhein et al., 2008),

throughout the whole breeding season at different times of day in a unique wild population of personality-typed great tits. Based on previous studies (Amrhein et al., 2008) we predicted the peak of singing not to be restricted to the fertile period and that faster explorers have a higher overall singing activity, as shown for song rate under aviary conditions (Naguib et al., 2010). Yet, we specifically predicted that personality traits interact with diurnal (dawn, morning, evening) and seasonal (mate reproductive stage) factors. Slower explorers have been shown to be more responsive to environmental changes so that we expected them to show larger diurnal and seasonal variation in singing than faster explorers and thus to stronger respond to expected changes in selection pressures. Moreover we expected an overall higher singing activity to have a positive fitness effect.

Methods

Study area

We collected the singing data from 20 March to 31 May 2012 from a nest box population of great tits in the study area Westerheide (52°01'00.0"N 5°50'20.0"E), The Netherlands, a mixed wood (120 ha) with approximately 200 nest boxes. Nest boxes were checked at least once weekly from early March onwards. We determined pair identity by catching individuals in the nest box during chick feeding following a standardized procedure (van Oers et al., 2008). We registered the day the first egg was laid, which allowed us to determine female reproductive status and we additionally recorded clutch size, hatch date, and number of offspring fledged as breeding data. The research complies with the current laws of the Netherlands and was conducted under the KNAW-DEC license (Dutch animal experimentation committee) NIOO 10.05.

Female reproductive stage

The fertile period in songbirds starts approximately a week before the start of egg laying and lasts until the laying day of the penultimate egg (Birkhead & Møller, 1992). Accordingly, we assigned six breeding stages to each nest: (1) pre-fertile period (21 to eight days before first egg); (2) pre-laying fertile (seven to one days before first egg); (3) egg laying fertile (first until penultimate egg); (4) incubation; (5) nestling (offspring in the nest being fed by the parents); (6) fledgling (offspring out of the nest being fed by the parents) stage.

Exploration behaviour

The great tit population at the study site is monitored all year. With exception of the breeding season, birds are caught at regular intervals all year round using mist

nets at feeder stations or via roost inspections of the nest boxes. During chick feeding, parents are caught in their nest boxes using spring traps. All individuals caught for the first time outside the breeding season are routinely taken to the avian indoor facilities and tested for exploration behaviour in a standardized context as a proxy for personality, using a novel environment test (Verbeek et al., 1994). On the morning after taking individuals from the wild, they are individually tested in a room (4.0 x 2.4 x 2.3 m) with five artificial trees. After birds enter the experimental room by themselves, we recorded the number of flights (movements between trees) and hops (movements within trees) within the first 2 min. The total number of flights and hops was used to calculate an exploration score. These scores have a high repeatability ($r = 0.48$, $P < 0.0001$, $N = 111$, (Dingemanse et al., 2002)) and have been shown in numerous studies to capture consistent and repeatable variation in behaviour in various contexts in the lab as well as in the field and thus are an established measure as personality trait (van Oers & Naguib, 2013). In total 95 males were used as subjects in this study, of which for 67 individuals we had an exploration score.

Singing activity

We determined singing activity three times per day: dawn (30 minutes before sunrise), morning (30 minutes after sunrise) and evening (two hours before sunset). We conducted systematic rounds by bicycle from 20 March to 31 May 2012 which were comprised of two different routes (passing different territories) alternated in two directions (both approximately 3.5 km long) and took about 35 minutes. Both routes were conducted on alternating sample days, each covering half of the territories. (total rounds per male, 78 ± 1 (*mean \pm SE*); rounds per male per time of day and season: dawn-pre-fertile, 7.1 ± 0.2 ; dawn-pre-laying fertile, 3.1 ± 0.2 ; dawn-egg laying, 3.7 ± 0.2 ; dawn-incubation, 6.8 ± 0.2 ; dawn-nestling, 6.7 ± 0.2 ; dawn-fledgling, 1.6 ± 0.3 ; morning-pre-fertile, 7.1 ± 0.2 ; morning -pre-laying fertile, 3.1 ± 0.2 ; morning -egg laying, 3.7 ± 0.2 ; morning -incubation, 6.8 ± 0.2 ; morning -nestling, 6.7 ± 0.2 ; morning -fledgling, 1.6 ± 0.3 ; evening-pre-fertile, 6.7 ± 0.2 ; evening -pre-laying fertile, 2.7 ± 0.2 ; evening -egg laying, 3.5 ± 0.2 ; evening -incubation, 5.9 ± 0.2 ; evening -nestling, 3.9 ± 0.2 ; evening -fledgling, 1.2 ± 0.3).

Previous studies on nightingales, blue tits, and great tits have shown that scoring singing this way, captures important variation in singing across time of day and season (Amrhein et al., 2002; Amrhein et al., 2004b; Amrhein et al., 2008). During each round the location of singing males was marked on a physical map. In R (v.2.15.0, R Development Core Team 2008 <http://www.R-project.org>), a tessellation procedure (Dirichlet tessellation; package 'deldir') of occupied nest

boxes was used to assess territories and assign singing locations to individual males. Singing activity is expressed as the proportion of rounds on which a certain male was heard singing, following established methods (Amrhein et al., 2002; Amrhein et al., 2004b; Amrhein et al., 2008). Binary data were derived from the three daily observation rounds (0 for not singing, 1 for singing) and used to calculate average proportions of singing across multiple days within each period. During part of this period (26 March until 7 April; mainly the pre-fertile period) playback experiments were performed after the morning rounds in the same area (**Chapter 7**). In order to exclude possible effects of subject and neighbourhood singing in response to playback, we excluded the evening rounds on the playback date and all rounds on the day after the playback for all subjects and their immediate neighbours, as previous studies have shown that playback can affect singing on the next day (Amrhein & Erne, 2006; Hall et al., 2006; Schmidt et al., 2007; Erne & Amrhein, 2008) and that neighbouring males can influence each other's singing behaviour (Naguib et al., 2004; Fitzsimmons et al., 2008a; Sprau et al., 2012; **Chapter 6**).

Statistical analysis

Data were analysed using generalized linear mixed-effect models (GLMM; GENLIMIXED procedure in SPSS 22.0) with the number of times a male was found singing over the total number of rounds as the response variable, a probit function and male ID (*Estimate*: 0.12 ± 0.03 , $P < 0.001$) as random factor. We also included the interaction between male ID and female period (*Estimate*: 0.05 ± 0.02 , $P = 0.01$) as random slope, to account for individual differences in slope (Schielzeth & Forstmeier, 2009). Factors tested were male age (2 calendar years or older), male condition (calculated as the residual of a linear regression of mass on tarsus), number of fledglings as measure of fitness, season (days since March 1), season2, time of day (dawn, morning, evening), exploration score, reproductive stage, and the two-way interactions: time of day and reproductive stage, time of day and exploration score, and reproductive stage and exploration score. Exploration score was analysed as a continuous variable. Yet, in order to better visualize the results in figure 2, and for the post-hoc contrast analysis including exploration behaviour, this variable was categorized into two categories with the individuals having the lowest 50% scores labelled as slow (*Mean \pm SE*; 16.0 ± 0.17 , *Range* 7.0 to 22.2, $N = 34$) explorers and the individuals with the 50% highest scores labelled as fast (*Mean \pm SE*; 32.3 ± 0.37 , *Range* 22.3 to 54.6, $N = 33$) explorers. We conducted stepwise backward elimination of non-significant terms, starting with the interactions to create a minimal adequate model. Variables were tested against this minimal

adequate model for hypothesis testing. Post-hoc analyses were conducted for significant variables and interactions using pairwise contrast analysis, where continuous predictors were set at fixed values using the GENLINMIXED procedure. P-values were corrected for multiple testing using least significance difference (LSD).

Results

Males varied their singing activity significantly across the time of day (Table 1; Figure 1) with a peak of singing at dawn (Figure 1). We show that variation in singing activity during the day also varied significantly across the reproductive stages of the female (reproductive stage * time of day; Table 1; Figure 1). The variation in singing activity across the season was observed only during dawn (contrast analysis; $F_{5,1012} = 8.44$; $P < 0.0001$) and during the evening (contrast analysis; $F_{5,1012} = 6.82$; $P < 0.0001$), while there was no variation in singing activity over reproductive stage for the morning song (contrast analysis; $F_{5,1012} = 0.81$; $P = 0.54$). Dawn singing activity peaked during key periods of female reproductive investment, egg laying (the main fertile period) and incubation (Table 1; Figure 1), thus did not decrease right after the fertile period. Variation in singing activity across the time of day was not affected by exploration behaviour (Table 1). Dawn singing activity during egg laying, measured as the proportion of rounds a male was heard singing, did not correlate with dawn song rate, measured as the number of songs per minute ($P = 0.19$, $r = 0.17$, $N = 64$; song rate data of the same individuals from Chapter 6).

Table 1 Results from the GLMM on singing activity at different times of day during the breeding season. Significant values are indicated in bold.

Fixed effects	<i>F</i>	<i>DF</i>	<i>P-value</i>
Season	11.9	1,246	<0.0001
Season ²	15.8	1,229	<0.0001
Time of day	17.4	2,1011	<0.0001
Reproductive stage	1.4	5,317	0.24
Exploration score	0.07	1,100	0.80
Condition	3.2	1,58	0.08
Time of day x reproductive stage	6.2	10,1011	<0.0001
Exploration score x reproductive stage	3.0	5,1011	0.01
Exploration score x time of day	0.4	2,1011	0.69
Number of fledglings	3.1	1,50	0.08
Age	1.1	1,53	0.30

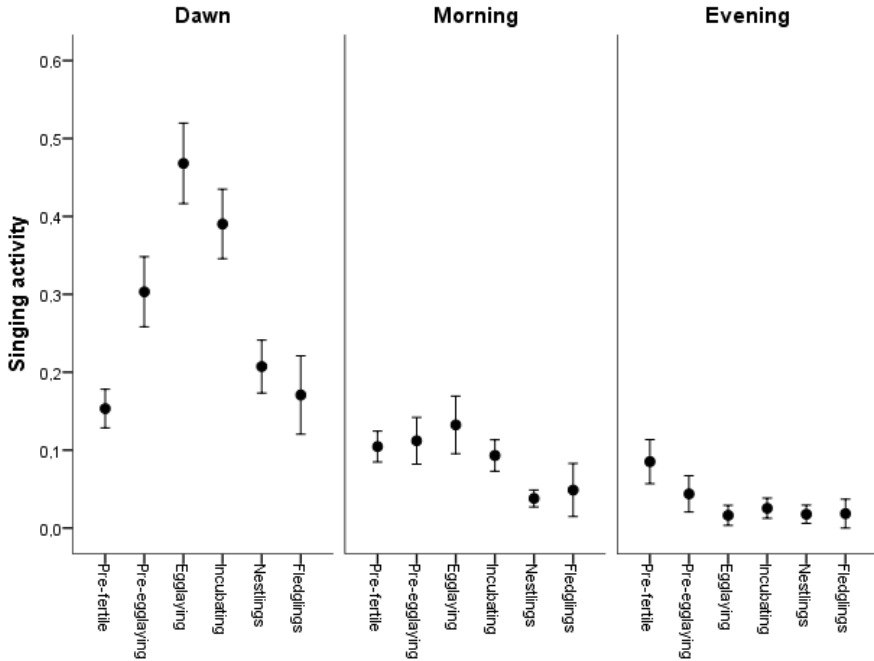


Figure 1. Singing activity at the different times of day during the reproductive stages. Average singing activity of all males at dawn, morning, and in the evening for all reproductive stages ($N = 91$ males; mean \pm se).

Males varied their singing activity across the reproductive stage of their mate but differently depending on their exploration behaviour (exploration score \times reproductive stage; Table 1; Figure 3). Slower exploring individuals had a more gradual pattern of singing activity over the season: they showed no significant differences between the reproductive stages (all pair-wise contrasts for slow explorers < 0.18 , $P > 0.12$). In contrast, faster explorers varied their singing more plastically with the changes in the female reproductive period and showed a significant peak in singing during female egg laying and incubation. Faster explorers significantly increased singing from pre-egg laying to egg laying (pair-wise contrast for fast explorers on pre-egg laying – egg laying, $P = 0.03$), and then significantly decreased singing activity from the incubation phase to the nestling phase (pair-wise contrast for incubating to nestling, $P = 0.001$). There was no association between exploration behaviour and the start of egg laying in our population ($r = 0.02$, $P = 0.86$, $N = 59$), thus potential personality-dependent differences in timing

of breeding cannot explain these differences in singing. Male singing also tended to be associated positively with condition (Table 1) and the number of fledglings raised (Table 1, Figure 3).

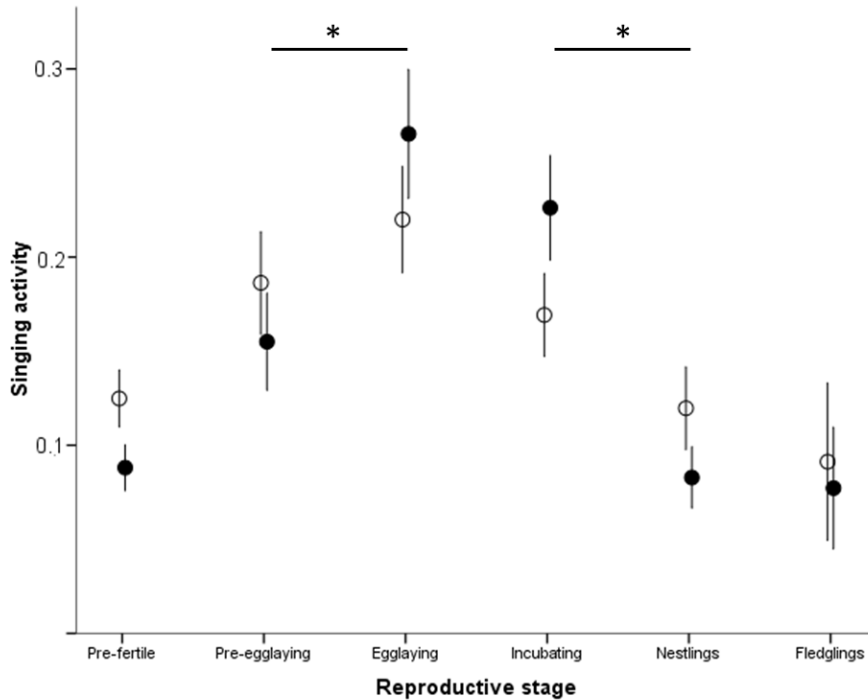


Figure 2. Singing activity associated with exploration behaviour in all reproductive stages. Singing activity for slow (○) and fast (●) explorers for all times of day. Exploration score was used as continues variable in the analysis (see methods). For graphical reasons, exploration behaviour here is split in slow (*Mean ± SE*; 16.0 ± 0.2 , *Range* 7.0-22.2, $N = 34$) and fast (*Mean ± SE* = 32.3 ± 0.4 , *Range* 22.3-54.6, $N = 33$) explorers. Significance (asterisk) refers to significant changes in singing activity by fast explorers over the respective reproductive stages.

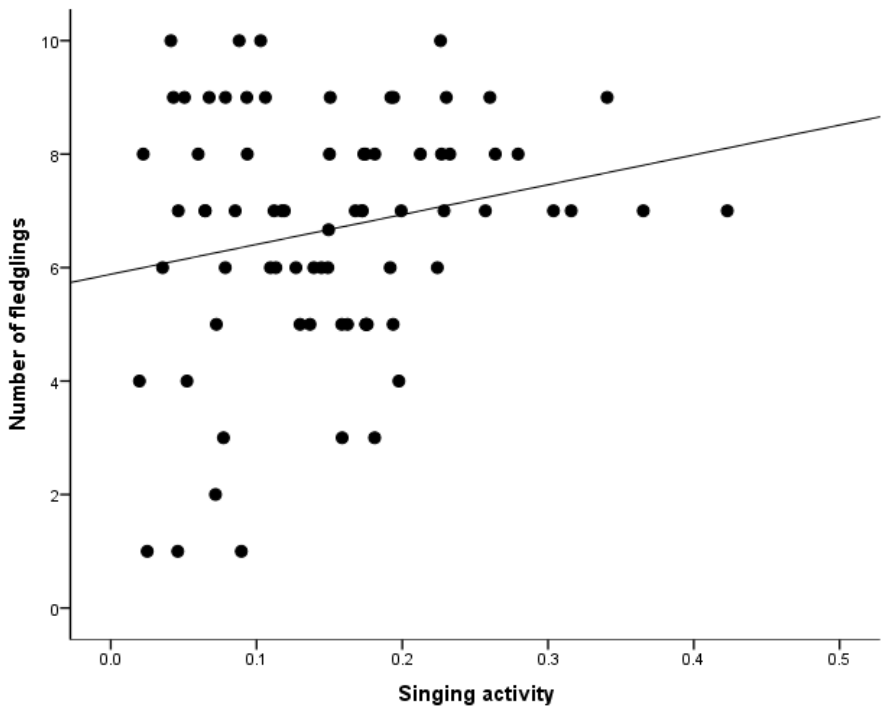


Figure 3. Relation between singing activity and the number of fledglings produced.

Discussion

This large scale sampling of natural singing activity in male great tits revealed that seasonal changes in singing varied with male exploration behaviour. Singing was most pronounced at dawn during their mates' fertile period and while she was incubating. This was specifically so in faster explorers who significantly increased in singing during the key periods of female reproductive investment, egg laying, and incubation. Moreover, males with higher singing activity tended to have a higher fitness, measured as the number of fledglings produced.

The finding that singing was most pronounced at dawn and peaked during egg laying, the fertile period of the female, and declined thereafter supports a previous study in great tits (Amrhein et al., 2008) and studies in other songbirds (Ballentine et al., 2003; Dalziel & Cockburn, 2008; Bruni & Foote, 2014) providing further correlative evidence that dawn song in great tits, as in other species (Gil et al., 1999), functions also beyond the fertile period. Even though increased singing at dawn during mate fertility had been suggested to be a handicap signal as only high

quality males would benefit from high singing activity (Møller, 1991), this idea is not generally supported due to evidence from several species showing that singing activity often is higher during other reproductive stages (Gil et al., 1999). Moreover, higher singing activity during egg laying might function likewise as a signal to the female triggering more hormonal investments in the eggs (Gil et al., 2004; Gil, 2008). More singing by males during incubation, thus after the fertile period, may still significantly affect fitness as it could act as continuing stimulus to signal male quality, possibly affecting female incubation behaviour and subsequent parental care as well as function in maintaining the territory. The trend that males which sang more produced more fledglings supports the general idea that male singing activity partly reflects aspects of male or territorial quality.

Most interestingly, this study shows that temporal variation in singing activity, besides reproductive stage and time of day, differed with male personality. We had predicted that faster explorers would sing more as shown for song rate in a laboratory study (Naguib et al., 2010), but overall this was not the case. Song rate and singing activity indeed are different non-correlating traits, as shown here, and thus are likely to have different signal values. Yet, as we also did not find a relation between personality and song rate in a field study on great tits (**Chapter 7**), the evidence is accumulating that singing in the wild is related to personality in more subtle or context-specific ways. This view is supported by our findings that only faster explorers significantly increased singing during the key periods of female reproductive investment, egg laying and incubation, indicating that males contrasting in personality vary their singing differently with changes in their mate's reproductive stage. Such a significant increase in singing during the egg laying and incubation period in faster explorers could reflect a greater effort in vocal mate guarding or mate stimulation and in attracting extra-pair females. Indeed faster explorers have been shown to have a higher probability of losing paternity than slower explorers (Patrick et al., 2012), even though a previous study on our population found that the combination of the personalities of both mates is related extra-pair paternity (van Oers et al., 2008). It may well be possible, however, that faster explorers have to invest more in signalling to not loose paternity.

In any case, the stronger increase in singing in fast explorers contrast our prediction that slower explorers rather than faster explorers would vary their singing more with changes in reproductive state by the female. The more pronounced variation in singing across the season in faster explorers could be interpreted as them being more rather than less responsive to environmental (social) change, contrasting the general view that faster explorers have more

routines and are less responsive to environmental and social changes (Carere et al., 2005; Wolf et al., 2008; de Lourdes Ruiz-Gomez et al., 2011; Herborn et al., 2014). Slower explorers have been shown to respond more to changes in environmental cues (Guillette et al., 2010; Titulaer et al., 2012), and to generally follow less routine-like behaviour (Benus et al., 1990; Verbeek et al., 1994). Thus our findings suggest that the more routine like behaviour in faster explorers is not expressed in all contexts and the personality-dependent differences in behavioural plasticity can be expressed differently in different contexts. The increase in singing with changing reproductive state of the female thus can be taken to suggest that the selection pressure for more song in faster explorers must be rather strong, especially in light of the positive trend between singing activity and number of fledglings. Yet, whether personality-dependent variation in singing optimizes personality dependent fitness remains to be shown.

Overall this study shows that singing activity signifies different aspects of a multifunctional signal during various times of the year and individuals do not all express the same seasonal pattern in singing activity. The significant interaction between reproductive state and personality might reflect different personality-dependent trade-offs leading to a temporal division of the acoustic space. Males differing in personality therefore partly use different signalling windows, possibly also to avoid direct social interactions with males of different personality (**Chapter 3**). These findings highlight that multiple factors including personality need to be integrated, when assessing causes of variation of highly variable sexually selected signals.

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6

Song trait similarity in great tits varies with social structure

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Abstract

For many animals, long-range signalling is essential to maintain contact with conspecifics. In territorial species, individuals often have to balance signalling towards unfamiliar potential competitors (to solely broadcast territory ownership) with signalling towards familiar immediate neighbours (to also maintain so-called “dear enemy” relations). Hence, to understand how signals evolve due to these multilevel relationships, it is important to understand how general signal traits vary in relation to the overall social environment. For many territorial songbirds dawn is a key signalling period, with several neighbouring individuals singing simultaneously without immediate conflict. In this study we tested whether sharing a territory boundary, rather than spatial proximity, is related to similarity in dawn song traits between territorial great tits (*Parus major*) in a wild personality-typed population. We collected a large dataset of automatized dawn song recordings from 72 unique male great tits, during the fertile period of their mate, and compared specific song traits between neighbours and non-neighbours. We show here that both song rate and start time of dawn song were repeatable song traits. Moreover, neighbours were significantly more dissimilar in song rate compared to non-neighbours, while there was no effect of proximity on song rate similarity. Additionally, similarity in start time of dawn song was unrelated to sharing a territory boundary, but birds were significantly more similar in start time of dawn song when they were breeding in close proximity of each other. We suggest that the dissimilarity in dawn song rate between neighbours is either the result of neighbouring great tits actively avoiding similar song rates to possibly prevent interference, or a passive consequence of territory settlement preferences relative to the types of neighbours. Neighbourhood structuring is therefore likely to be a relevant selection pressure shaping variation in territorial birdsong.

Introduction

Maintaining contact with conspecifics is important in many animal populations. Territorial animals are usually constrained to frequently closely approach conspecifics and therefore often maintain contact using long-range signals (McGregor, 2005; Peake et al., 2005), such as song in birds (Catchpole & Slater, 2008). Birdsong has been a key model in studies on territorial behaviour as it is not only important in territorial defence (Todt & Naguib, 2000; Catchpole & Slater, 2008), but also to establish and maintain relations with neighbours (Godard, 1991; Foote et al., 2008). Birds that possess long-term neighbouring territories can be “dear enemies”, showing reduced aggression towards each other to save time and energy, while still remaining potential rivals in competition for space and mates (Temeles, 1994). Neighbouring songbirds can even form alliances to expel common enemies, like predators (Grabowska-Zhang et al., 2012) and conspecific intruders (Goodwin & Podos, 2014). Although neighbours are known to be able to adjust their singing to each other during direct singing interactions (Todt & Naguib, 2000; Foote et al., 2008), little is known about whether territorial male songbirds affect each other’s song traits in more general terms. By understanding how signal traits vary overall, relative to the social environment, we gain greater insight into the potential selection pressures on territorial signal traits.

Territory advertisement (Krebs, 1977), along with inter-sexual advertisement (Staicer, 1996; Catchpole & Slater, 2008), is a well-established function of birdsong. For example, in great tits (*Parus major*) (Krebs, 1977) and song sparrows (*Melospiza melodia*) (Nowicki et al., 1998) broadcasting songs with speakers from inside a territory of which the owner has been removed delays intrusion of the territory by conspecifics. For many songbirds, dawn is a very active signalling period with many individuals singing at the same time, especially during the breeding season (Staicer, 1996; Gil & Gahr, 2002) when intruder pressure is high (Amrhein et al., 2004a). To repel potential intruders it is thus important for territory holders to frequently sing during dawn (Kacelnik & Krebs, 1983; Kunc et al., 2005). However, singing concurrently with similar song traits compared to immediate neighbours could lead to unintentional continuous song overlapping and song matching. For example, if several neighbours frequently sing concurrently with the same song rate, there is the potential that a part of a neighbour’s song will be unintentionally but continuously overlapped. This is undesirable, because systematic overlapping is often interpreted as an aggressive signal, eliciting strong responses by opponents (Todt & Naguib, 2000; Catchpole & Slater, 2008; Searcy & Beecher, 2009; Naguib & Mennill, 2010). Additionally, individual recognition through acoustic signals

(Myrberg jr & Riggio, 1985; Godard, 1991), for example by listening females (Hasselquist et al., 1996), might be compromised when using the same song traits as immediate neighbours. This is important, as immediate neighbours, can pose a high risk for males to lose paternity (Temeles, 1994; Hasselquist et al., 1996). Either way, in the absence of an immediate and direct conflict, such as between familiar neighbours during regular advertisement singing at dawn (Gil & Gahr, 2002; Catchpole & Slater, 2008), neighbours might benefit by not singing too similarly (Beecher et al., 2000).

Individual song traits can be predictable when they are to some extent repeatable (Wolf et al., 2011). However as long as the song traits are not perfectly repeatable, there can still be enough flexibility for slight adjustments, for instance at the start of the breeding season. In great tits, several song traits such as the proportion of time spent singing (Verhulst, 2003) and song consistency (Rivera-Gutierrez et al., 2010) are found to be moderately repeatable. Consequently, territorial great tits could anticipate the repeatable song traits of familiar immediate neighbours and moderately adjust their own song traits in order not to sing too similarly and so optimize signalling towards potential intruders and other eavesdropping conspecifics. In the breeding season, multiple neighbours will often be singing simultaneously for several weeks during dawn, and it would thus be very demanding for individuals to constantly dynamically adjust their singing behaviour to multiple individuals. Moderate repeatability of song traits would allow individuals to optimize signalling among neighbours by adjusting their song traits only a small number of times, for example during the beginning of the breeding season when new neighbours settle.

The aim of this study is to reveal whether or not song traits vary in relation to the song traits of neighbours on a population-wide scale. We therefore recorded the song of 72 unique male great tits during dawn, and examined whether song rate and start of dawn song differed more between immediate neighbours (sharing a territory boundary) than between non-neighbours. Next, we checked whether potential song trait differences between neighbours were not merely a consequence of avoiding overall signal interference. Immediate neighbours are usually singing spatially closer to each other and consequently could be hindering each other's signal transmission more than non-neighbours. Therefore we also analysed whether differences in song traits were related to the spatial proximity between the breeding nest boxes of the singing males.

Methods

Study population

The study was conducted in a long-term study population of great tits at Westerheide, The Netherlands (52°01'00.0"N 5°50'20.0"E). Westerheide is a mixed pine-deciduous wood with about 200 nest boxes attached to trees distributed within a 1000 m x 1200 m area (for further details see (Dingemanse et al., 2002)). Birds in this population are tested for exploration behaviour, an operational measure for personality, using an established and validated 'novel environment' test (Dingemanse et al., 2002; Drent et al., 2003), approved by the Institutional Animal Care and Use Committee: the Koninklijke Nederlandse Akademie van Wetenschappen - Dier Experimenten Commissie (KNAW-DEC licence NIOO 10.05 to MN and KVO). Exploration behaviour in great tits has been found to relate to social behaviour in wild great tits, both spatially (**Chapter 3**) and vocally (Amy et al., 2010; **Chapter 5 & 7**). Throughout the year, with the exception of the breeding season, newly caught individuals (captured either by mist-netting during the day or during a nest box check at night) are subjected to a novel environment test. Within 1.5 h of catching the great tits are brought to the testing facilities of the Netherlands Institute of Ecology (max 20 min by car), where they are housed individually and undergo the novel environment test the following morning (see (Dingemanse et al., 2002) for details). The birds were released again within 24 hours after initial capture.

During the breeding season, male great tits sing very actively just before sunrise and maintain high levels of singing activity throughout the pre-laying, laying and incubation period of their mate (Amrhein et al., 2008). Nest boxes were checked twice a week to record laying date (of the first egg), clutch size and hatching date. Following an established procedure, individuals in this study were identified during the chick rearing phase (when nestlings were approximately 7 days old) by catching them inside their nest boxes using spring traps (van Oers et al., 2008). We took biometry measures of the nestlings when they were 14 days old (with day 0 as hatching date). We measured both body mass and tarsus length on chicks and adults, and un-ringed birds were fitted with uniquely numbered aluminium leg rings.

Song recordings

Dawn song was recorded once for 72 males during their mate's fertile period between 1st April and 3rd May 2012. For 45 of these individuals we recorded dawn song twice during the fertile period; once during early egg laying (1-4 eggs) and once during late egg laying (5-9 eggs), with a mean interval of 4 days. Female

songbirds are fertile from approximately a few days before the start of egg laying until the day of the last egg (Birkhead & Møller, 1992). During the egg laying period, male great tits sing near the breeding nest box of their mate (Mace, 1986). Therefore, we hung programmable song recorders (Song Meters, Wildlife Acoustics or TASCAM DR-08) 1.5 m above nest boxes occupied by incubating females, the day before recording. Song recordings ran automatically from 1 h before sunrise to half an hour after sunrise.

We determined the start time of dawn song (the time before sunrise when the subject sings their first song) and calculated song rate (songs per minute) during the first 5 min using SASLab Pro (Avisoft, Berlin, Germany). For song recordings of lower quality only the start time was determined. We included males in our analysis only if we had recorded at least one of their five closest surrounding occupied nest boxes, in order to not bias our analyses with individuals who would only be compared to other males at far distances and to males with whom they did not share a territory boundary. Song rate was measured as number of songs per minute (*mean* = 9.1 songs, *SD* = 3.0, *Range* = 1.6-16.6) for 67 unique individuals, since for 5 recordings we could not accurately determine the song rate. Start time was quantified as song onset in minutes before sunrise (*mean* = 31.2, *SD* = 7.3, *Range* = 4.0-42.2) for 72 unique individuals. With the exception of the repeatability analysis, we always used the first available recording of a nest box for analysis (which was during early egg laying, except for three nest boxes), unless the quality of the first recording did not allow its use (*N* = 2).

Environmental variables

Sunrise, moonrise and moonset time data was retrieved from the KNMI (Koninklijk Nederlands Meteorologisch Instituut) and temperature data from the nearest KNMI weather station, Deelen (10 km). Percentage of moonlight, the fraction of the moon's surface illuminated (arcsine square root transformed), was taken as zero when the moon would not be above the horizon 43 min before sunrise (the earliest singing bird started 42.2 min before sunrise). We calculated distances between breeding nest boxes using GPS coordinates (Garmin GPSmap 62st). Subjects were on average 0.543 km (*SD* = 0.280 km) apart, with a minimum distance of 0.033 km and a maximal distance of 1.264 km.

Statistical analysis

For the 45 males we recorded twice during the fertile period of their mate, repeatability of song rate and start time was determined following Lessells and Boag (Lessells & Boag, 1987). The range of days between recordings was smaller

within individuals (3-6 days) than among individuals (0-32 days), raising the possibility that repeatability in song traits could be merely a reflection of more similar environmental conditions caused by the shorter time between recordings. To test this, we calculated the within- and among-days variation in song rate and start time of dawn song for both early and late egg laying, only including days on which more than one bird was recorded. Within-day variation in song rate was not smaller compared to among-day variation in song rate (Early egg laying, $F_{11,21} = 0.99$, $P = 0.48$; Late egg laying, $F_{11,22} = 0.89$, $P = 0.56$) and also within-day variation in start time of dawn song was not smaller compared to among-day variation in start time (Early egg laying, $F_{11,21} = 0.83$, $P = 0.62$; Late egg laying, $F_{11,22} = 1.68$, $P = 0.14$). Additionally, we conducted General Linear Mixed Models (GLMM) using SPSS 21.0 (IBM Corp., Armonk, NY, USA) to test for a possible linear effect of April date on song rate and start time of dawn song, including individual as a random factor to account for multiple measurements of the same individual. Residuals of both models followed a normal distribution, based on the Shapiro-Wilk test. We tested for a possible association between the condition of the bird and song rate or start time using a Pearson correlation test. Individual condition was calculated as the residual of the function of weight (body mass during chick rearing) against tarsus. We analysed whether there was a difference in song traits between one-year-old males and birds older than one year (2 years or older) using an Approximative Wilcoxon Mann-Whitney Rank Sum Test (10000 re-samplings) from the Coin R-package. Additionally, median song trait differences for one-year-old males were bootstrapped with the boot R-package to generate confidence intervals and so account for the difference in sample size, compared to the males older than one year, which could lead to spurious significant results. For all subsequent analyses we used R 2.15.2 (R Core Team, Vienna, Austria).

We calculated differences in song rate and start time between males for all comparisons (2211 $((67*66)/2)$ for song rate and 2556 $((72*71)/2)$ for start time). We conducted a tessellation using the AdeHabitat R-package. The Voronoi/Dirichlet tessellation procedure systematically divides the habitat into convex polygons (territories), each centred around the breeding nest box of a territorial male great tit. Each great tit territory has the property that every point within the polygon (territory) is nearer to its own nest box than it is to any of the other nest boxes. Birds were categorized as neighbours if they shared part of their territory boundary and their nest boxes were within 242 m of each other (twice the maximum estimated un-manipulated mean territory size (Both & Visser, 2000)). To account for the dependence of data when testing differences in similarity between

neighbours and non-neighbours, we employed the Approximative Wilcoxon Mann-Whitney Rank Sum Test (10000 re-samplings) from the Coin R-package. Additionally, median song trait differences for non-neighbours were bootstrapped with the boot R-package to generate confidence intervals and so account for the difference in sample size compared to the neighbour dataset.

Because we expected that a relationship between space and song traits would unlikely be linear, but fade with increasing distance around several centres in the population (like ripples caused by drops falling into water), we specifically tested for non-linear effects using several distance classes (Goslee & Urban, 2007). The Ecodist R-package tests for (dis)similarity of a certain feature within several distance classes (lags), by calculating a Mantel correlogram, a multivariate autocorrelation function. The Mantel r represents the (dis)similarity (of individual song trait in this study) at a certain lag distance. We used six distance lags, which are classes dividing the distance range between all possible pair combinations in six groups of equal distance intervals, ranging from close together (lag 1, midpoint 0.103 km) to exceedingly far apart (lag 6, midpoint 1.128 km). However, we only considered the first four lags when drawing conclusions, since an increasing number of birds was no longer represented in the last two lags (from 0.666 km onwards). We chose the number of six lags a priori, since a lower number would have resulted in too many distant pair combinations being included in the first (closest) distance lag (the closest pair combination was 0.033 km apart), and a higher number would have led to an increasingly smaller sample size for the closest distance lag. Increasing the number of lags (to 8 or 10) did not significantly change the outcome of the analysis.

When permutation or randomization procedures were employed, significance levels are shown using “<” or “>”, since in permutation models values for significance are based on approximation.

Results

Are great tit dawn song traits repeatable?

The song rate of individual birds was significantly moderately repeatable ($r = 0.45$, $SE = 0.12$, $F_{44,45} = 2.67$, $P = 0.001$; Figure 1a), as was the start time of dawn song ($r = 0.36$, $SE = 0.13$, $F_{44,45} = 2.10$, $P = 0.007$; Figure 1b). However, it should be noted that repeatability of start time decreased ($r = 0.21$, $SE = 0.14$, $F_{43,44} = 1.54$, $P = 0.078$), when leaving out the male with the latest start time (2.5 minutes before sunrise during late egg laying). There was no linear relationship between date and song

rate (GLMM, $F_{1,44} = 0.21$, $P = 0.65$) or start time of dawn song (GLMM, $F_{1,44} = 1.90$, $P = 0.18$). Additionally, there was no correlation between body condition and song rate (Pearson $r = 0.08$, $N = 41$, $P = 0.63$) or start time (Pearson $r = -0.13$, $N = 42$, $P = 0.42$). Finally, there was also no difference in song rate between one-year-old males and males older than one year ($Z = -0.89$, $N1 = 40$ and $N2 = 13$, $P > 0.3$), but there was a difference in start time of dawn song, with younger males starting to sing earlier than older males ($Z = 2.19$, $N1 = 43$ and $N2 = 15$, $P < 0.05$). But this significant effect of age was not reflected by the bootstrapped confidence intervals as the median start time of the older males (28.4 minutes before sunrise) did still fall within the 95 percentage bootstrapped confidence interval for the younger males ($CI = 26.9$ -37.2 min before sunrise; drawing 15 samples 10000 times without replacement).

Table 1. Non-linear spatial analysis of start time of dawn song similarity (minutes before sunrise) and temperature similarity (decimal degrees) using distance classes (lags).

		Lag 1	Lag 2	Lag 3	Lag 4
Start time of dawn song similarity	<i>Mantel r</i>	0.039	-0.04	-0.07	0.01
	<i>P</i>	<0.05	>0.1	<0.05	>0.5
Temperature similarity	<i>Mantel r</i>	0.09	-0.01	-0.05	-0.10
	<i>P</i>	<0.01	>0.7	<0.08	<0.001
Moonlight similarity	<i>Mantel r</i>	0.02	-0.001	-0.01	-0.05
	<i>P</i>	<0.23	>0.9	>0.6	<0.05
	<i>Midpoint</i>	0.103	0.308	0.513	0.718
	<i>N</i>	309	600	697	460

Does song trait dissimilarity correlate with sharing a territory boundary?

Neighbouring birds (114 out of 2211 pair combinations) were significantly more dissimilar in song rate than non-neighbouring birds were ($Z = -2.66$, $P < 0.01$; Figure 2a). The median difference in song rate for neighbours was 3.3 songs/min, falling outside the 95 percentage bootstrapped confidence interval for non-neighbours ($CI = 2.2$ -3.2 songs/min; drawing 114 samples 10000 times without replacement). Neighbours (123 out of 2556 pair combinations), however, were not more similar or dissimilar in start of dawn song compared to non-neighbours ($Z = 1.24$, $P > 0.2$; Figure 2b). The median difference in start time for neighbours was 6.4 min before sunrise, falling inside the 95 percentage bootstrapped confidence interval for non-neighbours ($CI = 6.0$ -9.2 min before sunrise; drawing 123 samples 10000 times without replacement).

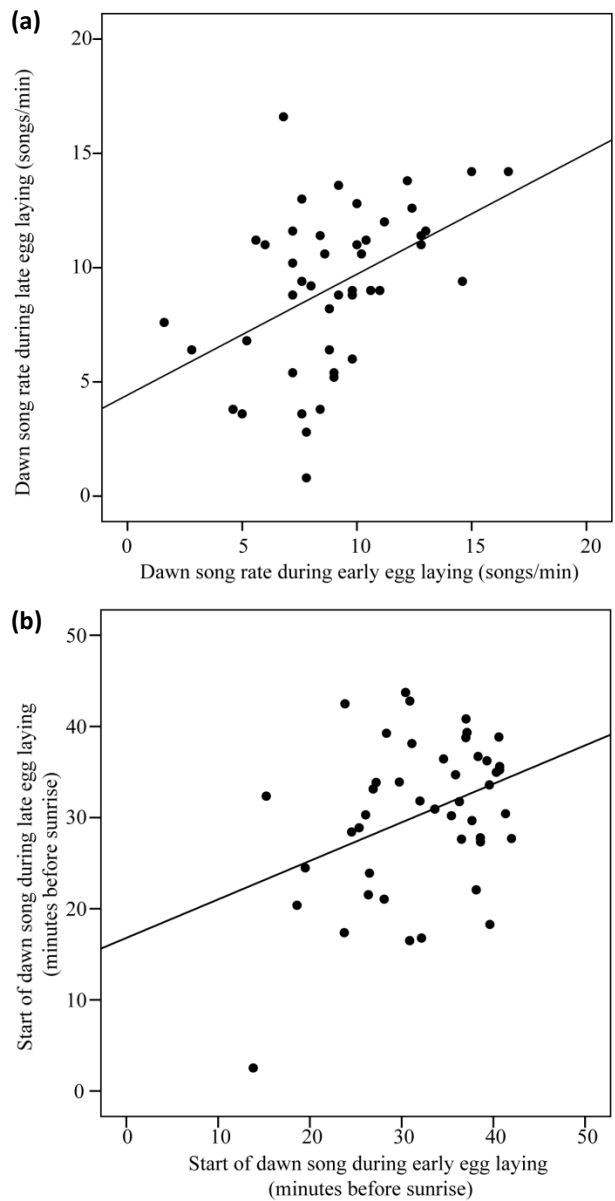


Figure 1. Male great tits are significantly repeatable in (a) song rate as well as (b) start time of dawn song. The song of 45 individual great tits was recorded twice in the fertile period of their mate, first during early egg laying (1-4 eggs) and second during late egg laying (5-9 eggs). Differences in these dawn song traits among individuals were significantly larger than within individuals.

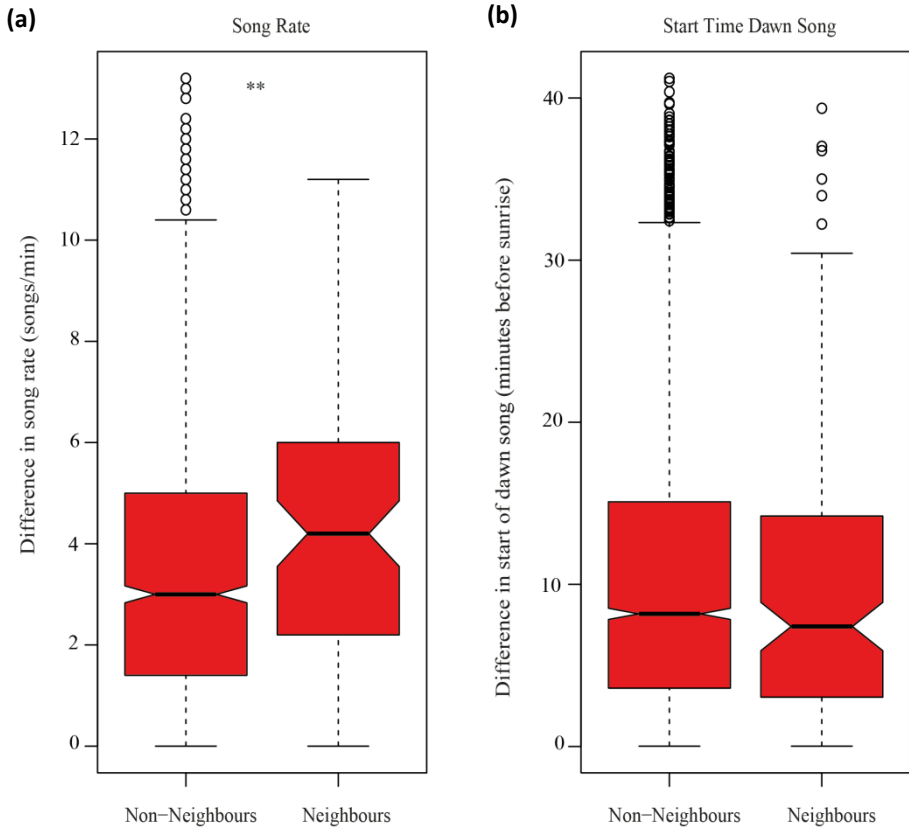


Figure 2. Notched box-plots reveal that (a) neighbours (birds sharing a territory boundary) differ more from each other in median song rate, (b) but not in median start time than non-neighbours do. Non-overlapping notches visualise a significant difference in medians.

Does song trait dissimilarity correlate with spatial proximity?

We found no evidence for a non-linear relationship between breeding distance and song rate, since birds that bred closest together did not significantly differ in song rate (Lag 1, midpoint: 0.103 km, 268 pair combinations, *Mantel* $r = -0.02$, $P > 0.3$) and there was only a trend for birds breeding very distant from each other to be more dissimilar in song rate (Lag 4, midpoint: 0.718, 419 pair combinations, *Mantel* $r = -0.04$, $P < 0.1$). However, birds breeding closest together (Lag 1) did have significantly more similar start times of the dawn song, while birds breeding one additional distance lag apart (Lag 2) did not have more similar start times (Table 1). Additionally, birds further away from each other (Lag 3) differed significantly in

start time, while there was no significant relation between birds even further apart (Lag 4; Table 1).

There was also no linear relationship between the breeding distance and the difference in song rate between males (*Mantel* $r = -0.05$, $P > 0.8$). When controlling for breeding distance, neighbours still tended to differ more in song rate than non-neighbours (Partial Mantel with Non-Neighbours and Neighbours, *Mantel* $r = 0.04$, $p < 0.06$). Likewise, there was no linear relationship between breeding distance and difference in start time (*Mantel* $r = -0.05$, $P > 0.8$) and when controlling for breeding distance, neighbours were not significantly similar or dissimilar in start time of dawn song compared to non-neighbours (Partial Mantel with non-neighbours and neighbours, *Mantel* $r = -0.04$, $P > 0.9$).

Could dissimilarity in song traits be explained by dissimilarity in personality or environmental conditions?

Neighbours (75 out of 1225 pair combinations) were not more similar or dissimilar in exploration behaviour compared to non-neighbours, for either song rate analysis ($Z = 0.33$, $P > 0.7$) or start time analysis (80 out of 1431 pair combinations, $Z = 0.53$, $P > 0.5$). However, although pairs of neighbours were not more similar in laying date ($Z = 1.34$, $P > 0.1$), pairs of neighbours in the analysis of song rate did have fewer days between recordings than non-neighbours ($Z = 2.05$, $P < 0.05$). Likely because of the shorter time between recordings, pairs of neighbours were also recorded during more similar temperatures compared to non-neighbours ($Z = 4.69$, $P < 0.001$). Neighbour recordings were not more similar, or dissimilar, in moonlight conditions compared to non-neighbours ($Z = 0.19$, $P > 0.8$). Still, similarities in environmental conditions during recordings of neighbours compared to non-neighbours cannot explain, and are in contrast to, the significant dissimilarity of song rate between neighbours.

For the analysis of start time, the closest breeding birds were more similar in laying date (Lag 1, midpoint: 0.103 km, 309 pair combinations, *Mantel* $r = 0.05$, $P < 0.01$) and were recorded with fewer days in between (Lag 1, midpoint: 0.103 km, 309 pair combinations, *Mantel* $r = 0.06$, $P < 0.005$), while there was a trend for very distant birds to be more dissimilar in laying date (Lag 4, midpoint: 0.718 km, 460 pair combinations, *Mantel* $r = -0.04$, $P < 0.1$) and to be recorded with more days in between (Lag 4, midpoint: 0.718 km, 460 pair combinations, *Mantel* $r = -0.05$, $P < 0.05$). Hence, likely because of fewer days between recordings, the birds breeding closest to each other (Lag 1) were also recorded with more similar temperatures, while very distant birds (Lag 4) were recorded with significantly dissimilar

temperatures and dissimilar moonlight conditions (Table 1). Yet, we did not find a linear relationship between similarity in start time and similarity in laying date (*Mantel* $r = -0.01$, $P > 0.5$), temperature (*Mantel* $r = -0.02$, $P > 0.5$) or moonlight (*Mantel* $r = -0.01$, $P > 0.5$).

Discussion

Our analysis of dawn song characteristics revealed that great tit song rate and start time during dawn song were significantly moderately repeatable, allowing neighbours to anticipate and respond by adjusting their own song traits accordingly (Wolf et al., 2011). Indeed, we found that sharing a boundary significantly related to the similarity in song rate of birds, in which neighbours differed more in song rate when compared to non-neighbours. We did not find an effect of neighbours on start time of dawn song, however we did find that males breeding close together started dawn song at more similar times than males breeding further apart, who started at more dissimilar times.

What could drive dissimilarity in song rate between neighbours?

The dissimilarity in song rate between neighbours could result from: (1) an active process of neighbouring males deliberately adjusting their song traits or (2) a passive process in which song rate differences result from different types of males settling nearby.

Neighbour-related differences in singing could result from familiar neighbours anticipating and actively averting the potential for unintentional continuous overlapping of each other (Maynard et al., 2012). By singing at different rates, males could be able to reduce signal interference and advertise territorial ownership without risking of being perceived as aggressive by their neighbours and so escalate an interaction (Todt & Naguib, 2000). For instance in black-capped chickadees (*Poecile atricapillus*) individuals breeding in the same neighbourhood frequency match each other (sang song types with similar frequencies) to a lesser degree when they are familiar with each other (in the same winter flock the previous winter) (Foote et al., 2008). Additionally, in this unique study using an acoustic location system to record dawn singing interactions, Foote et al. (Foote et al., 2008) also did not find an effect of neighbour proximity on the degree of matching, comparable to the lack of an effect of proximity on song rate that we found. A promising avenue for future work is further large-scale studies to examine if and how often similarity in song rate leads to consistent overlapping by concurrently singing neighbours and whether this would elicit aggressive interactions between them. Moreover, we recently showed that territorial male

great tits encounter each other non-randomly (**Chapter 3**), raising the question to what extent these social, possibly aggressive, encounters relate to the non-random variation in song trait differences we revealed here.

Besides avoiding neighbour aggression, singing distinctly from neighbours might also function in affecting behaviour of mates, who in song birds have been shown to use song information in extra-pair mating decisions (Mennill et al., 2002). Especially since the great tit is a socially, but not sexually, monogamous species (van Oers et al., 2008), it might be very relevant for males to distinguish themselves from neighbours using their song traits. Moreover, the difference in song rate could also be a consequence of neighbours distinguishing themselves by singing with different song types (that differ in duration), as individual male great tits can sing up to approximately seven different song types (Lambrechts & Dhondt, 1986). However, great tit males in close proximity actually tend to be more similar in their total song repertoire (McGregor & Krebs, 1982) and songs added to the song repertoire of individuals were found to mostly resemble, not differ from, those of newly arrived neighbours (McGregor & Krebs, 1989).

Here we show that song rate varies in relation to specific social relationships between territory owners, i.e. whether or not territory owners share a territory boundary, but next to active social processes our findings could also result from a more passive process. Song traits often link to individual quality, like age or condition (Cuthill & Macdonald, 1990; Poesel et al., 2006), hence neighbour-related differences in song might also be the result of territorial birds preferring to settle next to individuals that differ from themselves to increase contrast and recognisability (Gil & Gahr, 2002; Amy et al., 2010), to reduce (mate) competition (Qvarnström & Forsgren, 1998) or to form complementary alliances (Goodwin & Podos, 2014). We did not find neighbours to be more dissimilar in personality (exploration behaviour) than non-neighbours and there was no linear correlation between song rate and body condition, or any difference in song rate between young and older males. Although differences in personality, age and body condition could not explain the difference we found in the song rate between neighbours compared to non-neighbours, we cannot rule out the possibility that other (unmeasured) traits related to individual quality, like dominance rank (Otter et al., 1997), might explain the neighbour dissimilarity in song rate.

However, even when song traits are found to be honest indicators of individual quality, it does not rule out that there is still flexibility for birds to also moderately adjust these song traits in response to social stimuli. For example, in black-capped chickadees, both song rate and start of dawn song were found to be

honest indicators relating to dominance rank (Otter et al., 1997), yet territorial black-capped chickadees were still able to moderately advance their start time when provoked by a simulated intruder (Foote et al., 2011). Both the active and passive scenarios discussed above imply a significant role for neighbours in signalling, and an experiment in which the song rate of neighbours is manipulated could give valuable insight in the actual underlying mechanism.

What could drive similarity in start time of dawn song between males breeding close together?

In contrast to our findings on song rate, our results suggest that start time of dawn song is less influenced by specific social relations (sharing a boundary or not), and more by proximity. However, birds breeding close together were also recorded at more similar temperatures, which could have partly driven the spatial pattern found. In addition to sunrise (Catchpole & Slater, 2008), start time often associates with variable environmental factors like lunar phase (York et al., 2014), night temperature (Godfrey & Bryant, 2000) and in some cases also noise levels (Arroyo-Solís et al., 2013). This might also explain why the strength of the correlations with space, though significant, were rather small (Mantel R between -0.1 and 0.07). Yet, seasonal environmental factors are unlikely to explain the specific spatial pattern in this study, as within-day variation in start time of dawn song was not larger than among-day variation and there was no linear relation between start time similarity and temperature or moonlight similarity.

Alternatively, the spatial pattern we found could be caused by a social contagion effect, the spread of behaviour patterns in a group through imitation and conformity, or facilitation effect (Clayton, 1978; Videan et al., 2005). The start of dawn song by one bird could trigger nearby conspecifics to also start singing, while conspecifics further apart from this starting bird will respond to other, relatively closer, starting birds, creating a specific non-linear wave-like pattern of similarity and dissimilarity between close and more distant birds. This is in line with a previous study on nightingales (*Luscinia megarhynchos*) showing indeed that song originating from outside the territory triggers strong singing responses (Sprau et al., 2010) and that less degraded (near) song leads to stronger responses than more degraded (distant) song (Naguib & Wiley, 2001). Also a similar pattern was found in territorial chaffinches (*Fringilla coelebs*), in which the degree of similarity in singing activity decreased with distance in space (Halupka, 2014). Additionally, neighbouring black-capped chickadees slightly, but significantly, advanced the start of their dawn chorus after on the same morning a simulated unknown intruder started singing firstly from within a neighbour's territory (Foote et al., 2011).

Apart from social contagion, similarity in habitat structure and food availability (Kacelnik & Krebs, 1983; Smith et al., 2013) for birds breeding closer together could also explain the spatial effect on start time in great tits; for example if breeding near forest edges allows more natural and artificial light to penetrate during dawn (Kempenaers et al., 2010) and if spatial variation in local food availability during dawn affects the optimal time to start foraging (Kacelnik & Krebs, 1983). The combination of both habitat structure and social contagion to be driving the spatial pattern is also a possibility. The repeatability of start time implies that the same individuals generally start singing first, creating a relatively consistent order of starting males as was found in black-capped chickadees (Foote et al., 2011), which could consistently set off the rest of the neighbourhood.

Yet, as with the dissimilarity in song rate between neighbouring birds, similarity in start of dawn song could potentially be caused by non-random territory settlement when certain song traits relate to certain other individual characteristics (Cuthill & Macdonald, 1990) (Poesel et al., 2006). Although we did not find a correlation between start time and body condition, we did find indications that younger males start their dawn song earlier than older males, which could explain the spatial pattern if males of similar age settle closer together. However it is more likely that first year males settle in between older (resident) males, because site fidelity combined with age- and site-dependent dominance will presumably favour males from the previous year to win local disputes against other males in the process of establishing a breeding territory (Krebs, 1982), creating a mixed spatial pattern of old and young birds.

Future population-level studies of territorial bird song analysing the start time of dawn song on similar days are necessary to support the social contagion hypothesis for territorial great tits. Additionally, future studies on bird song traits incorporating both habitat and social structure (Boncoraglio & Saino, 2007) would be insightful to disentangle both of these possible selection pressures.

Concluding remarks

Our findings shed a new light on our understanding of communication networks, as they suggest that neighbours, and not solely spatial proximity, have a significant role in the use of signal space in territorial birdsong. Individual song is often part of a larger communication network and future studies on birdsong should thus take this social and spatial structure into account.

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7

Dawn song predicts behaviour during territory conflicts in personality-typed great tits

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Abstract

Territorial animals settle territory disputes and discourage conspecific intrusion via close-range confrontations as well as non-confrontational long-range signalling. Since individuals often differ consistently in general aggression and risk taking, the relative use of either close- or long-range territorial defence behaviour is likely to vary with the behavioural strategy of the territory owner. Here we quantified the relationship between dawn song, a well-studied long-range signal, and responses to a close-range confrontation as well as how individuals in a territorial population vary in this relationship. For this we recorded dawn song and experimentally simulated territory intrusions via playbacks in wild personality-typed male great tits, *Parus major*. We show that males that sang at a higher rate at dawn also showed stronger vocal responses towards a simulated intruder, but spent less time in proximity to the intruder. Moreover, males with a higher exploration score, an established proxy for personality traits, showed the strongest vocal and spatial responses during the confrontation, yet exploration behaviour did not predict the dawn song rate. These findings highlight the importance of both confrontational and non-confrontational territorial behaviours as well as personality for the social and territorial dynamics of animal populations.

Introduction

Individuals within a population often directly or indirectly affect each other's behaviour. In many group-living species (Couzin & Krause, 2003) and also in territorial species (Stamps, 1988) attraction to certain sites on a large spatial scale is positively influenced by the presence of conspecifics. Conversely, on a smaller spatial scale the presence of conspecifics will regularly repel territorial individuals from certain locations (Stamps & Krishnan, 2001). Territorial behaviour, including both close-range confrontations (Stamps & Krishnan, 1997) and long-range signalling (McGregor, 2005), often deters conspecifics from coming too close. These defence behaviours are crucial for territorial animal societies as they modulate the social dynamics and territory stability of a population (Beletsky, 1992; Bee & Gerhardt, 2002; Briefer et al., 2008). However, since close- and long-range territorial defence behaviours are often studied in isolation, it is still not evident whether and how they are related.

Territorial long-range signals keep rivals at a distance and hence can prevent close-range confrontations (Krebs, 1977; Bee & Gerhardt, 2002; Burmeister et al., 2002), inherently linking signalling and confrontational behaviour together. For example, in Pacific tree frogs (*Hyla regilla*) calling led to wider spacing of individuals than expected from random spatial settlement (Whitney & Krebs, 1975). Additionally, familiarity with the specific long-range signal characteristics of neighbours has been shown to reduce excessive aggression among territorial neighbours, in systems ranging from fish (Myrberg jr & Riggio, 1985) to frogs (Bee & Gerhardt, 2002) to birds (Falls & d'Agincourt, 1981; Briefer et al., 2008; Akçay et al., 2009). This link between long-range signalling and potentially aggressive close-range interactions becomes especially relevant when individuals within a territorial population vary in their propensity to avoid close-range confrontations with rivals, as such variation could also be reflected in individual differences in signalling behaviour. Indeed, the overall likelihood of territorial males being in proximity to a male conspecific differed between individual male great tits, *Parus major*, with respect to their personality (**Chapter 3**). Moreover, the intensity of response to a close-range confrontation with a simulated territorial intruder was similarly found to vary among territorial male great tits in relation to personality traits (Amy et al., 2010; Jacobs et al., 2014). Since male territorial songbirds connect by spatial proximity as well as by their song, a long-range signal (Peake et al., 2002; Amrhein et al., 2004a; **Chapter 3, 5, 6 & 8**), they are ideal models for studying how these close- and long-range territorial behaviours are related.

An important long-range signal of many territorial songbird species is the dawn song (Staicer et al., 1996; McGregor, 2005), a peak in singing activity just before sunrise. A predominant function of dawn song in territorial songbirds is territory advertisement to keep rivals at a distance (Kacelnik & Krebs, 1983; Kunc et al., 2005). Consequently, if individual songbirds vary in their motivation to avoid territorial confrontations, this should be reflected in their dawn singing behaviour. Certain dawn song traits have indeed been shown to predict various close-range behaviours, such as nest defence in willow tits (*Parus montanus*) (Welling et al., 1997) and territory defence in blue tits (*Cyanistes caeruleus*) (Poesel et al., 2004). Moreover, differences in dawn song rate in territorial great tits vary between males depending on whether or not they share a territory boundary (**Chapter 6**).

Although both personality (Amy et al., 2010) and long-range song (Poesel et al., 2004) can predict short-range territorial behaviour, it remains unclear whether personality could drive both short- and long-range territorial behaviour. This relationship is important because if personality drives territorial behaviour at both scales, eavesdroppers would be able to predict the personality of a potential competitor and hence their likely response to future close-range confrontations. Evidence that this might be the case comes from the finding that great tit neighbours that eavesdrop on a simulated territory intrusion adjust their spatial behaviour in relation to the personality of their intruded-upon neighbour (Amy et al., 2010). Consistency in behaviour mediated by personality differences makes individuals predictable and thus allows greater social responsiveness by conspecifics (Wolf et al., 2011).

Here, we examined how dawn singing, a long-range signal, is related to close-range territorial behaviour in a wild population of territorial great tits by simulating intrusions of rival males through song playbacks. The individuals in this population were also tested for exploration behaviour, an established proxy for personality traits in this species. We predicted that slower explorers would show weaker close-range territory defence behaviour, because slower explorers tend to take less risk and are generally less aggressive than fast explorers (van Oers & Naguib, 2013). This is also in line with the findings of Amy et al. (2010) in the same population. Moreover, we expected birds that showed a weak confrontational response to the playback (slower birds) to sing more actively during dawn to discourage possible intruders (Kacelnik & Krebs, 1983) and thus decrease the chance of risky confrontations. This prediction does differ somewhat from the outcome of an earlier aviary study in which faster explorers actually sang more (Naguib et al.

2010), but since the breeding pairs were housed in separate aviaries they did so while there was no risk of confrontations present. Additionally, we hypothesized that neighbourhoods (males living in close proximity to the playback subject) would react vocally more strongly to simulated intrusions in territories of those subject males that were generally more active singers at dawn, as we expected those males to increase singing activity the most and so trigger eavesdropping neighbours to also vocally defend their territories more intensely.

Methods

Study population

The study was conducted on our long-term nest box population of great tits at Westerheide near Arnhem (52°01'00.0"N 5°50'20.0"E), The Netherlands. Throughout the year, birds caught for the first time (outside the breeding season) are tested for exploration behaviour using a standard validated protocol. Birds are caught either from their nest box during a roost check at night or via mist netting during the day. After catching, the birds are immediately transported to the bird-housing facilities at the Netherlands Institute of Ecology (NIOO-KNAW) where they are weighed and subsequently individually housed in cages (0.9 x 0.4 m and 0.5 m high). The following morning, exploration behaviour is measured using the novel environment test following the procedure described in (Dingemanse et al., 2002). Birds are individually tested in a room (4.0 × 2.4 m and 2.3 m high) with five artificial trees. After birds enter the experimental room by themselves, we record the total number of flights (movements between trees) and hops (movements within trees) within the first 2 min. These are subsequently used to calculate an overall exploration score ranging from low (slow explorers) to high (fast explorers) (Dingemanse et al., 2002), which is known to be repeatable and to correlate positively with aggression, boldness, risk taking and approach of conspecifics in great tits (Groothuis & Carere, 2005).

General set-up

To simulate territory intrusions we conducted playback experiments in the mornings between 0800 and 1115 hours at the nest boxes of 37 male great tits in the Westerheide study site. Playbacks, broadcasting songs of an unfamiliar male great tit, were performed from 26 March until 7 April 2012, and were conducted near nest boxes with nest-building activity but without eggs (with the exception of one bird). On a given day, playbacks were conducted several territories apart following a procedure similar to (Amy et al., 2010). To quantify the relation between dawn song and responses to territory intrusion, song recordings were

made during dawn on the day before the playback experiment using automatic song recorders. Additionally, song recordings were made during dawn after the intrusion to assess repeatability of the dawn singing behaviour. Neighbourhood dawn singing activity was scored daily throughout the breeding season (see below).

Dawn song recording

We recorded dawn song using time-programmable song recorders (Wildlife Acoustics Inc., Maynard, MA, U.S.A.; SM2 song meter and TASCAM DR-08) placed above nest boxes with nest-building activity. We were able to collect good-quality dawn song recordings for 23 playback subject males the dawn before the playback (for 22 playback subjects the dawn song was successfully recorded on both the dawn before and after the playback). Recordings were then analysed with Avisoft SASlab Pro (R. Specht, Berlin, Germany). As a standardized measure, the first 5 min from the time the bird started singing before sunrise was quantified. We measured song rate in number of songs ('strophes') per second and start time of dawn song in minutes before sunrise. Repeatability of start time of dawn song and song rate was tested, in accordance with (Lessells & Boag, 1987), using song recordings of two consecutive mornings for 27 male great tits (22 playback subjects and five additional males). Both the start time of dawn song ($r = 0.53$, $SE = 0.14$, $F_{26,27} = 3.30$, $P = 0.001$) and the song rate ($r = 0.49$, $SE = 0.15$, $F_{26,27} = 2.93$, $P = 0.004$) were highly and significantly repeatable. This repeatability of dawn song traits before egg laying is in accordance with the previously revealed repeatability of the same traits later in the season, during the egg-laying phase (**Chapter 6**).

Simulated territory intrusions

To attract attention, we used a 1 min lure with common goldcrest (*Regulus regulus*) and long-tailed tit (*Aegithalos caudatus*) calls, followed by a pause of 20 s, then 2 min of great tit song comprising a systematic repetition of one song type of a great tit recorded at least 2 years before. It is very unlikely that the subjects were familiar with the playback song given the short average life expectancy of wild great tits (1–2 years). To prevent pseudo-replication, each subject received a song recording from a different male (with two exceptions). The playback files were constructed using Adobe Audition by repeating one song of a unique bird with intervals of 4 s to 2 min and normalizing the peak amplitude to the same level for all songs. Songs were broadcast at 84 dB (measured at a neutral site at 1 m from the loudspeaker with a Voltcraft Digital sound-level meter 322) using a Yamaha NX-U10 loudspeaker (frequency range 90 Hz – 20 kHz), connected with a 25 m cable to a media player (Archos 405, 30 GB). Subject responses were recorded using two Sennheiser

ME66/K6 microphones (frequency range 40 Hz – 20 kHz \pm 2.5 dB) and a Marantz PMD660 solid-state recorder (sampling frequency 44.1 kHz, frequency range 16 kHz \pm 0.5dB) until 2 min after the playback had ended. From these recordings and from simultaneously recorded spoken notes we quantified two spatial response measures, (1) latency to approach within 5 m (s) and (2) total time spent within 5 m of the loudspeaker (s), as well as four vocal response measures: (3) number of song overlaps, (4) total song rate (songs/s during total observation time), (5) song rate during the actual playback (song rate (songs/s)) and (6) total singing duration (s). All playback experiments were performed by the same observer (M.H.) and all subjects received only one playback.

An additional seven playback experiments were performed, but were left out of the analysis because of technical problems during the execution of the experiment ($N = 3$) or a total absence of noticeable vocal and spatial presence ($N = 4$). By excluding birds that did not show any visual and vocal presence during the experiment we minimized the possibility of wrongfully assigning a weak playback response to individuals that were out of hearing range at the time of the experiment. For 16 of the playback subjects the exploration score was available, but unknown to the observer at the time of the playback.

Neighbourhood singing activity

We conducted observation rounds of 30 min, in which we assessed singing activity in the whole study site, following methods given in (Amrhein et al., 2008). These rounds were made every morning during the 2-week playback period. They began half an hour before sunrise and consisted of two fixed routes, which were alternated each day. The observer cycled along the fixed route (alternating start and end points within routes) and recorded on a map where a great tit was singing. For each playback subject the proportion of total singing points within 100 m of the playback location for the morning after the playback and during a control round were calculated, to determine overall neighbourhood singing activity. A control round was conducted within 4 to 2 days before the playback ($N = 6$) or 2 days after the playback ($N = 5$), restricted to days when no other playback trials were performed nearby. Owing to the alternating routes in combination with the fact that playback locations on the same morning were as far apart as possible, we could only calculate a neighbourhood singing activity measure for 11 (of 23) subjects.

Subject identification

During the breeding season we checked nest boxes twice a week using a standardized procedure (van Oers et al., 2008). Playback subjects were identified by catching them inside their nest box using spring traps on approximately the 7th day after chicks hatched. Playback subjects that we trapped during chick feeding, to establish their identity, were not inside their nest box for longer than 15 min. We fitted un-ringed individuals with uniquely numbered aluminium leg rings and measured body mass and tarsus length within approximately 5 min.

Statistical analysis

Latency to approach within 5 m was set to 320 s for the five birds that did not approach within 5 m of the loudspeaker, but that did respond vocally. We conducted a principal component analysis on the six behavioural measures comprising the total response of the subject during the playback experiment. Two principal components (Varimax rotation with Kaiser normalization; rotation converged in three iterations; Appendix Table A1) had an eigenvalue larger than 1. Since our main focus in this study was on the close-range territory defence behaviour we also independently analysed the two spatial response behaviours: (1) latency to approach within 5 m (s) and (2) total time spent within 5 m of the loudspeaker (s).

Since availability of exploration scores significantly reduced our sample size, correlations were tested first excluding exploration behaviour as a variable ($N = 23$ for playback response and dawn song) and subsequently including it ($N = 16$ for playback response and exploration; $N = 17$ for dawn song and exploration). Because a complete data set (good-quality dawn song recording, playback response and exploration score) was not available for several subjects, sample size differs between models ($N = 14$ for all variables combined). Age of the subject (as a categorical factor: Independent samples t test of second calendar year or older: $1.6 > \text{all } t > -0.6$, all $P > 0.1$) and number of days to the first egg of the mate (as a continuous variable: Spearman correlation: $0.3 > \text{all } r > -0.2$, all $P > 0.3$) did not significantly influence the dawn song measures or either of the main response components during the playback experiment, and were a priori left out of the model. Additionally, minor variations in number of stimulus songs ($\text{Mean} = 18.5$, $SD = 2.1$, $N = 37$; $0.06 > \text{all } r \text{ or } r_s > -0.25$, all $P > 0.1$) and total playback duration (mean = 319.35 s, $SD = 4.86$ s, $N = 37$; $0.35 > \text{all } r \text{ or } r_s > -0.35$, all $P > 0.05$) did not significantly bias the two main response components or the six response measures individually.

Whether territory intrusions resulted in persisting effects on the use of dawn song was tested with two paired-samples t tests comparing start time of

dawn song and song rate during dawn before and after the playback ($N = 22$). To examine whether typical dawn singing behaviour (before playback) was associated with neighbourhood vocal response, we quantified the change in relative neighbourhood singing activity by calculating the proportion of neighbours singing during the control round and subtracting the proportion of neighbours singing the morning after the playback. Calculated proportions for neighbourhood singing activity were arcsine transformed and zero values were replaced by $(1/4N)$ (Zar, 1999). General linear models were used to analyse whether or not dawn signalling traits from the dawn before the playback (still undisturbed by potential playback effects) predicted longer term changes in (relative) neighbourhood singing activity after a simulated territorial confrontation for 11 males. Possible effects on relative neighbourhood singing activity caused by the territorial defence behaviour of the territory owner were tested in a separate linear model to prevent collinearity problems with the dawn song traits.

A backward model selection procedure was conducted for all models, removing the least significant terms, until only factors with $P < 0.1$ remained (final model). Residuals of the models were normally distributed according to the Kolgomorov–Smirnov test. All statistical analyses were performed using IBM SPSS Statistics 21 (SPSS, Inc., Chicago, IL, U.S.A., www.spss.com).

Ethical Note

Birds caught for personality testing were transported from the field to the research institute by car within 1.5 h after the first bird was caught (the car ride lasted a maximum of 20 min). During transport we housed the birds individually in a compartment (10 x 10 x 10 cm) of a standard darkened wooden carrier. We measured tarsus and weight (handling time of 5 min) before individuals were housed in the individual cages. The birds were provided with water and food *ad libitum*, including sunflower seeds, mealworms, fat balls and apple. We conducted the novel environment test between 0800 and 1200 hours. The indoor cages connected to the room via a 20–20 cm sliding door so a bird could enter the experimental room without being handled, following darkening of the individual cage. The test was terminated after at most 10 min, after which the experimental room was darkened and we caught the bird by hand to release it back to its cage within 1 min. Individuals do not usually lose weight outside their natural range (Dingemanse et al., 2002) during this routine procedure. Within 24 h we released birds back in the field close to their nest box. This procedure was approved by the Institutional Animal Care and Use Committee: the Koninklijke Nederlandse

Akademie van Wetenschappen—Dier Experimenten Commissie (KNAW-DEC license NIOO 10.05 to M.N. and K.V.O.).

Results

Response to a territory intrusion

The main vocal component (PC1: higher value reflects stronger response) including all four song measures explained 52% of the total variation. The main spatial component (PC2: lower value reflects stronger response) including the two approach measures explained 29% of the total variation (Supplementary Table S1).

The total approach response (PC2) was negatively predicted by the dawn song rate (Pearson correlation: $r = 0.50$, $N = 23$, $P = 0.015$; Figure 1a), with birds singing at the highest dawn song rate showing the weakest approach response. Considering the spatial territorial response behaviours separately, males with a higher dawn song rate spent significantly less time within 5 m of the simulated intruder (Pearson correlation: $r = -0.55$, $N = 23$, $P = 0.007$), but there was no correlation with the latency to approach (Spearman correlation; $r_s = 0.28$, $N = 23$, $P = 0.20$).

When exploration behaviour was included in the model ($N = 14$), it significantly positively predicted the total spatial response, with faster explorers showing a stronger response (Table 1, Figure 1b). When we again considered the spatial territorial response behaviours separately, fast explorers spent more time within 5 m of the loudspeaker, but there was no effect on latency to approach (Table 1). When we included exploration behaviour there was no longer a significant effect of the dawn song rate on the total spatial response (PC2) but the significant correlation between the time spent within 5 m and the dawn song rate of a territory owner remained (Table 1).

The total vocal response (PC1) during the simulated intrusion was predicted by both the dawn song rate and the exploration behaviour of territory owners, with strong vocal responders also having the highest dawn song rate (Pearson correlation: $r = 0.51$, $N = 23$, $P = 0.014$) and fastest exploration score (Table 1). Start time of dawn song was not predictive for any of the close-range territorial defence behaviours, both when excluding (all $P > 0.1$) and when including exploration behaviour (Table 1).

Dawn singing behaviour

The start time of dawn song (paired-samples T Test: $t_{(20)} = -0.22$, $N = 22$, $P = 0.83$) and the song rate ($t_{(20)} = -1.50$, $N = 22$, $P = 0.15$) did not differ significantly between the dawn following a playback and the dawn before the playback.

The exploration behaviour of the territory owner did not correlate with the start time of dawn song (Pearson correlation: $r = -0.17$, $N = 17$, $P = 0.53$) or dawn song rate ($r = -0.21$, $N = 17$, $P = 0.41$; Figure 1c) on the day before the playback or with the change in start time of dawn song ($r_{10} = 0.26$, $P = 0.39$) and song rate ($r_{10} = -0.12$, $P = 0.71$) the morning after a playback.

Table 1. GLM results for subject playback response components and two spatial playback response behaviours individually as a function of dawn song traits and exploration score

Response variable	Independent	Test statistic	<i>P</i>	<i>N</i>
PC2 (spatial response)	Dawn song rate	$F_{1,10} = 1.36$	0.27	14
	Dawn song start time	$F_{1,11} = 3.07$	0.11	14
	Exploration	$F_{1,14} = 6.53$	0.02	16
Total time spent within 5 m (s)	Dawn song rate	$F_{1,11} = 5.55$	0.04	14
	Dawn song start time	$F_{1,10} = 0.56$	0.47	14
	Exploration	$F_{1,11} = 5.04$	0.046	14
Latency to approach within 5 m (s)	Dawn song rate	$F_{1,10} = 0.50$	0.50	14
	Dawn song start time	$F_{1,10} = 1.65$	0.23	14
	Exploration	$F_{1,10} = 1.49$	0.25	14
PC1 (vocal response)	Dawn song rate	$F_{1,11} = 19.39$	0.001	14
	Dawn song start time	$F_{1,10} = 2.02$	0.19	14
	Exploration	$F_{1,11} = 10.25$	0.008	14

Significant values are in bold.

Neighbourhood response

The relative neighbourhood singing activity the morning after the playback significantly decreased when the territory owner's undisturbed dawn song rate (the morning before the playback) was higher (general linear model: $F_{1,9} = 5.22$, $P = 0.048$) but was unrelated to the start time of dawn song ($F_{1,8} = 2.48$, $P = 0.15$). This negative correlation between dawn song rate and change in relative neighbourhood singing activity was unlikely to have been mediated by the playback response of the territory owner as the total vocal response (PC1) and total spatial response (PC2) during the territorial confrontation were both unrelated to the change in relative neighbourhood singing activity (PC1: $F_{1,14} = 0.004$, $P = 0.95$; PC2: $F_{1,14} = 0.62$, $P = 0.45$). Finally, relative neighbourhood singing activity was unrelated to the exploration behaviour of the eight males for whom we had data on both measures (Pearson correlation: $r_8 = 0.53$, $P = 0.18$).

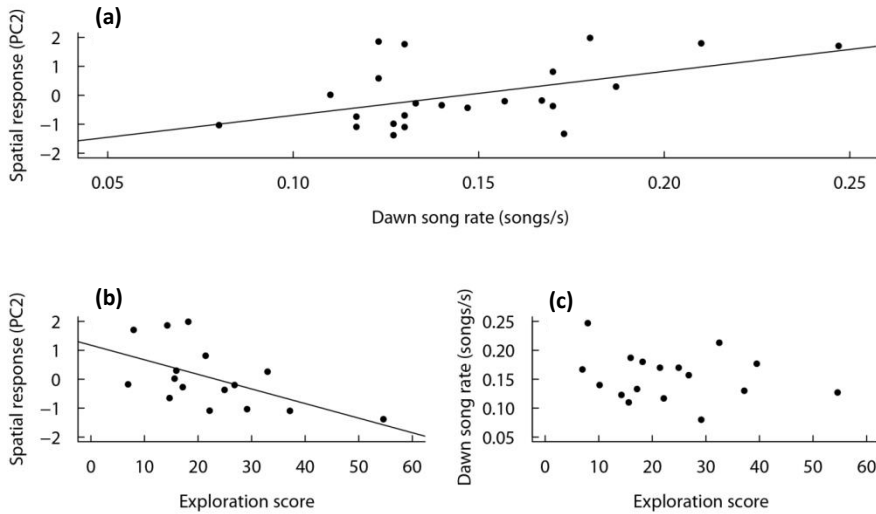


Figure 1. Relationships between dawn song rate, spatial response to the playback and exploration score. Spatial response (lower value reflects stronger response) as a function of (a) dawn song rate and (b) exploration score. (c) Dawn song rate as a function of exploration score. Lines represent significant correlations.

Discussion

Here we have shown that (1) a long-range territorial signal correlates negatively with the close-range territorial defence to a simulated intruder, (2) exploration behaviour can predict the strength of both the close-range vocal and spatial response to an intruder, but not the typical long-range signalling behaviour and (3) a long-range territorial signal can predict the vocal neighbourhood response after a territory intrusion.

The role of personality in territory defence behaviour

Dawn song, a long-range signal, predicted the spatial response during a simulated intrusion, a behaviour that is likely to be risky in a territorial population. Indeed, our finding that faster explorers, which are generally seen as being more risk taking, spent significantly more time in proximity to a simulated intruder, confirms similar findings by Amy et al. (2010) in this study population. One likely explanation is that individuals that take lower risks in spatial confrontations signal more actively in a non-confrontational setting, to keep rivals at a distance. Long-range vocalizations as stay-away signals are widespread among many diverse territorial

species, such as Lusitanian toadfish (*Halobatrachus didactylus*), Pacific tree frogs (Whitney & Krebs, 1975), mantled howling monkeys (*Alouatta palliata palliata*) (Whitehead, 1987) and great tits (Krebs, 1977). Furthermore, a radio tracking study on nightingales (*Luscinia megarhynchos*), showed that males with higher daily song output received fewer intrusions by neighbouring males (Naguib et al., 2001; Naguib et al., 2011b).

Exploration behaviour has been linked to several forms of risk taking in the field, such as nest defence in great tits (Hollander et al., 2008), song post height in male collared flycatchers (*Ficedula albicollis*) (Garamszegi et al., 2008) and aggressive territory defence by helpers in a cooperative breeding cichlid (*Neolamprologus pulcher*) (Bergmüller & Taborsky, 2007). However, exploration behaviour as a correlate of risk-taking behaviour can only partially support our findings. While we found a positive correlation between exploration behaviour and the time spent close to the simulated intruder, we did not find a relationship between exploration behaviour and the dawn singing behaviour. Although we initially predicted slow birds to be more active singers at dawn, in a study concerning singing activity in captive great tits fast-exploring birds sang more (Naguib et al., 2010). However, the captive fast birds in this study sang more throughout the whole day, not just dawn, which could also be a reflection of fast birds generally being more active in captivity (Carere et al., 2001). Comparison between behaviour indoors and territorial behaviour in the wild remains challenging, since the constraints on the time and energy budgets are very different. In the wild, birds have a much larger area to defend and have to spend significantly more time and energy on finding food while avoiding predation, resulting in a very different trade-off in time and energy expenditure. Studies including additional personality traits that relate more directly to the tendency to avoid or seek out confrontations, such as boldness, aggression or risk taking, might give more insight into whether long-range signalling, such as dawn singing behaviour, and proximity during confrontational territory conflicts are correlated via variation in risk-prone personalities.

In contrast to this study and the study of Amy et al. (2010), a recent study by Jacobs et al. (2014) revealed that slower explorers show a stronger spatial response to a simulated intruder. This confirms previous studies that showed that behavioural variation between personality types is often context or population dependent (Dingemanse & de Goede, 2004; van Oers et al., 2005). This study and the study by Amy et al. (2010) were conducted before egg laying, while Jacobs et al.'s (2014) study also included the egg-laying phase. The female is fertile during the egg-laying

phase and as a consequence males are highly engaged in mate guarding and thus have to make different trade-offs when responding to territorial intrusions. Moreover, the frequency distribution of exploration scores in Jacobs et al.'s (2014) great tit population included many slow explorers, while the distribution in our study was more uniform. Differences in the frequency distribution of personality types in a population can be the result of natural selection since fitness consequences of certain personality types are known to vary in contrasting ways possible due to specific environmental conditions (Dingemanse et al., 2004). When there are many slow explorers in a population, slow individuals might benefit from taking more social risks as the chance of encountering fast explorers (which are generally more aggressive, (Groothuis & Carere, 2005)) is lower. In contrast, fast explorers, living in a population with many slow explorers, might have experienced that a less spatial and a more vocal defence (song type switching) is enough to settle disputes. Indeed, exploration behaviour itself has been shown to be adaptively plastic when environmental circumstances are changed (Nicolaus et al., 2012). Jacobs et al. (2014) raised the variation in the signal strength of the playbacks as a potential explanation for varying results between playback subjects. This could indeed explain some of the differences between the three playback studies. For example, the study by Amy et al. (2010) consisted of a 1 min playback of non-interactive songs, followed by a 2 min interactive playback from another loudspeaker, while we conducted a non-interactive playback for 2 min and the Jacobs et al. (2014) study broadcast non-interactive songs for only two times 45 s. Thus variation in perceived intruder determinedness (signal strength) could be influencing the defence strategy of the playback subjects, with fast explorers responding more aggressively to a more persistent intruder in Amy et al.'s (2010) study and slow explorers taking more risks with a less persistent intruder in Jacobs et al.'s (2014) study. Certainly, great tits are known to decrease the intensity of their response when an intruder is likely to be perceived as less threatening (Peake et al., 2001).

The predictive value of a long-range signal

Our findings that dawn song predicted behaviour during a territory intrusion underlines the signal value of dawn song, as it is not only consistent (**Chapter 6**) but also predictive for other contexts. As we anticipated, more active long-range signallers (i.e. males with a higher dawn song rate) showed a weaker spatial response, but a stronger vocal response during the intrusion. The finding that dawn song behaviour can predict behaviour in other contexts conforms to studies of long-range song in other bird species (Welling et al., 1997; Naguib et al., 2001;

Poesel et al., 2004). Also, territorial song in general can provide important predictive information. Male song sparrows, *Melospiza melodia*, responded adaptively by varying their response to the playback of songs of conspecific males that were known to differ in their aggressiveness (Hyman & Hughes, 2006). Moreover, long-range territorial signalling behaviour can predict fitness in both territorial bats (Behr et al., 2006) and birds (Hasselquist et al., 1996; Mennill et al., 2002). Predictive information transfer of individual quality and motivation via long-range territorial signalling therefore seems to be a common phenomenon in territorial systems.

In contrast to song rate, the start time of dawn song, although repeatable, did not predict behavioural responses to intrusions. Several studies have shown that the start time of dawn song correlates with components of individual quality, for instance earlier-singing kingbirds (*Tyrannus tyrannus*) and blue tits experience certain fitness benefits (Poesel et al., 2006; Murphy et al., 2008). Nevertheless, individual variation in the start time of dawn song might be restricted by the environment as start time is also known to be driven by environmental factors such as lunar phase (York et al., 2014), artificial light (Kempnaers et al., 2010), night temperature (Godfrey & Bryant, 2000) or noise levels (Gil et al., 2015). Moreover, start time might be affected by the social environment through social facilitation (Clayton, 1978), as it was recently shown that similarity in start time was higher for males breeding in closer proximity (**Chapter 6**).

In several different fish and bird species conspecifics are known to react to information indicating relative quality and motivation of signallers gathered from eavesdropping (Naguib & Todt, 1997; Oliveira et al., 1998; Peake et al., 2001; Mennill et al., 2002; Fitzsimmons et al., 2008a; Webster & Laland, 2013). Next to signal interactions, long-term changes in individual signalling behaviour could signal relevant information. Similar playback studies on winter wrens (*Troglodytes troglodytes*) (Amrhein & Erne, 2006) and black-capped chickadees (*Poecile atricapillus*) (Foote et al., 2011) found long-term changes in dawn song traits of the territory owner after a simulated intrusion. These changes can be detected by all conspecifics in hearing range and could change their behaviour. However, we did not find evidence for such a significant long-term change in the subject's song traits after the intrusion in this study. Possibly the changes in signalling behaviour are only short term in great tits or the long-term changes in signalling are too subtle to detect as there are many different individual and environmental factors influencing dawn song behaviour (Foote et al., 2011).

Surprisingly, relative neighbourhood singing activity was lower the morning after the simulated territory intrusion for subjects with a higher dawn song rate. We expected neighbours of birds with a higher dawn song rate to respond by increasing rather than decreasing their singing activity, especially since these birds also showed a stronger vocal response during the intrusion. Possibly birds with a lower song rate, which also showed a stronger spatial response during the intrusion, became more spatially active by defending their territory boundaries and so arousing their neighbours to vocally defend their own territories more intensively during the next dawn chorus. Replicate studies tracking playback subjects and their neighbours following a territory intrusion would be necessary to confirm this hypothesis. Insights into long-range territorial signalling behaviour, including consistent signal characteristics, signal interactions and changes in signalling behaviour, are very relevant in social behaviour studies as long-range signals reveal information about the signaller not only to the intended receiver but also to all other conspecifics in range (McGregor, 2005). Territorial long-range signals thus have the potential to affect the social dynamics of many individuals within a population simultaneously.

Conclusion

Our findings provide new insights into the links between individual long-range and close-range territorial behaviour. We have shown that individuals varying in personality use a different mix of negatively correlated territorial behaviours. Therefore studies examining the social dynamics of territorial populations, for example social network studies, would benefit from integrating both close- and long-range social connectivity. The rapid technological developments in recording specific signal interactions in small animals (Anisimov et al., 2014) will make this goal more achievable in the near future.

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Supplement to Chapter 7

Table S1

Table S1. Rotated component matrix with component loadings of the measured playback response behaviours

Playback response measures	Component	
	1	2
Latency to approach within 5 m (s)	0.06	0.92
Total time within 5 m of speaker (s)	-0.04	-0.93
Number of overlaps	0.84	0.23
Total song rate (songs/s)	0.92	0.00
Song rate during playback (songs/s)	0.90	0.06
Total singing duration (s)	0.87	-0.04

The Kaiser–Meyer–Olkin measure of sampling adequacy = 0.68 and Bartlett’s test of sphericity were significant ($P < 0.001$). The highest component loading for each playback response behaviour is in bold.



8

Neighbourhood watch: The spatial response of the neighbourhood following territory intrusions in a wild songbird

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To be submitted

Abstract

Social connectivity plays a key role in animal populations, influencing processes such as resource acquisition, sexual selection and competition. In many species, individuals are socially connected to each other both within proximity networks and communication networks. Yet, how these social networks interact remains poorly understood. For example, certain vocal characteristics in territorial songbirds are expected to attract females while repulsing males. Exploratory behaviour, an established personality trait, is known to associate with an individual's position in a proximity network, but also with the behaviour of individuals in a communication network. Here we tested whether or not the proximity network positions of territorial male great tits (*Parus major*) change in relation to their vocal response and their exploratory behaviour, following a simulated territory intrusion. Unlike previous studies we did not find a positive correlation between exploration and vocal response. However, the vocal response of the territory owner positively associated with body condition and age. Interestingly, males with a stronger vocal response attracted neighbouring females at closer distances to the intrusion site, while neighbouring males stayed further away in such cases. Yet, a stronger vocal response did not predict the number of female compared to male associates after an intrusion. Finally, there was no significant relation between the exploratory behaviour of the subject and the neighbour distance to the intrusion site, but faster males, compared to slower males, tended to associate more with females compared to males after the intrusion. These findings suggest that proximity and communication networks are linked and that perturbations to one social network type can have direct consequences for the other.

Introduction

Social connectivity is crucial for individuals in animal societies. The number and nature of social relationships can even predict the evolution of behavioural strategies (Ohtsuki et al., 2006). In many populations individuals indeed do not associate at random, resulting in social network structures, in which individuals differ in their degree of social connectivity (Croft et al., 2008). These differences in social network position can have both advantageous and disadvantageous consequences for social stability (Flack et al., 2006), sexual selection (Oh & Badyaev, 2010), social status (McDonald, 2007), access to social information (Aplin et al., 2012; Allen et al., 2013; Aplin et al., 2015a) and spread of disease (Bull et al., 2012; Weber et al., 2013).

Social connectivity can take many forms. In various species, individuals are socially connected to each other within proximity as well as communication networks (McGregor, 2005; Croft et al., 2008). In communication networks conspecifics use long-range signal interactions to connect, often over large distances. In the case of disease transmission (Bull et al., 2012; Weber et al., 2013) and social learning of foraging techniques (Allen et al., 2013; Aplin et al., 2015a; St Clair et al., 2015), proximity networks will be most relevant. Yet, in many proximity networks communication can also play a significant role (Hughes et al., 2012; Krams et al., 2012), since animals frequently use long-range signals (McGregor & Dabelsteen, 1996) to facilitate (Maynard et al., 2014), maintain (Garland et al., 2011), but also discourage (Whitney & Krebs, 1975; Naguib et al., 2001) spatial associations. Long-range communication and proximity networks are therefore often inherently linked (Waser & Wiley, 1979; Garland et al., 2011; **Chapter 6 & 7**).

A potential key player determining exactly how spatial and communication networks link together is personality. Personality differences relate to differences in proximity network positions and overall network assortment in a variety of species (Pike et al., 2008; Croft et al., 2009; Krause et al., 2010; Aplin et al., 2013; Aplin et al., 2014; **Chapter 3**). But also in communication networks, personality is likely to be an important factor (Amy et al., 2010; Matessi et al., 2010). For example in great tits (*Parus major*) personality predicts the main vocal response (Amy et al., 2010; **Chapter 7**) and the likelihood of switching songs (Jacobs et al., 2014) in response to a simulated intruder, but also explains variation in general singing activity (Naguib et al., 2010; **Chapter 5**) and use of visual signals (Carere et al., 2005).

Conspicuous long range signals can be used as source of information by a larger audience, and specifically gaining information from others' signalling interactions by conspecific "eavesdroppers" is seen as a key component of communication networks (McGregor, 2005). Eavesdropping on signals in a communication network is known to occur in many species, ranging from fish (Oliveira et al., 1998; Earley & Dugatkin, 2002) to bats (Cvikel et al., 2015a) to birds (McGregor & Dabelsteen, 1996; Mennill et al., 2002; Naguib et al., 2004). For example, eavesdropping on courtship displays led to local enhancement in male guppies (*Poecilia reticulata*) (Webster & Laland, 2013) and male great tits that overheard another male lose a territory conflict reduced their song output in response to the losing male (Peake et al., 2001). Eavesdropping can even facilitate a response to a disturbance of the whole neighbourhood (Fitzsimmons et al., 2008a).

The signal characteristics on which conspecifics eavesdrop during signalling interactions, both absolute signal traits (song rate) and signal patterns (song overlapping), have the potential to give valuable information on the signaller's quality (Otter et al., 1999; McGregor & Peake, 2000; Mennill et al., 2002), including traits such as body condition, fighting ability and age or experience (Davies & Halliday, 1978; Halperin et al., 1998; Cate et al., 2002; Gil & Gahr, 2002). Reliability of absolute signal traits can be maintained by physical production costs, while reliability of signal patterns could be maintained by social costs, such as receiver retaliation (Gil & Gahr, 2002; Anderson et al., 2012). Females in several songbird species use vocal signals to assess males, approaching or even copulating with better vocal performers (Ballentine et al., 2004; Maynard et al., 2014), but also see (Holveck & Riebel, 2010). In contrast, males can be repelled by specific vocal performances (Krebs, 1977; Nowicki et al., 1998), for example, male nightingales (*Luscinia megarhynchos*) that sang with a higher song rate received fewer territory intrusions (Naguib et al., 2001). In summary, vocal signals and signalling interactions can attract and repel females and males in a context-specific way.

Signals transmitted in communication networks thus have the potential to influence proximity networks of territorial songbirds. Still, if and how these networks interact remains unclear. To advance our understanding of these network interactions, it is necessary to experimentally manipulate a network or perform external perturbations and study the consequences for social connectivity (Flack et al., 2006; James et al., 2009; Krause et al., 2013; Firth & Sheldon, 2015).

We here study if individual social connectivity within proximity networks changes after perturbations in the communication network, using a novel automatic tracking technology. Specifically, we examined whether after a territory intrusion, the number of unique social connections of male great tits would change in relation to their vocal response and personality. We simulated territory intrusions (playback experiments) by playing the song of an unfamiliar male in the territories of male great tits. Subsequently we monitored the spatial response of the territory owner and the surrounding male and female conspecifics using the tracking system Encounternet (Mennill et al., 2012; Rutz et al., 2012; **Chapter 3**). Territorial great tits are an ideal model to study responses of both neighbouring females and males since great tits are social but not sexually monogamous songbirds. Exploration, an individual's response to a novel environment (Verbeek et al., 1994; Huang et al., 2015), is a validated personality trait in this species and predictive for behaviour in many other contexts (Groothuis & Carere, 2005; van Oers & Naguib, 2013).

We expected faster exploring males to show an overall stronger vocal response to a simulated intrusion (Amy et al., 2010; **Chapter 7**), as shown earlier in our study population. Moreover, after the intrusion we expected neighbouring females to be more attracted to stronger vocal responders and conspecific male neighbours to be more repelled, since a strong vocal response could indicate that the resident male is in a good condition (Cate et al., 2002; Gil & Gahr, 2002; Buchanan et al., 2003) and potentially aggressive (Searcy et al., 2014). But a strong vocal response could also indicate that the intruder is persistent and may pose a stronger threat to the larger neighbourhood (Naguib et al., 2004), motivating the neighbouring males to go and defend their own territory after the intrusion. Indeed in an earlier study in our population, neighbouring males tended to move a greater distance when the resident male had responded vocally stronger to a simulated intruder (Amy et al., 2010). Also, in black-capped chickadees (*Poecile atricapilla*) the neighbourhood song output was higher if a simulated intrusion of a neighbour involved more song overlapping and song matching, which are considered stronger and more aggressive signals (Fitzsimmons et al., 2008a). Following simulated territory conflicts with a high level of song overlapping, territorial males sang more actively (Fitzsimmons et al., 2008a; Amy et al., 2010) and male neighbours more closely bordering the intruded resident most strongly increased their territorial song output (Fitzsimmons et al., 2008a). Because exploration in great tits is commonly positively related to both vocal response (Amy et al., 2010; **Chapter 7**) and male-male aggression (Verbeek et al., 1996; Carere et al., 2005), we expected females to also be more attracted to faster explorers (strong vocalizers during conflicts) and

males to be more evasive of faster explorers (more aggressive). A correlation between the response of neighbouring males and the resident exploratory behaviour was revealed in an earlier study during which neighbouring great tit males reached their closest song post much later if the intruded resident was a faster explorer (Amy et al., 2010).

Methods

Study population

We conducted this experiment in a long-term study population of great tits at Westerheide near Arnhem, The Netherlands (52°01'00.0"N 5°50'20.0"E). Westerheide is a mixed pine–deciduous wood with approximately 200 nest boxes distributed within a 1000 m x 1200 m area (see Dingemanse et al. (2002) for details). Using a routine procedure, newly caught birds are tested for exploratory behaviour using a novel environment test (Verbeek et al., 1994; Dingemanse et al., 2002; Groothuis & Carere, 2005), an established operational measure for personality traits (Réale et al., 2007).

Exploratory behaviour

We caught the birds via mist netting during the day or took them from nest boxes during a roost check in the evening. The birds were subsequently transported to the facilities at the Netherlands Institute of Ecology (NIOO-KNAW), where we weighed them and individually housed them in cages (0.9 x 0.4 m and 0.5 m high). The following morning, we tested for exploration using a novel environment test following the procedure described in (Dingemanse et al., 2002). We tested the birds individually in a room (4.0 x 2.4 m and 2.3 m high) with five artificial trees. After birds enter the novel environment room by themselves, the total number of flights (movements between trees) and hops (movements within trees) within the first 2 min are recorded. We used these measures to calculate an overall exploration score ranging from low (slow explorers) to high (fast explorers) (Dingemanse et al., 2002). This score is known to be repeatable in great tits and correlates positively with aggression, boldness, risk taking and approach of conspecifics (Groothuis & Carere, 2005).

General set-up

We used the automatic tracking system Encounternet (Encounternet LLC, Portland, OR, U.S.A.) to track 44 birds (21 males, 23 females) in March 2014. Encounternet consists of transmitters (tags fitted to the birds) and receiver stations distributed in a 40 m grid across the study site. Additionally, handheld antennas were used to

manually track birds and to download data from the receivers. This unique spatial data allowed us to simultaneously monitor the response of multiple individuals to territory intrusions in their neighbourhood. To simulate territory intrusions we performed playback experiments, broadcasting songs of an unfamiliar male great tit, between 0900 and 1130 AM at the roost boxes of male great tits. Playbacks, were performed from 12 March until 20 March 2014. We performed 1 to 4 playbacks on a given day, several nest boxes apart, following a procedure similar to that described in Chapter 7, but without use of a lure. All playbacks were conducted before the female fertile period; the first egg was laid on March 31. Permission for this study was granted by the Dutch legal entity: KNAW Dier Experimenten Commissie (DEC) no. NIOO-10.05 to M.N and K.v.O and no. NIOO-12.02 to K.v.O and M.N.

Spatial tracking

On March 10, we caught all birds that were roosting in the area covered by the receiver grid ($N = 44$). This triangular 40 m grid (Mean \pm SD: 41 ± 6 m) consisting of 166 receiver stations was put up the afternoon before the roost check. The birds were equipped with an Encounternet tag of approximately 1.3 g, using a leg-looped backpack harness. These tags did not have negative effects on the likelihood of breeding or the apparent survival of the subjects (**Chapter 2**). The Encounternet tags are active radio-transmitters, set to transmit every 5 s (see **Chapter 3** for more details). Previous analyses revealed that birds caught and tagged via this procedure will represent approximately half of the territorial individuals (**Chapter 3**). We only analysed spatial data from the second morning onwards following the evening of tagging (12–20 March 2014).

We retrieved the locations of these birds via triangulation, combining all the ID-coded received signal strength indicator (RSSI) values for each bird within the same half-minute for at least three receivers (see **Chapter 3** for more details). Signal strength detection accuracy was quantified for all the retriever stations by conducting calibration measurements at the end of the tracking period. We approached each receiver up to a two-meter distance (measured with a Leica Range finder) while carrying an Encounternet tag on a two-meter wooden pole. We adjusted the RSSI values for all receiver stations that deviated more than one standard deviation from the average (based on all 166 receivers) maximum RSSI value (14 ± 4 RSSI). Thus for example, for a receiver which logged a RSSI of 9 when the test tag was at a 2 m distance, we added +5 to all its RSSI values. Next to these signal strength calibration tests, we also quantified the accuracy of the system in

detecting spatial associations. We did this by simulating seven spatial associations throughout the tracking area. During these simulated associations, we moved and turned test tags on two two-meter wooden poles for at least 2 minutes (within 1 m of each other). These tags were located (triangulated) on average 10.8 ± 7.5 m of each other. This is less accurate compared to our calibration tests in 2012, in which test tags were located 7.5 ± 6.5 m apart (**Chapter 3**). This discrepancy was possibly caused by the fact that we substantially moved and turned our test tags more in 2014 compared to 2012 (likely more than real birds would have moved and turned). For the actual bird tracking data, following (**Chapter 3**), we thus a-priori defined a spatial association as two individuals localized within 10 m of each other.

Simulated territory intrusions

The roost boxes of the 21 males, which were caught and tagged during the roost check on March 10, were selected as playback locations. We played back a great tit recording of approximate 2 min comprising a systematic repetition of one song type of a great tit recorded at least 4 years before (see **Chapter 7** for details). To prevent pseudo-replication, each subject received a song recording from a different male. The playback files were constructed using Adobe Audition, by repeating one song of a unique bird with intervals of 4 s for 2 min (126.8 ± 15.2 s) or 20 songs (19.3 ± 1.4) and normalizing the peak amplitude to the same level for all songs. Songs were broadcasted at 79 dB (measured at a neutral site at 1 m from the loudspeaker with a Voltcraft Digital sound-level meter 322) using a Yamaha NX-U10 loudspeaker (frequency range 90 Hz – 20 kHz), connected with a 25 m cable to a media player (Archos 405, 30 GB). During the playback, two observers were at least 15 m away from the speakers (measured with a Leica RangeMaster 900). To obtain an indication of whether the intended subject was in the vicinity at the time of playback, we used a handheld antenna to detect the presence of the intended subject' signal-ID. One observer would conduct the playback while the other observer monitored the signal strength of the intended subject. We recorded subjects using two Sennheiser ME66/K6 microphones (frequency range 40 Hz – 20 kHz ± 2.5 dB) and a Marantz PMD660 solid-state recorder (sampling frequency 44.1 kHz, frequency range 16 kHz ± 0.5 dB) until 2 min after the end of the playback. From the recordings and from simultaneously recorded spoken notes we quantified four vocal response measures: (1) number of song overlaps, (2) total number of songs during total observation time (playback + 2 min), (3) number of songs during the actual playback and (4) the total singing duration (s), as well as two spatial response measures: (5) total time spent within 5 m of the loudspeaker (s) and (6) latency to approach within 5 m (s) (**Chapter 7**).

We did not observe a visual or vocal response during several of the 21 playbacks. Additionally, the signal strength of the intended subject was so low, or the signal disappeared in total just before the start of the playback, that these subjects almost certainly did not hear the playback ($N = 6$). Three of these failed playbacks were repeated but only during one of these repeats the intended subject responded. The five non-responding and absent subjects did not breed in or near the playback location and were therefore excluded from analysis. Also, a different (untagged) male responded instead of the intended (tagged) male during two of the original playbacks. Because we could not positively identify these responding males or have tracking data for these birds, these playbacks were also excluded from the analysis. In summary, we collected a dataset of playback responses for 14 tagged males. Unfortunately, because of an unintended reset of the tracking system on the final playback day, we could not collect the spatial neighbourhood data for one of these 14 males.

Data analysis

Response to simulated intrusions

Latency to approach within 5 m was set to 240 s for four birds that did not approach within 5 m of the loudspeaker. We conducted a principal component analysis (Varimax rotation with Kaiser normalization; rotation converged in three iterations) for all six response behaviours. Two principal components had an eigenvalue larger than 1. The first component represented the four vocal response behaviours (PC1-vocal; eigenvalue = 3.6; Supplementary Table S1), while the second component represented the two spatial response behaviours (PC2-spatial; eigenvalue = 1.9; Supplementary Table S1). The number of stimulus songs played back (*Mean* \pm *SD*: 19.3 \pm 1.4) did not influence any of the quantified subject responses (Supplementary Table S2). Also stimulus duration (126.8 \pm 15.2s) did not correlate with the subject response, with exception of latency to come within five meters (Supplementary Table S2). This is likely a statistical artefact, because approaches usually happened before the stimulus ended. We used correlation tests and group comparison test to examine whether age and condition correlated with any the playback response variables and exploration score. We categorized age as one year or older and condition as the residual from a regression of weight over tarsus length.

Changes in social and spatial structure

To examine potential effects of territory intrusions on neighbour spatial behaviour, we analysed the distance of the closest male and closest female to the location of

the simulated intrusion, comparing the 60 min directly before with the 60 min after the simulated intrusion.

To examine potential changes in social structure following the simulated territory intrusions we compared the number of spatial association partners (within 10 m) and the average time spent with each association partner (number of half minute locations that association partners were within 10 m) of the 60 min before the intrusion with the 60 min after. We hereby distinguished between male and female association partners ('associates' from now on). Mates of the subjects ($N = 3$) were excluded from the female associates. Mates of male great tits will behave differently from other females, responding in a specific defensive way to a simulated territory intrusion (Otter et al., 1999).

Statistical analysis

Correlations were examined using the Pearson correlation test (parametric) or the Spearman correlation test (non-parametric). We conducted paired group comparisons with the Wilcoxon signed rank test (non-parametric). For non-paired group comparisons we used the Welch Two Sample t-test (parametric) and the Wilcoxon rank sum test (parametric).

We used linear mixed effect models (R package lme4) for analysing the changes in the social structure and the spatial behaviour. The difference in minimum distance to the location of the simulated intrusion, number of unique associates and average time spent with associates were the three dependent variables. For each dependent variable two models were constructed, one with the interaction of the sex of the associates and the subject exploration score as predictor and a second model with the interaction of the sex of the associates and the main vocal response as predictor. We fitted interactions with sex since we expected any effect of song or personality to be contrasting for females (attraction) compared to males (avoidance). We used the subject ID as a random effect. The best fitting of two models was selected based on the lowest *AIC*. The final models were compared to the null-model using likelihood ratio tests. We separately added condition and age and their interactions with the sex of the associates as control variables to the final models, since also here we expected any effect of condition and age to be contrasting for females and males. Control variables were added later to save degrees of freedom for the variables of interest. Due to an outlier in the models for average time spent with associates (*Standardized Residual* > 2 & *Cooks D* $>> 4$ / ($N = 13$)) we rank-transformed the average time spent with associates. We investigated

the specific vocal response variables separately when effects of the main vocal response (PC1-vocal) were $P < 0.1$.

Normality of residuals was evaluated using the Shapiro-Wilk test ($P > 0.05$). The principal component analysis was conducted in IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp, Armonk, NY). All other statistical test we conducted in R 3.2.1. (R Core Team, Vienna, Austria) using RStudio Version 0.99.489 (RStudio Inc., Boston, MA). Graphs were constructed with the package `ggplot2`. For visibility purposes, we used the `"location_dodge(width = 0.4)"` function to prevent overlapping data points in final two figures.

Results

Subject responses to simulated territory intrusions

The main vocal component (PC1-vocal), including all four song measures, explained 59% of the total variation. The main spatial component (PC2-spatial), including the two approach measures, explained 31% of the total variation (Supplementary Table S1).

Both age and body condition of the subject did not associate with the exploration score of the subject (exploration score; Welch Two Sample t-test age: $t_{(11.46)} = 0.01$, $P = 1.00$; Pearson Correlation test condition: $r = -0.07$, $N = 14$, $P = 0.81$) or with the subject's main spatial response (PC2-spatial; Wilcoxon rank sum test age: $Z = 0.31$, $P = 0.75$; Spearman Correlation test condition: $r_s = 0.14$, $N = 14$, $P = 0.64$). But body condition did specifically positively predict the time spent close to the simulated intruder ($r = 0.59$, $N = 14$, $P = 0.03$). Moreover, age and condition were significant predictors of the subject's main vocal response (PC1-vocal; Welch Two Sample t-test age: $t_{(11.99)} = -3.10$, $P = 0.01$; Pearson Correlation test condition: $r = 0.59$, $N = 14$, $P = 0.02$; Figure 1). Specifically, all vocal response variables, with exception of number of overlaps, were significantly stronger for subjects older than one year (Table S3). And all vocal response variables, with exception of total singing duration, were significantly stronger for subjects in better condition (Table S3). Older birds tended to be in better condition (Welch Two Sample t-test age: $t_{(8.43)} = -1.91$, $P = 0.09$).

Exploratory behaviour of the subject did not significantly predict the main vocal response during the intrusion (PC-vocal: $r = 0.16$, $N = 14$, $P = 0.16$) or the main spatial response (PC-spatial: $r_s = 0.36$, $N = 14$, $P = 0.21$). There were also no

correlations between the subject exploration score and the individual response variables (Table S4).

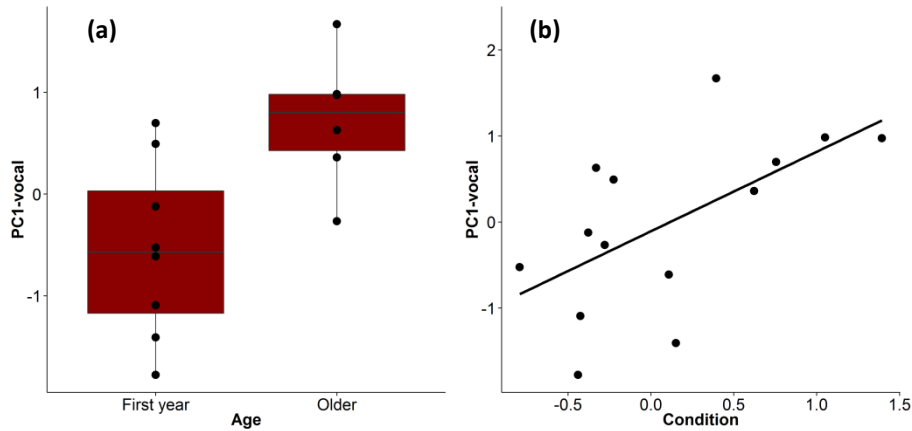


Figure 1. The main vocal response (PC1-vocal) of the subjects ($N = 14$) during the playback experiment and two minutes thereafter in relation to (a) age and (b) condition.

Effects of simulated territory intrusions on neighbour spatial behaviour and social structure

There was no main change in the closest visitor distance to the playback after a simulated intrusion (Paired Wilcoxon signed rank test male visitors: $Z = 0.14$, $P = 0.89$; female visitors: $Z = 1.09$, $P = 0.27$). There was also no main change in the number of male or female associates a subject had after a simulated intrusion (Paired Wilcoxon signed rank test male associates: $Z = 0.24$, $P = 0.22$; female associates: $Z = 1.18$, $P = 0.81$) or in the average time spent with the associates (Paired Wilcoxon signed rank test male associates: $Z = 0.05$, $P = 0.96$; female associates: $Z = 0.59$, $P = 0.55$), regardless of location.

Neighbour spatial behaviour in relation to subject vocal response and personality

The exploratory behaviour of the subject did not predict whether neighbouring conspecifics came closer to the playback location after the playback (sex*exploration score: $\beta = 0.96$, $F_{1,11} = 0.25$, $P = 0.624$, $AIC = 231.0$). Yet, males with a stronger vocal response during the playback tended to draw neighbouring females closer to the playback location while neighbouring males tended to move further away after the playback (sex*PC1-vocal: $\beta = 13.52$, $F_{1,11} = 4.27$, $P = 0.06$, $AIC = 226.8$; final model comparison to null-model: $P = 0.06$; Figure 2a). Specifically, the number of song overlaps and the total number of songs sung contrastingly

influenced the minimum approach distance of the closest female and the closest male (number of overlaps: $\beta = 6.10$, $F_{1,11} = 12.59$, $P = 0.005$, $AIC = 218.7$; final model comparison to null-model: $P = 0.002$; total number of songs: $\beta = 1.34$, $F_{1,11} = 11.04$, $P = 0.007$, $AIC = 220.4$; final model comparison to null-model: $P = 0.003$; Figure 2b,c). The number of songs sang during the vocal intrusion and the total singing duration of the subject tended to point in the same direction (number of song during: $\beta = 1.80$, $F_{1,11} = 4.13$, $P = 0.07$, $AIC = 226.9$; final model comparison to null-model: $P = 0.06$; singing duration: $\beta = 0.17$, $F_{1,11} = 3.34$, $P = 0.095$, $AIC = 227.8$; final model comparison to null-model: $P = 0.096$). These effects were not directly associated with the condition or age of the bird (final model comparison to null-model with singing responses replaced by condition: $P = 0.08$; age: $P = 0.20$).

Change in social structure in relation to subject vocal response and personality

The number of unique associates a territorial male had after a simulated territory intrusion tended to change as function of the exploration score of the territory owner in interaction with the sex of the associates, however this model was not significantly better than the null-model (sex*exploration score: $\beta = -0.23$, $F_{1,11} = 4.30$, $P = 0.06$, $AIC = 89.8$; final model comparison to null-model: $P = 0.16$, Figure 3). Condition and age of the subject did not have a significant effect, when added to the final model (sex*condition, sex*age, $P > 0.70$). Finally, the main vocal response of the subject did not predict the number of female and male associates (sex*PC-vocal: $\beta = -0.22$, $F_{1,11} = 0.21$, $P = 0.65$, $AIC = 92.7$).

Subjects that had more unique male associates after the intrusion also increased the average time they spent with their male associates ($r_s = 0.87$, $N = 13$, $P = 0.0001$). This was not the case for unique female associates ($r = 0.11$, $N = 13$, $P = 0.72$). The average time subjects spent with male and female associates was not related to their exploratory behaviour (sex*exploration score: $\beta = -1.41$, $F_{1,11} = 3.08$, $P = 0.11$, $AIC = 186.1$; final model comparison to null-model: $P = 0.31$). There was also no significant effect of the main vocal response on the average time associated with males and females (sex*PC-vocal: $\beta = -2.76$, $F_{1,11} = 0.94$, $P = 0.35$, $AIC = 184.2$).

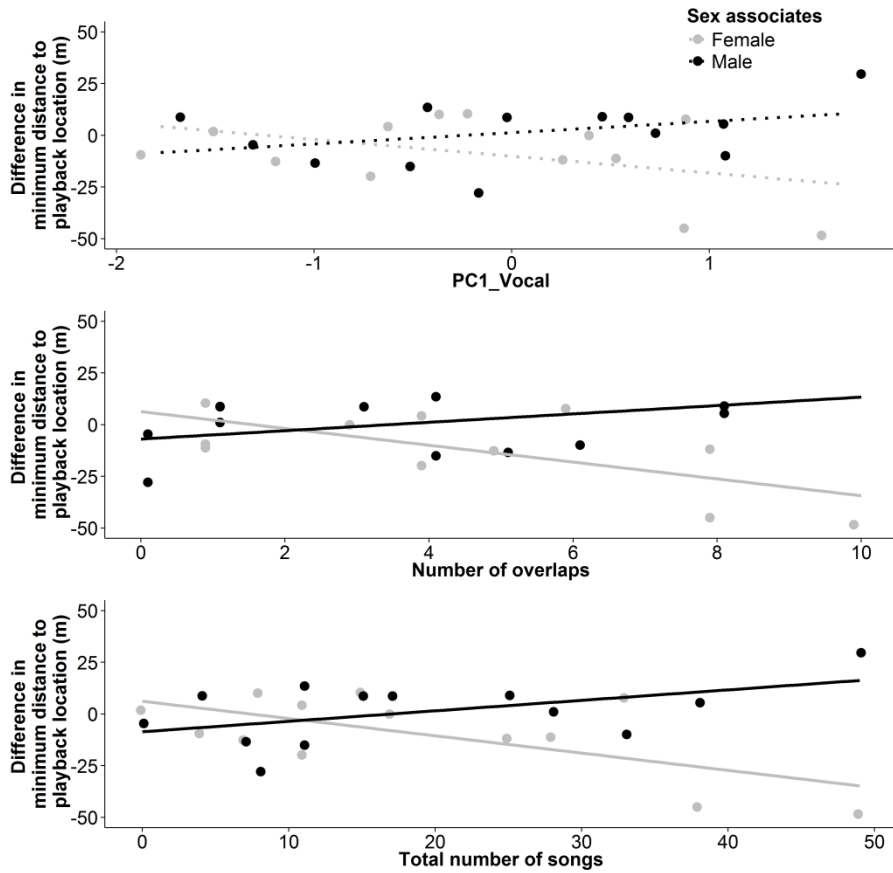


Figure 2. The difference between before and after the territory intrusion in the minimum distance for the closest male (black) and female (grey) came to the playback locations ($N = 13$) after the playbacks in relation to (a) the main vocal response of the subject (PC1-vocal) during the playback experiment and two minutes thereafter, (b) the number of song overlaps by the subject and (c) the total number of songs the subject sang. When the difference in minimum distance is negative, conspecifics came closer.

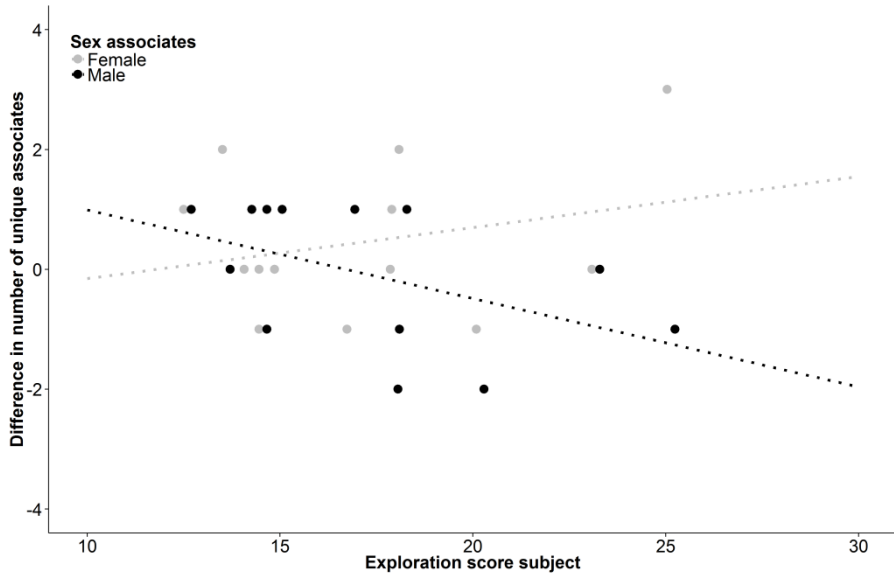


Figure 3. Difference between before and after the territory intrusion in the number of unique neighbouring male (black) and female (grey) associates a subject ($N = 13$) encountered after the playback in relation to (a) the exploration score of the subject. When the difference in unique associates is negative the subject encountered fewer associates after the playback.

Discussion

We here showed, following an experimental perturbation in the communication network, that males which vocally responded with more songs and more song overlaps, attracted neighbouring females closer to the territory intrusion site while neighbouring males stayed further away. The vocal response of the territory owner was positively correlated to his body condition and age, but in contrast to previous studies and our predictions we did not find a relation between his exploratory behaviour and the main vocal response. After the intrusion, faster explorers tended to spatially associate with more females and with fewer males compared to slower explorers.

To our knowledge this is one of the first studies spatially tracking the response of female songbirds to a simulated territory intrusion in the wild. Our findings agree with the hypothesis that females are attracted to strong vocal responders because they signal good quality. Stronger vocal responses were in significantly better body

condition and the vocal response positively predicted whether females, compared to males, were attracted to the intrusion site. A recent study in the same population which revealed that more active singers tend to be in better body condition and to raise more fledglings (**Chapter 5**), also supports vocal performance to be a signal of quality in great tits. Neighbouring females are likely attracted to males of good quality, while neighbouring males might prefer to keep their distance. In nightingales, males that overlapped more during agonistic interactions experienced a higher mating success (Kunc et al., 2006), while neighbouring great tit males tended to move greater distances when the resident male vocally responded stronger (Amy et al., 2010). Likewise, contrasting responses by males and to the same song trait were observed in chaffinches (*Fringilla coelebs*) (Leitão & Riebel, 2003). Yet, our findings seem to be in contrast to earlier results in nightingales where male neighbours intruded the territory of a resident quicker when the resident had vocally responded stronger (Naguib et al., 2004), but are in line with another nightingale study in which neighbour distance was taken into account, revealing that closer male neighbours responded more cautiously after eavesdropping on an aggressive (overlapping) singing interaction ((Naguib et al., 2004; Fitzsimmons et al., 2008a; Sprau et al., 2012). Whether a certain type of vocal response signals physical quality, motivation or arousal is thus likely to be song trait dependent and species specific.

Especially the number of overlaps was a strong predictor of female attraction and male repulsion in this study. Also in other animals, including anurans, overlapping appears to be a strong signal for eavesdroppers (Naguib & Mennill, 2010). The number of overlaps positively correlated with body condition in this study, yet any signaller should be physically capable of performing overlaps. Overlapping would thus be expected to be a signal of intent rather than physical quality. But, if overlapping is indeed a signal of aggressive intent (Naguib & Mennill, 2010; Maynard et al., 2012; Naguib et al., 2004; Fitzsimmons et al., 2008a; Sprau et al., 2012), this could evoke fights that might be too risky for individuals in lower body condition. Indeed, males in lower body condition spent significantly less time close to the simulated intruder. In this way social retaliation could thus maintain correlations between physical traits and song traits (Gil & Gahr, 2002; Anderson et al., 2012).

If neighbouring females were indeed attracted to stronger performers because they signal good quality we would also expect strong vocal performers to associate more with females at close-range. However, stronger vocal responders, which were overall also males in good body condition, did not increase in their number of female associates. Male-female spatial associations during the day-time

might not be the best indicator of female interest in (extra-pair) males with strong vocal responses. Therefore it would be very interesting to investigate if strong vocal performers eventually sire more extra-pair offspring, especially since more active singers also tended to raise more within-pair fledglings (**Chapter 5**). Indeed high-ranking male black-capped chickadees were more likely to lose paternity if their mates had heard them lose a singing contests to a simulated intruder (Mennill et al., 2002) and nightingales that responded stronger to playback were more likely to be mated later in the season (Kunc et al., 2006). Furthermore, male sac-winged bats (*Saccopteryx bilineata*) sired more offspring if they were stronger territorial singers (Behr et al., 2006). Yet, male great tits that were allowed to win in simulated territory intrusions were not less often cuckolded than males that lost the vocal interaction (Otter et al., 2001).

Surprisingly, we did not find a relation between exploratory behaviour and the vocal response to a simulated intruder. This is in contrast to two earlier studies in the same study area that revealed such a relationship (Amy et al., 2010; **Chapter 7**). However, while in the study by Amy et al. (2010) and **Chapter 7** fast explorers responded stronger, in a study by Jacobs et al. (2014) slow explorers appeared to be stronger responders. Different playback studies vary in subtle ways such as in whether or not lure playbacks are used (Amy et al., 2010; **Chapter 7**), the exact timing of playback in the breeding season or in more general ecological or social conditions within a year or population (Jacobs et al., 2014). Personality-dependent vocal responses thus seem to be strongly context dependent (**Chapter 5**). The current social environment, such as the population distribution of personality types varying in aggressive behaviour, could influence whether male song traits will mostly relate to motivation (when there is a low chance on a fight) or to physical capabilities (high chance of a fight).

Slower explorers tended to increase in the number of male associates, compared to female associates, after a territory intrusion. However, the effect of exploratory behaviour on the number of female compared to male associates was not significant. Even though inferences need to be cautiously drawn, also because of the relatively small sample size, the observed trend is interesting. If we assume this trend to be biologically meaningful, it might be explained by the fact that neighbouring males are attracted to territory owners that are expected to not be very effective in defending their territory. Neighbouring males indeed showed a shorter latency to reach the closest song post if the intruded resident was a slower explorer (Amy et al., 2010). Yet these resident males could also be actively seeking

out association themselves. Slower exploring great tits males are more sensitive to the behaviour of male conspecifics (van Oers et al., 2005), show more social exploration of unfamiliar male conspecifics (Carere et al., 2001) and behave more collectively (Aplin et al., 2014). Because they are commonly also physically less aggressive during direct confrontations (Verbeek et al., 1996) and simulated territorial confrontations (Amy et al., 2010; **Chapter 7**) they might seek out alliances more, or re-confirm their relations with other territory holders, after a territory intrusion. The formation of alliances is not uncommon in territorial songbirds (Olendorf et al., 2004; Templeton et al., 2012; Goodwin & Podos, 2014). Great tits join each other in nest defence more when they are familiar to each other (Grabowska-Zhang et al., 2012). And late arriving great tit males increase their chance on establishing a territory when they associate with other late arriving males (Farine & Sheldon, 2015). Neighbouring males might at the same time try to avoid fast explorers, since they are commonly more aggressive. Fast explorers might redirect their aggression after a territory intrusion or might want to re-establish their territory boundaries. However, future studies are needed to test whether exploratory behaviour indeed significantly influences social associations after a territory intrusion.

One major challenge with studying social behaviour from spatial tracking data remains the identification of the initiators of a spatial association. It is likely that both the behaviour of the territory owner (seeking out associations) as well as the behaviour of neighbouring conspecifics (attraction or avoidance of territory owner) play a key part in the structuring of this social network. Males staying further away from the intrusion site of strong vocal responder could imply that they tried to actively avoid subsequent encounters with the resident male. But at the same time neighbouring males could also have moved away to defend their own territory.

A second challenge in spatial tracking is the choice of the distance threshold to define associations. However, based on our previous results (**Chapter 3**) we are convinced that a 10 m cut-off captures substantial valuable information on spatial associations. Territorial birds within 10 m of each other will likely be aware of each other. Moreover, birds that were actually 20 m or more apart (and possibly unaware of each other) were very unlikely found to be located within 10 m of each other by the system. For example, during calibration tests in December 2012, conducted in the same study area and using a similar grid, test tags that were 20 m or more apart were never located within 10 m of each other (4 transects, each: 20, 25, 30, 50 m).

We here showed that perturbations in the communication networks can lead to changes in the spatial behaviour, and possibly social proximity networks, of territorial songbirds. There is also evidence that perturbations in the social proximity network in turn have direct consequences for the communication network. Social instability led to an increase of signalling levels in Siamese fighting fish (*Beta splendens*) (Matessi et al., 2010). And removal of a mate, or removal of all male neighbours, stopped day and dawn time singing respectively in male chipping sparrows (Liu, 2004). Together these findings suggest that proximity and communication networks are closely linked.

In some cases communication networks can actually be used to directly infer proximity networks. For example song sharing positively relates to spatial associations in juvenile male song sparrows (Templeton et al., 2012) and European starlings (*Sturnus vulgaris*) (Hausberger et al., 1995). Bottlenose dolphins use signature whistle matching to address social companions (King et al., 2014) and populations of humpback whales (*Megaptera novaeangliae*) share vocal traditions (Garland et al., 2011; Allen et al., 2013). Similarities between vocal communication networks and proximity networks become especially interesting when it involves species in which social networks are easier to study vocally than spatially. Tapping into the social networks of vocal signals can eventually lead to important insights into animal culture and sociality (Garland et al., 2011; Krams et al., 2012).

Acknowledgements

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Supplement to Chapter 8

Table S1

Table S1. Rotated component matrix showing component loadings of the playback response variables.

Playback response variable	Component	
	1	2
Number of overlaps	0.759	0.535
Number of songs during playback	0.924	0.302
Total number of songs	0.939	0.114
Singing duration (s)	0.939	-0.100
Time spent within 5 m (s)	0.547	0.765
Latency to approach 5 m (s)	0.087	-0.934

The Kaiser–Meyer–Olkin measure of sampling adequacy = 0.77 and Bartlett’s test of sphericity were significant ($P < 0.001$). The highest component loading for each playback response behaviour is in bold.

Table S2

Table S2. Playback response variables in relation to playback stimulus characteristics.

Stimulus variable	Response variable	Test statistic	P-value
Number of songs	Number of overlaps	$r_{s(12)} = 0.12$	0.68
	Number of songs during playback	$r_{s(12)} = 0.18$	0.55
	Total number of songs	$r_{s(12)} = 0.10$	0.74
	Singing duration (s)	$r_{s(12)} = 0.17$	0.56
	Time spent within 5 m (s)	$r_{s(12)} = 0.12$	0.69
	Latency to approach 5 m (s)	$r_{s(12)} = -0.19$	0.52
Duration	Number of overlaps	$r_{s(12)} = 0.317$	0.27
	Number of songs during playback	$r_{s(12)} = 0.10$	0.75
	Total number of songs	$r_{s(12)} = -0.03$	0.93
	Singing duration (s)	$r_{s(12)} = -0.10$	0.74
	Time spent within 5 m (s)	$r_{s(12)} = 0.39$	0.16
	Latency to approach 5 m (s)	$r_{s(12)} = -0.63$	0.02

Table S3

Table S3. Vocal playback response variables in relation to subject body condition and age.

Stimulus variable	Response variable	Test statistic	P-value
Condition	Number of overlaps	$r_{(12)} = 0.65$	0.01
	Number of songs during playback	$r_{(12)} = 0.71$	0.005
	Total number of songs	$r_{(12)} = 0.67$	0.01
	Singing duration (s)	$r_{(12)} = 0.51$	0.06
Age	Number of overlaps	$t_{(7.05)} = -1.36$	0.21
	Number of songs during playback	$t_{(9.98)} = -2.84$	0.02
	Total number of songs	$t_{(8.43)} = -2.84$	0.02
	Singing duration (s)	$t_{(11.78)} = -2.79$	0.02

Table S4

Table S4. Playback response variables in relation to subject exploration score

Response variable	Test statistic	P-value
Number of overlaps	$r_{(12)} = 0.18$	0.54
Number of songs during playback	$r_{(12)} = 0.06$	0.84
Total number of songs	$r_{(12)} = 0.11$	0.70
Singing duration (s)	$r_{(12)} = -0.12$	0.68
Time spent within 5 m (s)	$r_{(12)} = 0.47$	0.21
Latency to approach 5 m (s)	$r_{s(12)} = -0.34$	0.23

Figure S1



Figure S1. Three tagged birds, two males and one female, during an “encounter”.



9

General discussion

General discussion

Understanding of the role of personality in social structuring is rapidly developing. Social network studies focus especially on the social dynamics of fission-fusion societies, yet the social bonds in territorial systems might be even more intriguing. In many territorial populations, neighbours are both potential allies as well as rivals (dear enemies), creating a distinct cost-benefit trade-off for social associations. With this thesis I aimed to further the understanding of the role of personality in the social associations of territorial animals. Since personality is directly related to the risk individuals are willing to take, I expected personality to play a key role in whether or not individuals would risk associating with potential rivals. In this final chapter I synthesize and discuss my main findings, but I will also dive further into what is known about the interaction between personality and the social environment and why it is important to understand how animals within wild populations are connected.

Personality in the social environment

“Dogs got personality, personality goes a long way.”

-Jules, Pulp Fiction-

In **Chapter 3** I reveal that the pro-active personality type (fast explorer) has the most central social network position and tends to have the most unique social connections. In **Chapter 4** the re-active personality type (shy individual) spends the most time with a simulated unfamiliar conspecific. Who is more social, the one with the most social connections or the one with the best social connections? And why would different personality types differ in this respect? In the winter flocks of great tits (*Parus major*), re-active individuals (slow explorers) have more stable connections and behaved more collectively by staying within their groups, while the pro-active individuals (fast explorers) have more but weaker connections and moved between the groups (Aplin et al., 2013; Aplin et al., 2014). A similar pattern was discovered in three-spined sticklebacks (*Gasterosteus aculeatus*), shy fish showed stronger connections and are less likely to move away from an association (Pike et al., 2008). Furthermore, in guppies (*Poecilia reticulata*) reactive individuals showed stronger social connections, but in this instance also more unique connections (Croft et al., 2009). To gain insight into the role of personality in social networks I believe it is important to consider three questions:

- (1) Are social connections intentional?
- (2) What is the nature of the connections?
- (3) Do the consequences differ for frequency compared to strength of social connections?

(1) Are social connections intentional?

For some transmission processes, such as disease transmission, it will not matter much whether associations were intentional, as long as they were made in very close proximity. Yet for other transmission processes, such as information flow via the active sharing of information, the intention of the association might be very important for the likelihood of the information being shared. Irrespectively, it would certainly further our understanding of social structuring, as well as the understanding of cost and benefits of personality differences, to know if specific personality types show a stronger (intentional) social preference (**Chapter 4**). However, in a non-manipulated situation it is difficult to draw conclusions about intention from spatial associations. Pro-active individuals, such as fast exploring great tits and bold three-spined sticklebacks, tend to also show a higher level of activity (Réale et al., 2007; Pike et al., 2008; Chapman et al., 2011; David et al., 2011) and this could influence how many social partners they meet by chance. In **Chapter 3** I examined this alternative explanation by testing if average activity (speed in m/s) was related to exploratory behaviour and I found no such correlation. Although exploratory behaviour did not correlate to average activity in terms of speed, fast explorers might still visit different areas more often. However, in a previous study the home ranges (50 & 95 percent kernels) of great tits in summer, personality did not correlate to exploratory behaviour (van Overveld & Matthysen, 2010). Furthermore, the Minimum Convex Polygons (50 percent) based on our own spatial tracking data of 2014, did not suggest a correlation between exploration score and space use (Figure 1 and 2). These combined findings thus suggest that a potential higher level of activity in the wild is not a passive driver of the personality effect on the proximity networks in this thesis.

Bold three-spined sticklebacks were more likely to move to a new location compared the shy three-spined sticklebacks, either when alone or with a partner (Pike et al., 2008). However, when associating with a social partner, shy fish were even less likely to move to a different location compared to when they were alone. These simple behavioural differences can lead to complex social patterns. Indeed seemingly complex social structures can often be explained by simple local rules (Couzin & Krause, 2003; Fewell, 2003; Hemelrijk & Wantia, 2005; Ohtsuki et al., 2006; Gross & Blasius, 2008). To make causal inferences about the role of

personality in social networks it is therefore necessary to rule out alternative explanations or to perform a controlled experiment. For example, individuals with stronger social connections might indeed actively prefer to stay with certain social partners (Croft et al., 2004a; Croft et al., 2004b; Croft et al., 2006) or they might just prefer similar habitats (Webster et al., 2009). When drawing conclusions on social preferences, this makes a substantial difference.

The influence of the social partner is also relevant when making inferences about social preferences based on spatial associations. You cannot as easily associate with someone who does not want to associate with you. Thus, individuals that show few social connections might still have a relatively strong preference for those social

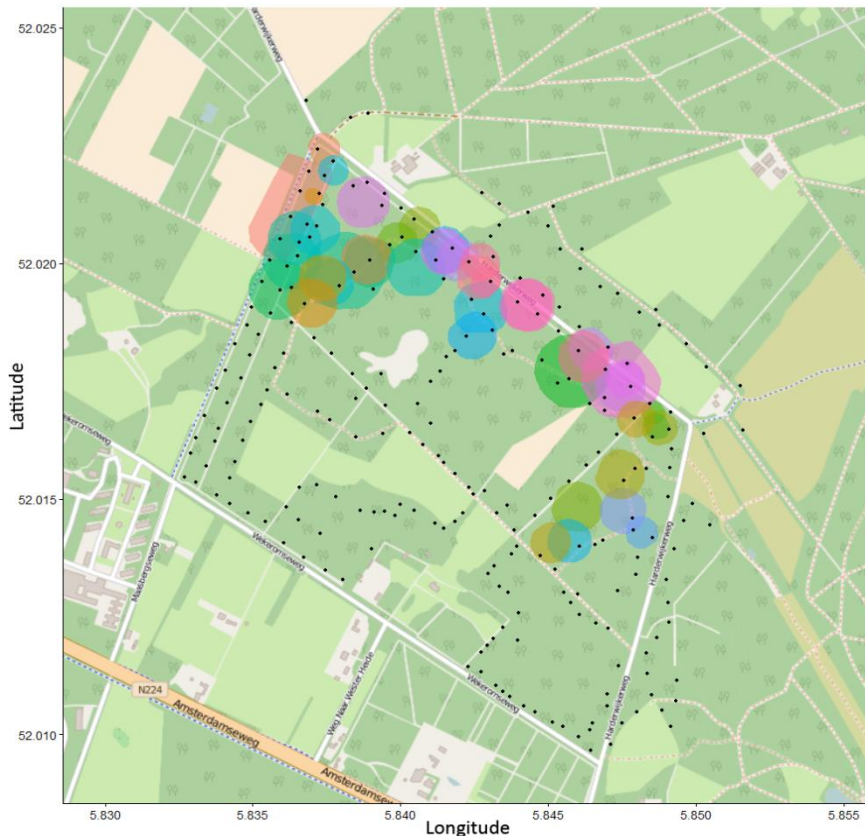


Figure 1. The 50% Minimum Convex Polygons for all 44 tagged birds from March 12 until March 19 2014.

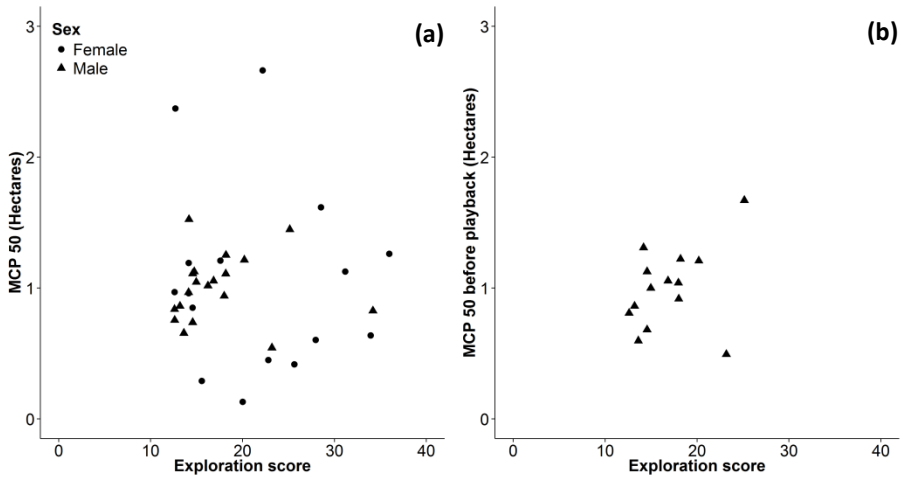


Figure 2. The 50% Minimum Convex Polygons (circle = female, triangle = male) in relation to exploration score for (a) 40 tagged birds from March 12 until March 19 2014 for which we retrieved at least 75% of the number of expected locations (based on a regression of the total number of locations retrieved and the total number of days the tags were active) and (b) a subset showing the 14 playback subjects until the playback experiment.

associations. General familiarity and previous cooperation or defection can have a significant influence on whether a potential social partner will associate (Ward & Hart, 2003; Croft et al., 2004a; Croft et al., 2005; Croft et al., 2006; Grabowska-Zhang et al., 2012; Carter & Wilkinson, 2013). And of course the individual characteristics of a partner could influence how likely this individual will be to associate (Carere et al., 2001; Croft et al., 2009; Aplin et al., 2014; Geffroy et al., 2014; Jolles et al., 2015; **Chapter 4**). Ideally one would therefore test the social characteristics of one individual by placing it several times in different group compositions and in different contexts. Something which is difficult to achieve with wild animals or when only a few animals are available in captivity. By using proper randomization techniques in social network analysis somewhat more informative conclusions can be drawn, since this analysis technique tests the likely influence of certain individual traits, given the current group composition (Croft et al., 2008; James et al., 2009). Additionally, replication of correlative studies would help generate more robust conclusions on the active role of personality in social networks.

Spatial associations alone thus may not reflect intentional social associations and, consequently, social preference. Spatial associations can (1) overestimate social preference when associations are unintentional, or (2) underestimate social preference when associations are highly dependent on a small number of social partners. Yet, collecting data for a social network analysis usually means making a trade-off between these two potential biases. It will depend on the type of question the researcher aims to answer what methodology is preferred: a proximity-based often large sample size network, usually gathered via the tracking of spatial associations or a detailed but often lower sample size network, usually gathered via direct observations that can infer intention from associations. For some questions less is perhaps more.

(2) What is the nature of the connections?

In flocking, swarming or schooling populations spatial associations will most likely reflect neutral or affiliative relationships. However in competitive environments (limited resources), such as in territorial systems, associations can frequently be aggressive. The question is: does it matter? The few studies that have compared different types of networks indeed found that different networks have different implications (Helliwell & Huang, 2013; Boogert et al., 2014; Castles et al., 2014). Not very surprisingly, for humans, real-life friends are much more important for happiness, especially for single people, than on-line friends (Helliwell & Huang, 2013). In captive starlings (*Sturnus vulgaris*) the perching network, much more than the foraging network, predicted whether individuals would acquire a new foraging task (Boogert et al., 2014). And in chacma baboons (*Papio ursinus*) proximity networks were overall not comparable to affiliative interaction networks when inferences were made about the global network properties (Castles et al., 2014), but see (Farine, 2015).

The use of proximity networks is frequent in animal social network analyses (Whitehead et al., 2005; Croft et al., 2008; Farine, 2015), but often does not allow identification of the nature of spatial associations. Despite this downside, in this thesis I used the technique of automatically and continuously tracking spatial associations (**Chapter 2, 3 & 8**), because the technique is not biased towards certain contexts in which social interactions and associations are more easily observable or measurable (Dingemanse & de Goede, 2004; Psorakis et al., 2012). My first aim was to get a general idea of social connectivity. An alternative to automated tracking would be to make behavioural observations. However, especially in spring, great tits are difficult to observe and identify and it is likely that

the behaviour of the birds would be influenced by people continuously following them around. Since I was tracking in a period during which great tits are territorial (**Chapter 3 & 8**, just before the fertile period) I assumed that any spatial association between males would be risky. Although during my time in the field I have encountered males engaging in aggressive interactions and sometimes even in physical fights, it would be valuable to find out what proportions of associations are indeed aggressive and what neutral or affiliative. Additionally, examining if aggressive associations have certain spatial and temporal characteristics could help filter these specific types of interactions from future spatial association data.

Being able to distinguish between the different types of association will be valuable, especially for the study of personality in great tits. Pro-active great tits seem more likely to initiate aggressive or competitive interactions, while re-active great tits seem more likely to form neutral or affiliative associations (Verbeek et al., 1996; Carere et al., 2001; Carere et al., 2003; Amy et al., 2010; **Chapter 3, 4 & 7**). Additionally, the nature of the associations could differ between contexts (territorial or in winter flocks), which in turn could have a significant influence on whether pro-active or re-active personality types are better socially connected. For great tits, context-dependence frequently seems to play an important role in revealing effects of personality (Dingemanse et al., 2004; Dingemanse & de Goede, 2004; van Oers et al., 2005; Jacobs et al., 2014). For example, whether males were territorial significantly influenced conclusions about whether the relationship between personality and dominance in great tits was positive or negative (Dingemanse & de Goede, 2004). How to determine the nature of spatial associations without observers following birds continuously remains a challenge (but see Otter et al. (1999)). However, at the very least an approximation can be made by taking the context (competitive, risky, neutral, affiliative) and qualitative information on relationships between individuals (mates) into consideration when studying social networks.

(3) Do the consequences differ for frequency compared to strength of social connections?

To gain benefits from social connections, either the quality (strength) of the bond or the number of direct and indirect connections may be most important. Simple social information on novel food patches can be gathered by short-term (weak) associations and in this case it will pay to associate with as many different groups or individuals as possible (Aplin et al., 2012), just like pro-active personality types seem to do (Pike et al., 2008; Aplin et al., 2013; Aplin et al., 2014; **Chapter 3**). The

benefits of having many weak associations probably depend on the competitiveness of the context. Pro-active types likely have more competitive ability, since in several species, including ants, birds and fish, a positive correlation exists between boldness or exploration and physical aggression towards unfamiliar conspecifics (Verbeek et al., 1996; Bell & Stamps, 2004; Réale et al., 2007; Chapman et al., 2011; David et al., 2011; **Chapter 7**). Competitive environments can be created by limited access to high quality food. In wild great tits, personality indeed correlates with competitive advantages, as fast exploring territorial great tits are more dominant at feeders (Dingemanse & de Goede, 2004) and show more competitive ability (time spent on feeder)(Cole & Quinn, 2012). A similar pattern was revealed in captive female zebra-finches (*Taeniopygia guttata*) (David et al., 2011). Pro-active types, in competitive environments, thus seem to have less to lose when switching between different and novel social partners or groups. Re-active types in contrast would have a higher chance of losing conflicts over food (or other) resources. Moreover, pro-active great tits were more likely to copy successful foraging choices of a tutor (Marchetti & Drent, 2000), suggesting that they will not only have less to lose, but also have more to win than re-active types by having many weak associations.

At least in a competitive context, having many superficial connections hence seems to be more beneficial for pro-active than for re-active personality types. This suggests that bridges between distinct subgroups or populations are usually formed by pro-active individuals, which is important information for predicting disease transmission and cultural transmission (Croft et al., 2008). Whether social groups are indeed mostly connected via pro-active individuals is also relevant information for predicting the likelihood of connectivity between reintroduced or invasive populations and the resident populations. Indeed, there is accumulating theoretical and empirical evidence for pro-active personality types as bridges between populations; pro-active individuals tend to disperse more and socially embedded individuals tend to disperse less (Cote et al., 2010; Sih et al., 2012; Canestrelli et al., 2016). Interestingly, Blumstein et al. (2009) found evidence suggesting that it is not just the number of affiliative connections, but more how socially embedded individuals are in the current group, that influences the likelihood of dispersal. This supports the *social cohesion hypothesis*: individuals who socialize more are less likely to disperse (Bekoff, 1977). There might thus be one important link between personality, social connectivity and general dispersal patterns in wild animal populations.

Many types of “social benefits” require strong stable social bonds. For example, helping in nest defence (Olendorf et al., 2004; Grabowska-Zhang et al., 2012) or forming a coalition against conspecific competitors (Getty, 1987; Lusseau, 2007; Schülke et al., 2010; Gilby et al., 2013). Female chacma baboons who form more stable, and stronger, social bonds with other females live significantly longer compared to female baboons who’s social bonds are less stable, irrespective of dominance (Silk et al., 2010). In male Assamese macaques (*Macaca assamensis*) the strength of social bonds directly predicts the number of sired offspring (Schülke et al., 2010). As in chimpanzees (*Pan troglodytes*) these fitness benefits were achieved by forming coalitions (Schülke et al., 2010; Gilby et al., 2013). In several species familiarity with certain conspecifics is known to have fitness benefits (Beletsky & Orians, 1989; Grabowska-Zhang et al., 2011; Carter & Wilkinson, 2013). On an individual level strong connections, rather than many connections, might thus be most important in predicting fitness effects of social network positions, which would explain why we did not find an effect of network centrality on reproductive success in **Chapter 3**. However, in house finches (*Carpodacus mexicanus*), the duller males that frequently switched between groups (high ‘betweeness’) were the ones to enhance their mating chances (Oh & Badyaev, 2010). And in long-tailed manakins (*Chiroxiphia linearis*), information centrality (taking also indirect connections into account) predicted whether young males would later rise in social status and become the alpha male in male-male partnerships (McDonald, 2007).

An interesting avenue for social network research is thus to analyse under which (social) conditions and with which individual states or characteristics, certain social network positions lead to significant fitness benefits. When social network positions are consistent over time, as they appear to be in great tit winter flocks (Aplin et al., 2015b), they can become targets for natural and sexual selection. A large-scale analysis of social network fitness consequences could help explain the evolution and stability of a variety of animal social systems (Krause et al. 2007) as well as provide insight into the maintenance of personality variation. When personality types consistently differ in social network position, personality may have significant consequences for key ecological and evolutionary processes, provided specific social network positions lead to important social and sexual advantages. The link between personality and social structure and especially its consequences for ecological and evolutionary processes, such as dispersal, provides many interesting research opportunities for the future (Cote et al., 2010; Sih et al., 2012; Kurvers et al., 2014; Wolf & Krause, 2014; Canestrelli et al., 2016).

Can social networks also influence personality?

The link between social phenotype and personality is not a one way street (Krause et al., 2010), as the social environment can influence personality differences in several ways. Social experience, competition, social responsiveness and 'social' niche specialization can influence the variation and consistency of personality differences (Dingemanse et al., 2004; Hemelrijk & Wantia, 2005; Rutte et al., 2006; Bergmüller & Taborsky, 2010; Wolf et al., 2011; Nicolaus et al., 2012; Laskowski & Pruitt, 2014). One of the most well-known examples of how social experience can influence the behaviour of an individual is the winner-loser effect (Hsu & Wolf, 1999; Rutte et al., 2006). Individual aggression and the likelihood of winning or losing a conflict often depends on whether an individual won or lost its previous conflict. In addition, knowing if an opponent has won or lost a previous contest seems to influence how an animal behaves in a conflict situation (Peake et al., 2001; Earley & Dugatkin, 2002). Social experience can thus influence observed aggressiveness.

Many personality traits are characterized by the way animals cope with (mildly) stressful situations. However, how individuals cope with stress can be strongly influenced by their social environment. Social partners can increase or decrease an individual's stress response. Especially in captivity the potential negative effects of social partners are well-known (Rodenburg & Koene, 2007), but social partners can also lower stress (Rault, 2012), a process known in humans and primates as social support (Silk et al., 2003; Charuvastra & Cloitre, 2008). There is some evidence that variation in early social conditions directly relate to individual stress responses and behavioural traits (Lantová et al., 2011; Naguib et al., 2011a; Geffroy et al., 2014).

Confusingly, in some species it seems dependent on certain personality traits whether social experience and social support influence an individual's behaviour (van Oers et al., 2005; Jolles et al., 2014). In great tits, slow males adjusted their risk-taking behaviour, a repeatable personality trait (van Oers et al., 2004), to the behaviour of a social partner (van Oers et al., 2005). In three-spined sticklebacks, bold fish, but not shy fish, adjusted their role as leader and their time spent out of cover when they had previously been paired to a bolder social partner (Jolles et al., 2014). Finally, the role of a fish as a follower was mostly influenced by the personality of the current social partner, with shy fish showing the most adjustment to the current social context (Jolles et al., 2014). Certain personality traits can thus influence if and how the social context affects (the measurement of) other personality traits.

In the wild it is almost impossible to control or quantify social experience, but captivity offers more possibilities for controlling and manipulating the social context. In **Chapter 4** the captive subjects were very similar in their social background, which will have strongly contributed to uncovering the dominance effect on social preference. Experiments in captivity can thus give important information on potential mechanisms. However, whether such mechanisms are indeed relevant in nature or are instead totally overshadowed by other factors, such as social experience, remains an important open question for which (again) an observational study in the wild will be necessary to answer it.

The social environment can also indirectly influence personality traits by influencing survival chances. Higher population densities or changes in the sex-ratios of the social environment will lead to more competition for resources, decreasing future expectations. When there is less to lose individuals can afford to take more risks and thus adaptively behave more boldly (Wolf et al., 2007; Nicolaus et al., 2012). Wild great tits indeed change in their exploratory behaviour in accordance to a change in future fitness expectations, modulated by an experimental change in the local social breeding environment (Nicolaus et al., 2012).

Next to the direction of personality traits (i.e. becoming bolder or shyer), social factors are also expected to influence the stability of personality traits. Wolf et al. (2011) presented an intriguing social explanation for the observed limited behavioural plasticity (i.e. personality) of individuals. Individuals could benefit from being consistent and thus predictable for conspecifics, but only if being predictable cannot be exploited. Consistency will be favoured in situations where both individuals benefit from behavioural coordination or avoiding costly outcomes. When there is some variation and consistency already present in a population, possibly via mutations, this consistency may select for social responsiveness, since individuals can benefit from taken a social partner's previous response into account, and this social responsiveness can again favour consistency if it benefits coordination or prevents particular negative outcomes, creating a positive self-reinforcing feedback loop. In this way a population can arise with both responsive individuals and unresponsive, but behavioural consistent, individuals (Wolf et al., 2011). This is interesting, since especially in great tits there is evidence that individuals consistently differ in how responsive they are to changes in the environment (Verbeek et al., 1994; Marchetti & Drent, 2000).

The social environment can be a particularly strong influence when the environment is very competitive. To avoid competition it might therefore be beneficial to specialize. The social niche hypothesis proposes that the existence of various consistent behavioural responses among individuals is similar to the existence of ecological niches among species (Bergmüller & Taborsky, 2007). This mechanism is most likely relevant in animal populations in which individuals repeatedly encounter each other, i.e. stable social groups, and thus become familiar with each other's behaviour. Moreover, such populations should consist of responsive individuals (Wolf et al., 2011). To avoid competition, individuals in groups which have many repeated interactions should thus exhibit more variation among individuals and less variation within individuals (Laskowski & Pruitt, 2014). This expectation was not met when comparing the behaviour of familiarized and unfamiliar three spine stickle backs (Laskowski & Bell, 2014). However, in a social spider (*Stegodyphus mimosarum*), between-individual variation in behaviour increased and within-individuals variation decreased the longer the spiders were in the same social group (Laskowski & Pruitt, 2014). The difference in results between these species is hypothesised to relate to the fact that social spiders live in much more stable social groups, also under natural conditions, whereas social organization in sticklebacks is much more plastic.

In various ways, depending on the characteristics of the social systems, the social environment can thus promote, change and maintain individual differences in behaviour. Because social systems between different animal species are so diverse, social network analysis might be a key tool for quantifying and comparing the frequency and stability of social interactions between populations and the associated variation and consistency in personality traits.

The social networks of signals

"Everything becomes a little different as soon as it is spoken out loud."

-Hermann Hesse-

Besides proximity networks I also investigated the communication networks of great tits, to get a second measure, or at least an indication, of social connectivity (**Chapter 5, 6 & 7**). The technology for collecting informative data on vocal signalling behaviour is strongly advancing. Automated song recorders (**Chapter 6 & 7**), miniature microphones (Cvikel et al., 2015b) and acoustic location systems

(Fitzsimmons et al., 2008b; Foote et al., 2008) are promising tools for quantifying communication networks in much more detail in the near future. Signal characteristics are interesting for the study of social networks because they can influence as well as reveal spatial association patterns.

How signals influence social networks

Signals function to transmit information from a signaller to a receiver, usually benefitting both the signaller and intended receiver (Bradbury & Vehrencamp, 1998; McGregor & Peake, 2000). For example, aggressive signalling strategies exist because both the signaller and the receiver have a common interest in avoiding injuries (Enquist et al., 1998). On a large spatial scale conspecifics are often attracted to each other (Stamps, 1988; Couzin & Krause, 2003), but on a smaller scale, especially territorial, individuals discourage each other from coming too close (Stamps & Krishnan, 2001). Coming to close can lead to costly close-range confrontations (Stamps & Krishnan, 1997). Hence, animals can use long-range signals to keep same-sex conspecifics at a distance (McGregor, 2005). Signals in territorial animal societies, ranging from scent markings to bird song, are often essential in modulating the social dynamics and territory stability of populations (Beletsky, 1992; Bee & Gerhardt, 2002; Briefer et al., 2008).

In **Chapter 7** I found evidence that individuals that are less prone to close-range confrontation of an intruder signal more actively (higher dawn song rate). Interestingly, dawn song rate did not correlate with the measure of singing activity we used in **Chapter 5**. Although we know that territorial song *per se* can keep rivals at a distance (Krebs, 1977; Nowicki et al., 1998), the interpretation of specific varying signal traits is more difficult. Signal traits that vary between individuals could just be a reflection of the variation in physical abilities. Singing activity in **Chapter 5** tended to relate to body condition, and individuals with a stronger vocal response in **Chapter 8** (but not **Chapter 7**), were in significantly better condition. However, the repeatable song rate in **Chapter 6** was not related to body condition. Singing with a higher song rate in a non-confrontational context, the dawn chorus, could also indicate a higher motivation to avoid close-range confrontations. Yet this would only make sense if a higher song rate in great tits is also more effective in keeping conspecifics at a distance, such as higher song rate seems to be for nightingales (Naguib et al., 2001). Playback experiments by broadcasting songs with varying song traits from unoccupied territories would be necessary to test this assumption. If certain signal traits are indeed more effective in keeping rivals at a

distance, it would be very interesting to analyse if individuals with such dawn song traits also occupy the least central male-male proximity network positions.

Next to spacing individuals out (Waser & Wiley, 1979), signals can also influence social dynamics by attracting conspecifics. Certain signals can attract potential sexual partners (Ryan et al., 1981; Leitão & Riebel, 2003; Holveck & Riebel, 2010; Maynard et al., 2014; **Chapter 8**). Males with a higher singing activity in **Chapter 5**, tended to raise more fledglings, which could be indicative of higher female investment in relation to singing activity, especially since males sang most actively in the fertile and incubation period. However, signalling can also be used to contact and attract social (non-sexual) partners, especially in group living or (non-territorial) solitary species. For instance, when separated, grey wolves (*Canis lupus*) howl more to keep contact with affiliated partners (Mazzini et al., 2013). The quality of the social relationship rather than the cortisol levels predicted the howling response. Animals that move over large distances, such as some marine mammals, effectively use long-distance vocal signals to keep contact and to find each other (Rendell & Whitehead, 2004; Garland et al., 2011; Marcoux et al., 2012). But also African elephants (*Loxodonta Africana*), can recognize a contact call as belonging to a certain social partner over distances of 2.5 km (McComb et al., 2003). Thus for some social systems long-range signals are the key enablers of social networks.

How signals reveal social networks

Beyond attraction and repulsion, signals have the potential to reveal the nature of social connections, and hence whether connections are pre-dominantly aggressive or affiliative. For example, in primates certain visual signals give reliable indications of dominance relationships (Flack et al., 2006). Not only what you signal but also how you signal it can give information about the nature of the relationship (Todt & Naguib, 2000). Changes in call frequency in male cricket frogs (*Acris crepitans*), seemed to signal information about agonistic intent; temporal call characters predicted whether a resident would tolerate an opponent (Burmeister et al., 2002). Moreover, in a number of territorial songbirds certain singing patterns, such as overlapping, are regarded as aggressive (McGregor, 2005; Naguib & Riebel, 2014).

Since signal patterns, such as overlapping or matching, are often not directly dependent on the physical ability of the signaller, signal patterns are especially relevant for researchers interested in the intention of social associations. Some signal traits require a certain body size or body condition for an individual to

consistently produce them, yet any signaller should be physically capable of overlapping another signaller. Signal patterns could thus very well be a signal of intent rather than quality. Seemingly in contrast, in **Chapter 8** I found a significant positive correlation between the number of overlaps and body condition. But, if overlapping is indeed a signal of aggressive intent (Naguib & Mennill, 2010; Maynard et al., 2012), this would evoke physical confrontations which might turn out to be very costly for individuals in lower body condition. Independent of the underlying mechanism, signal patterns can reveal the nature of social relationships. For example, male long-tailed manakins more often overlapped simulated rivals than neighbours (Maynard et al., 2012).

Repertoire sharing is another way signals can reveal social connections. Juvenile male song sparrows (*Melospiza melodia*) with strong social bonds shared more song types (Templeton et al., 2012). In bottlenose dolphins (*Tursiops truncatus*) direct whistle matching was shown to be a directed affiliative signal, driven by temporal association (Watwood et al., 2004; King et al., 2014). Also, parrots, orange-fronted conures (*Eupsittula canicularis*), imitate each other's contact calls exceptionally well, which allows them to address specific individuals in fission-fusion flocks (Balsby et al., 2012). In addition to dyadic connections, membership of specific social units can be revealed by shared repertoires, as in several animal species, social groups share specific vocalizations. Corn bunting (*Emberiza calandra*) in Britain were shown to live in distinct dialect populations, with all males within a dialect population singing the same form and number of song types (McGregor, 1980). Moreover, populations of very mobile species can also share distinct vocalizations, such as in humpback whales (*Megaptera novaeangliae*), sperm whales (*Physeter macrocephalus*) and narwhals (*Monodon monoceros*) (Rendell & Whitehead, 2004; Garland et al., 2011; Marcoux et al., 2012). Knowing how signal characteristics vary with social structure, either via signal types or via signal traits (see also **Chapter 6**), has the potential to increase qualitative information on the nature of social relationships and to facilitate the study of social networks in species that have little spatial associations but do use long-range signals.

Personality and signalling

Until now, the link between established personality traits and signal characteristics has been surprisingly weak. In collared flycatchers (*Ficedula albicollis*), more explorative and risk-taking individuals sang at lower song posts in the presence of a human observer (Garamszegi et al., 2008). In captive great tits, faster explorers

sang with a higher song rate (Naguib et al., 2010). Yet, in my research with wild birds I only found correlations between song traits and personality in confrontational contexts, i.e. simulated territory intrusions. None of the dawn song analyses revealed a direct relationship with exploratory behaviour, although there was a negative correlation between dawn song rate and the time spent near an intruder, a behavioural response commonly linked to exploratory behaviour (Amy et al., 2010 ; **Chapter 7**). However, personality effects on communication being mostly revealed in confrontational contexts makes sense intuitively, since many established personality traits, such as exploratory behaviour, boldness, risk-taking and aggressiveness reflect how individuals cope with stressful situations.

Slow explorers in captivity used more aggressive display signals during confrontations and took longer to attack, while faster explorers attacked more often (Carere et al., 2005). These findings agree with my findings in **Chapter 7** and suggest a relationship between certain signalling characteristics and the tendency to avoid aggressive confrontations. In an earlier study, slow explorers also responded to a simulated intrusion with a higher song rate (Amy et al., 2010). Yet, the overall vocal response during the simulated confrontation was stronger for fast explorers (Amy et al., 2010; **Chapter 7**), so more information is necessary on which specific song traits are most effective in keeping rivals at a distance.

It is well-established that fast explorers are more aggressive (Verbeek et al., 1996; Carere et al., 2005). Interestingly, slow explorers in captivity spent more time on social exploration of unfamiliar conspecifics (Carere et al., 2001; **Chapter 4**). It would thus be interesting to test if social preference and aggressiveness, next to exploratory behaviour, directly link to certain signalling strategies. Signal strategies are only effective if receivers are responsive. Since certain personality types seem to differ in their responsiveness (Verbeek et al., 1994; Marchetti & Drent, 2000; Guillelte et al., 2010) and in their motivation to avoid aggressive physical interactions, the re-active personality types in particular might be essential for the maintenance of both flexible signalling strategies and repeatable signalling traits (Wolf et al., 2011). Yet, in **Chapter 5**, we found evidence that faster explorers (pro-active types), not slower explorers (re-active types), responded to changes in the breeding cycle of their mate by significantly altering their singing activity. Future research, via empirical studies and modelling approaches (Hemelrijk & Wantia, 2005; Wolf et al., 2011) could give some valuable insights into the evolution of signalling strategies (Enquist et al., 1998; Schmidt et al., 2007; Maynard et al., 2012) by examining not only the role of personality in signalling behaviour, but also

the responsiveness of different personality types to varying signal traits and patterns.

Connecting in a changing world

“Pull a thread here and you’ll find it’s attached to the rest of the world.”

-Nadeem Aslam-

I personally find the study of social connectivity in animal populations fascinating, but I also think it is important, for example for effective conservation of animal populations. In his book “The song of the Dodo”, David Quammen shares an analogy which accurately reflects the relevance of social network analysis for nature conservation: *Slice up a fine Persian carpet into a few dozen neat rectangular pieces. The swatches may together occupy the same area as they did before. But your carpet no longer exists. You’re left with a pile of worthless tatters and scraps.* He concludes by saying: *An ecosystem is a tapestry of species and relationships. Chop away a section, isolate that section, and there arises the problem of unraveling.* In this final part of my synthesis I therefore discuss the value of social network analysis for nature conservation. The fact that social network analysis is relevant for predicting and managing disease transmission is well established (Hamede et al., 2009; Bull et al., 2012; Hirsch et al., 2013; Rushmore et al., 2013), but here I briefly focus on three other (anthropogenic) threats in nature conservation for which network analysis could provide valuable insights:

- 1) Habitat fragmentation
- 2) Anthropogenic noise
- 3) Selective removal/harvesting

(1) Habitat fragmentation

Habitat fragmentation disrupts spatial connectivity between individuals, which can have severe consequences for population viability (Debinski & Holt, 2000; Frankham et al., 2002; Banks et al., 2007; Tuomainen & Candolin, 2011). When encounter rates and group sizes change as a result of structural changes in the environment, this can have significant consequences for social behaviour, anti-predator behaviour, parasite transmission and mate choice behaviour, thus potentially influencing individual fitness (Banks et al., 2007). Fragmentation can decrease conspecific densities due to an overall decrease in resources, but it can

also result in an increase in conspecific density because individuals clump together in the small patches of suitable habitat that are left (Banks et al., 2007; Tuomainen & Candolin, 2011). In some species, such as Eurasian badgers (*Meles meles*), increases in density can lead to more aggressive encounters (Macdonald et al., 2004). Structural changes to the complexity of the environment of sleepy lizards (*Tiliqua rugosa*) result in greater social connectivity and stability, but also in a higher level of aggressive interactions (Leu et al., 2016). Without knowing how individuals are socially tied we can never accurately predict what the consequences will be when we substantially change those ties by changing the habitat. This is especially relevant for highly threatened animal populations. When the social structure is quantified, for example via radio tracking (but see **Chapter 2** for specific considerations), we can model how global properties of the social structure change when certain links are physically broken or when the physical structure of the habitat is otherwise altered (Leu et al., 2016). Moreover, social network analysis can also be relevant from the opposite perspective, for example by identifying important bottlenecks in social connectivity and design management projects such as ecological corridors accordingly (Debinski & Holt, 2000). In reintroduction programs individuals are often radio-collared, making tracking the social connectivity between individuals or groups of individuals living in fragmented landscapes feasible. Practical examples of studies in which this approach would fit are the conservation projects for golden lion tamarins (*Leontopithecus rosalia*) (Bales et al., 2006) and the Californian condors (*Gymnogyps californianus*) (Hunt et al., 2007).

(2) Anthropogenic noise

Communication networks can strongly influence the proximity networks of animals and hence disturbances in the communication network may lead to significant perturbations in the proximity network. Animal populations encounter many anthropogenic disturbances that could disrupt social connectivity and stability. In addition to causing stress, anthropogenic noise can impact animal behaviour by acoustic masking, which reduces effective communication (Brumm & Slabbekoorn, 2005; Wright et al., 2007; Slabbekoorn & Ripmeester, 2008) or eavesdropping (Jones, 2008). Some animals seem to adapt to the background noise by changing their vocal behaviour (Slabbekoorn & Peet, 2003; Foote et al., 2004; Francis et al., 2010; Arroyo-Solís et al., 2013; Gil et al., 2015), but this can also lead to the loss of signal specific functions (Halfwerk et al., 2011). If masking cannot be avoided it can lead to severe problems, for example in wide ranging animals, such as marine mammals, that rely on communication to exchange information, stay connected to

family and to attract potential mates (Wright et al., 2007). By comparative analysis of social networks of populations with and without certain forms of acoustic disturbance, we can gain relevant insights into how anthropogenic noise influences the social dynamics of specific species. Furthermore, experiments introducing noise and monitoring the social responses can be crucial for understanding noise effects. For example, effects of noise on provisioning behaviour in great tits were revealed to depend on the personality of the parents and not on the masking of communication (Naguib et al., 2013). Understanding the mechanisms of increasing anthropogenic impacts on animal behaviour contributes to the development of mitigation measures to reduce or prevent such negative effects.

(3) Selective removal

Lastly, the removal of specific individuals from the wild, for human consumption, entertainment or wildlife trafficking, has the potential to destabilize entire social groups (Flack et al., 2006; Williams & Lusseau, 2006; Wey et al., 2008) and potentially populations. A 'knock-out' experiment in which certain pigtailed macaques (*Macaca nemestrina*) were taken away from their (captive) group, based on their policing function, showed that removal of key individuals can have important consequences for the stability in social groups (Flack et al., 2006). Selective removal of specific individuals or locations that facilitate social association can also be simulated to generate predictions about the effects of real-life removal (Williams & Lusseau, 2006; Silvis et al., 2014), provided the social network of wild populations is accurately quantified. When the social network of wild populations is accurately quantified, the simulated removal of specific individuals or locations that facilitate social association can be used to generate predictions about the effects of real-life removal. For example, the social network of North-eastern Pacific killer-whales appeared to be robust to random removals, but during simulations that mimicked historic live-captures the network was likely to break into isolated parts. Not only the effects of removing individuals can be experimentally and virtually simulated, also the potential effects of introducing new animals can be studied (Jacoby et al., 2010; Ilany et al., 2013).

Insights into the social connectivity of animal populations can thus be vital for predicting how resilient populations are to disturbances (Lusseau, 2003; Williams & Lusseau, 2006; Jacoby et al., 2010; Firth & Sheldon, 2015). Social network analyses can help to understand how social fragmentation and instability can be prevented, but possibly also how social stability could be restored.

Conclusion

To conclude, in this thesis I showed the importance of personality in the social networks of great tits. Additionally I investigated and reviewed the relevance of communication for social networks. Social connectivity is vital for individuals as well as populations. My findings strongly suggest that individual personalities should be taken into account when studying, and especially when changing, the social dynamics of animal populations.



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Summary

Many animals are social and **social connectivity**, how an individual is directly and indirectly connected to conspecifics, regularly plays a key role in animal societies. Not only the direct social bonds between individuals are important, also the position an individual animal has in the **social network** can be critical. This social network position determines social connectivity, which can influence the likelihood of an individual finding new food, acquiring novel foraging techniques, rising in social status, acquiring a mate, but also vulnerability to diseases and parasites. Having many social interactions thus not only has **benefits**, it also has **costs**. Next to exposure to diseases, social connectivity can make individuals more vulnerable to social stress and increase the likelihood to receive aggression from conspecifics.

Animals in groups and populations do not interact at random; some individuals have significant more social interactions than others. A potential explanation for this is that not all individuals weigh the cost and benefits of social interactions in the same way. How animals weigh and experience the benefits of social interactions is likely modulated via **personality differences**, for example the access to social information compared to the level of social stress. If this is true, personality will play a significant role in social networks. In this thesis I therefore examined the role of personality in the social networks of the **great tit** (*Parus major*), a **territorial songbird**. The territorial great tit was a good study system for this research for three reasons. First, in a territorial system, the costs and benefits of social interactions are more pronounced than in group living animals. Second, the influence of personality traits on great tit ecology is well known. Third, to socially connect great tits do not only approach conspecifics, they also sing, providing me with the opportunity to study how social connectivity is modulated via both **proximity networks** as well as **communication networks**.

The big challenge in quantifying animal social networks is following multiple individuals simultaneously; something which is crucial for determining a social network structure. **New technologies** allow us to study social networks of animals in more detail than ever before. Thus to study the proximity networks of territorial great tits, I used an automatic tracking system: *Encounternet*. *Encounternet* has two exceptional characteristics: this system can automatically track many individuals at the same time and it can be used to track small songbirds. However, giving a small bird a radio-transmitter to carry around for several days or weeks is not trivial. In **Chapter 2** I therefore analysed the potential consequences of this technique for the life-histories of great tits. In this chapter I conclude that especially the context and

timing of attaching a transmitter are important for whether to expect any negative consequences. There was no apparent effect on survival, but in some years there were negative effects on breeding success (there were no negative transmitter-effects observed for the years presented in **Chapter 3** and **Chapter 8**). I speculate that the presence of already existing stressors, such as cold and a large number of chicks, is the main trigger that influences negative **effects of transmitter attachment** on breeding success in great tits. These findings are important to put results obtained with certain tracking technologies into perspective.

In **Chapter 3** I revealed that **exploratory behaviour**, an established personality trait in great tits, indeed predicts social network position; slow explorers occupy the least **central network positions**. Exploratory behaviour relates to aggression and the experience of social stress. Fast explorers, that are commonly more aggressive and risk-taking, are probably less inhibited to approach other males. Slower explorers, in contrast, are known to be more vulnerable to social stress. This could make slower males more reluctant than faster males to seek out male-to-male encounters. However, there is a disadvantage of studying encounters via spatial associations: it is impossible to conclude who initiated the association. In **Chapter 4** I therefore examined if individual traits, like exploratory behaviour, boldness and dominance, influenced the preference for social associations. I used video-playback stimuli, so that an encounter could only take place when a subject initiated it him or herself. I actually discovered that exploratory behaviour did not influence **social preference**, the frequency and duration of time spent near a conspecific (video). Surprisingly, when they saw the stimulus bird for the first time, shy individuals showed a stronger social preference compared to bold individuals. And birds with a low dominance rank continuously showed the highest social preference, also when the dominance ranks of individuals were changed following group rearrangements. By using video playback stimuli, there was no opportunity for aggressive interactions and **shyer and low ranking individuals** might have recognized these safe circumstances quickly. Since there was no chance for fights, the balance for these birds between the potential benefits of staying close to an unfamiliar conspecific (safety in numbers) and the potential costs (social stress) could have shifted compared to real-life confrontations. Combining the results from **Chapter 4** with the results of **Chapter 3** shows that we should not be too eager to draw general causal conclusions on the drivers of social behaviour. Social associations in **Chapter 3** were riskier to initiate than in **Chapter 4**, which might have shifted the cost/benefit ratio for the birds and thus the relationship between personality and social preference.

When spatial associations are risky, individuals could use communication instead. Great tits use song to attract females and to advertise their territories, keeping male rivals at a distance. Proximity networks are thus expected to partly vary with communication networks. Hence, **Chapter 5** examined if, similar to the proximity networks in **Chapter 3**, personality has a significant influence on the communication networks. There was no effect of exploratory behaviour on overall singing activity, but faster explorers, more than slow explorers, increased their singing activity during the main periods of fertility and maternal investment (egg laying and incubation). Personality thus might relate to how great tit males communicate, although the effects seem more subtle in communication networks than in proximity networks. Also in **Chapter 6** I discovered that differences in how individuals communicate can be very subtle. **Dawn song rate**, a repeatable trait in great tits, varied with the social structure of the territorial population. Males that shared territory boundaries differed more in song rate than males that did not share a boundary, while spatial proximity did not have a significant effect on song rate. Male great tits might slightly adjust their singing behaviour during **territory settlement** to avoid neighbour conflicts or to differentiate themselves better. The difference in dawn song rate could also be the consequence of non-random territory-settlement driven by individual characteristics that correlate with certain song traits. However, age, condition and exploratory behaviour did not differ between the neighbours.

That exploratory behaviour in the wild is a more straight-forward predictor for spatial behaviour than for communication is strengthened in **Chapter 7**. Exploratory behaviour did not predict dawn song rate or the start time of dawn song, but it did predict the spatial **approach of simulated intruders**. In accordance to a previous study, faster explorers spent more time close to the simulated intruder. But males that spent the least amount of time close to the intruder sang with the highest song rate during dawn. Dawn is the main territory advertisement period for great tits, thus males that are more inhibited to approach male rivals, commonly slow explorers, might defend their territories more fiercely via the dawn song. Together these chapters thus strongly suggest that proximity networks and communication network in wild territorial great tits are linked. Yet, if changes in one network can actual change dynamics in the other remains unknown. In **Chapter 8** I therefore introduced a perturbation into the communication network, by simulating territory intrusions, and monitored the subsequent changes in the number of **spatial associations** for the intruded males. Great tits that decreased in the number of male associates also associated with them for shorter periods. The

vocal response of the intruded male significantly influenced whether neighbours would come close to the intrusion site. Yet, in contrast to previous studies and **Chapter 7**, there was no relation between the exploratory behaviour of the intruded male and his vocal response. Vocal response and exploratory behaviour did not significantly predict changes in the number and duration of spatial associations, although there was a tendency for slower males to increase in the number of neighbouring male associates while faster males tended to increase in neighbouring female associates. The findings in this thesis suggest that communication and social networks are closely linked, but that effects of personality on communication and proximity networks can be context-dependent. In the general discussion of this thesis (**Chapter 9**) I examined the role of personality in social networks in more detail. Also I discussed the various ways spatial and communication networks can be linked in more depth. Not only in song birds, but also in a variety of other animal species. It is especially relevant to realize that **perturbations** to the communication network, for example via anthropogenic noise, could have significant consequences for the social connectivity of animals.

To conclude, in this thesis I revealed the role of personality in the social networks of great tits. Additionally I investigated and reviewed the relevance of communication for social networks. Many animals are socially connected. My findings suggest that individual personalities should be taken into account when studying, and especially when changing, the social dynamics of animals, both in the wild and in captivity. **There can be consequences for the social network in its entirety when the smallest components are changed.**

*Nature is to be found in her entirety nowhere more
than in her smallest creatures.*

Plinius

Samenvatting

Veel dieren zijn sociaal en **sociale verbondenheid**, hoe een individu direct en indirect met soortgenoten is verbonden, speelt vaak een belangrijke rol in hun populaties. Niet alleen directe sociale banden tussen individuen zijn relevant, ook de positie die een dier inneemt in **het sociale netwerk** kan van belang zijn. Deze sociale netwerk positie beïnvloedt het vinden van nieuwe voedselbronnen, technieken om voedsel te verkrijgen, het verkrijgen van sociale status en het vinden van een partner, maar het beïnvloedt ook de kwetsbaarheid voor besmettelijke ziektes en parasieten. Veel sociale contacten hebben, heeft dus niet alleen voordelen maar ook nadelen. Naast kwetsbaarheid voor ziektes, kunnen sociale contacten ook stress en agressie opleveren.

Dieren in groepen hebben niet zomaar willekeurig contact met elkaar, sommige dieren hebben vaker sociale interacties dan andere dieren. Een mogelijke verklaring hiervoor is dat niet alle individuen de **kosten en baten** van sociale interacties op dezelfde manier afwegen. Hoe individuen deze sociale kosten en baten afwegen wordt waarschijnlijk beïnvloedt door hun **persoonlijkheid**, bijvoorbeeld de baten van sociale informatie tegenover de kosten van stress en agressie. Als dit inderdaad zo is dan zal persoonlijkheid een significante rol spelen in de vorming van sociale netwerken. Daarom heb ik in dit proefschrift de rol van persoonlijkheid in de sociale netwerken van **koolmezen** (*Parus major*), **territoriale zangvogels**, onderzocht. De territoriale koolmees was een geschikt studie model voor drie redenen. Ten eerste zijn de kosten van sociale interacties in territoriale systemen vaak duidelijker dan in groepsdieren. Ten tweede is er veel informatie beschikbaar over de rol van persoonlijkheid in het leven van de koolmees. Ten derde interacteren koolmezen niet alleen door elkaar op te zoeken maar ook via zang. Op deze manier kon ik dus goed onderzoeken hoe sociale verbondenheid zowel door **ruimtelijke netwerken** maar ook door **communicatie netwerken** wordt verkregen.

De grote uitdaging in het bestuderen van sociale netwerken in dieren is het volgen van meerdere dieren tegelijkertijd. Dit is echter wel essentieel voor het bepalen van de netwerkstructuur. Recente **technologische ontwikkelingen** hebben het mogelijk gemaakt om meerdere dieren continue en tegelijkertijd te bestuderen. Om de ruimtelijke netwerken van territoriale koolmezen te bestuderen heb ik daarom dan ook gebruik gemaakt van een nieuw automatisch zendersysteem: *Encounternet*. *Encounternet* heeft twee uitzonderlijke eigenschappen: het systeem kan meerdere individuen tegelijkertijd volgen en het kan gebruikt worden voor de

studie van kleine zangvogels. Maar om een kleine zangvogel een zendertje te geven dat het meerdere dagen mee zal dragen is niet zomaar iets. In **Hoofdstuk 2** heb ik daarom geanalyseerd of er mogelijke negatieve gevolgen waren van deze methode voor de vogels. In dit hoofdstuk concludeer ik dat voornamelijk de context en de timing van het zenderen belangrijk zijn in het voorspellen van effecten. Ik vond geen effect van zenderen op de kans om een vogel terug te vangen, maar in sommige jaren was er wel een effect op het broedsucces (ik heb geen negatieve effecten gevonden in de jaren beschreven in **Hoofdstuk 3** en **Hoofdstuk 8**). Ik speculeer dat de aanwezigheid van al bestaande stressoren, zoals een lage omgevingstemperatuur en een groot aantal kuikens om te voeden, de grootste factors zijn in het bepalen van **zender-effecten** in koolmezen. Deze bevindingen zijn belangrijk om uitkomsten van sommige type zenderonderzoeken in perspectief te kunnen plaatsen.

In **Hoofdstuk 3** onthul ik dat **exploratiedrag**, een bekend persoonlijkheidskenmerk in koolmezen, inderdaad de positie in een sociaal netwerk voorspelt: dieren met een lage exploratie score hebben de minst **centrale netwerk posities**. Exploratiedrag correleert met agressie en het ondervinden van sociale stress. Dieren met een hoge exploratiescore zijn vaak agressiever en nemen meer risico's en zijn daarom mogelijk minder terughoudend in het benaderen van mannelijke soortgenoten. Dieren met een lage score zijn echter vaak gevoelig voor sociale stress, wat ze juist weer meer terughoudend kan maken om associaties aan te gaan met mannelijke soortgenoten. Maar het probleem met ruimtelijke associaties is dat je als onderzoeker niet weet wie van de twee de associatie is begonnen. Dus of vogels met een hogere exploratiescore ook werkelijk een sterkere voorkeur hebben om associaties met soortgenoten aan te gaan of dat ze veel andere vogels aantrekken. Daarom heb ik in **Hoofdstuk 4** onderzocht of individuele kenmerken, zoals exploratiedrag, durf en dominantie de voorkeur voor sociale associaties beïnvloedt. Ik maakte gebruik van stimulus vogels op video, zodat een associatie alleen plaats kon vinden als de vogel waarin ik geïnteresseerd was de associatie zelf initieerde. Ik kwam er achter dat vogels met een hogere exploratiescore geen sterkere **sociale voorkeur** hadden, met sociale voorkeur gedefinieerd als: het aantal keer en de tijd die ze dichtbij een soortgenoot (op video) doorbrachten. Wanneer ze de stimulus vogel voor het eerst zagen, lieten verrassend genoeg de schuwere vogels een sterkste sociale voorkeur zien dan brutalere vogels. En dieren met een lage dominantie rang lieten in alle gevallen de sterkste sociale voorkeur zien, ook nadat de dominantie rangen waren veranderd als gevolg van groepswissels. Doordat ik video's gebruikte, was er vanzelfsprekend

geen kans op agressieve interacties en deze veilige omstandigheden hebben **schuwe en laag-gerankte individuen** misschien heel snel doorgehad. Omdat er geen kans was op gevechten, is de balans tussen de voordelen van sociale associaties (veiligheid voor mogelijke roofdieren) en de nadelen (stress) mogelijk verschoven vergeleken met situaties in het wild. Wanneer ik de resultaten van **Hoofdstuk 4** combineer met de resultaten van **Hoofdstuk 3** laat dit zien dat we niet te snel conclusies moeten trekken over welke factoren sociaal gedrag beïnvloeden en in welke richting. Sociale associaties in **Hoofdstuk 3** waren risicovoller om aan te gaan dan in **Hoofdstuk 4**, dit heeft mogelijk de kosten/baten verhouding verschoven en dus mogelijk ook de relatie tussen persoonlijkheid en sociale voorkeur.

Wanneer sociale associaties risicovol zijn, kunnen individuen ook communicatie gebruiken. Koolmezen gebruiken zang om vrouwtjes aan te trekken en hun territorium te verdedigen, door rivalen op afstand te houden. Ruimtelijke netwerken zouden dus gedeeltelijk kunnen variëren met communicatie netwerken. Daarom is in Hoofdstuk 5 onderzocht of, net zoals in de ruimtelijke netwerken van **Hoofdstuk 3**, persoonlijkheid een bepalende invloed heeft op communicatie netwerken. Er was geen effect van exploratiegedrag op de mannelijke zangactiviteit in het algemeen, maar vogels met een hogere exploratiescore verhoogde hun **zangactiviteit** meer tijdens de vruchtbare periode van hun vrouwtje en tijdens incubatie van de eieren dan vogels met een lagere score. Persoonlijkheid kan dus mogelijk beïnvloeden hoe koolmeesmannen communiceren, hoewel de effecten wat subtieler lijken te zijn in communicatie netwerken dan in ruimtelijke netwerken. Ook in **Hoofdstuk 6** kwam ik erachter dat verschillen in hoe individuen communiceren heel subtiel kunnen zijn. Hoeveel liedjes een koolmees per minuut zingt in de vroege ochtend, een voorspelbaar gedrag, varieerde met de sociale structuur van de territoriale populatie. Mannen die een territoriumgrens deelden verschilden meer in het aantal liedjes dat ze per minuut zongen dan mannen die geen grens deelden, onafhankelijk van de absolute afstand tussen de territoria. Koolmeesmannen proberen mogelijk hun zanggedrag enigszins aan te passen wanneer ze zich **vestigen in hun territorium** om zo conflicten te vermijden of om zichzelf beter herkenbaar te maken. Het verschil in zanggedrag kan ook veroorzaakt zijn doordat koolmezen zich niet willekeurig vestigen in hun territoria maar dat deze vestiging wordt bepaald door bepaalde eigenschappen die ook met zang correleren. Leeftijd, lichaamsconditie en exploratiegedrag verschilden in ieder geval niet tussen de burens.

Dat exploratiegedrag in het wild een duidelijkere voorspeller is voor ruimtelijk sociaal gedrag dan voor communicatiegedrag wordt nog eens bevestigd in **Hoofdstuk 7**. Exploratiedrag voorspelde niet het aantal gezongen liedjes per minuut en ook niet de starttijd van zingen in de vroege ochtend, maar het voorspelde wel hoe dicht een koolmeesman **een gesimuleerde indringer benaderde**. In overeenstemming met een eerdere studie, besteedde mannen met een hogere exploratiescore meer tijd in de nabijheid van de indringer. Maar mannen die de minste tijd doorbrachten in de buurt van een indringer zongen het actiefst in de vroege ochtend. De vroege ochtend is de voornaamste periode voor koolmeesmannen om hun territoriumbezit te verkondigen, dus mannen die minder geneigd zijn mannelijke koolmezen fysiek weg te jagen, vaak mannen met een lage exploratiescore, verdedigen mogelijk hun territorium daarom extra actief via zang in de ochtend. Gezamenlijk suggereren deze hoofdstukken dus dat er inderdaad een link is tussen ruimtelijke netwerken en communicatienetwerken in territoriale koolmezen. Maar of veranderingen in één netwerk ook de dynamiek in het andere netwerk kan beïnvloeden is niet zeker. Daarom heb ik in Hoofdstuk 8 verstoringen in het communicatie netwerk geïntroduceerd, wederom door het indringen van een onbekende koolmeesman in het territorium te simuleren, en heb ik vervolgens geobserveerd of er verandering optraden in het aantal **ruimtelijke associaties** tussen de koolmezen. Koolmezen die minder ruimtelijke associaties aangingen brachten ook minder tijd door in de associaties die ze nog wel hadden. De vocale reactie (zang) van de koolmeesman op de indringer beïnvloedden of vrouwelijke burens dichtbij de locatie kwamen waar de indringer was geweest en of mannen verder weg bleven. Maar in tegenstelling tot een eerdere studie en mijn bevindingen in **Hoofdstuk 7** was er nu geen relatie met de persoonlijkheid van de koolmees en zijn vocale reactie op de indringer. De vocale reactie en het exploratiegedrag voorspelden niet of er veranderingen optraden in het ruimtelijke netwerk. Hoewel er de neiging was voor koolmeesmannen met een lagere exploratiescore om, na de verstoring, meer ruimtelijke associaties met mannelijke soortgenoten te hebben terwijl mannen met een hogere score juist meer associaties hadden met vrouwelijke soortgenoten. De bevindingen in mijn thesis suggereren dus dat communicatie en ruimtelijke netwerken met elkaar verbonden zijn, maar dat de mogelijke effecten van persoonlijkheid op deze netwerken afhangen van de specifieke context. In de algemene discussie van mijn thesis (**Hoofdstuk 9**) heb ik de rol van persoonlijkheid in sociale netwerken in meer detail onderzocht. Ook heb ik in het laatste hoofdstuk meerdere manieren besproken hoe ruimtelijke netwerken en communicatie netwerken verbonden kunnen zijn, niet alleen in zangvogels maar ook in andere diersoorten. Het is naar mijn mening

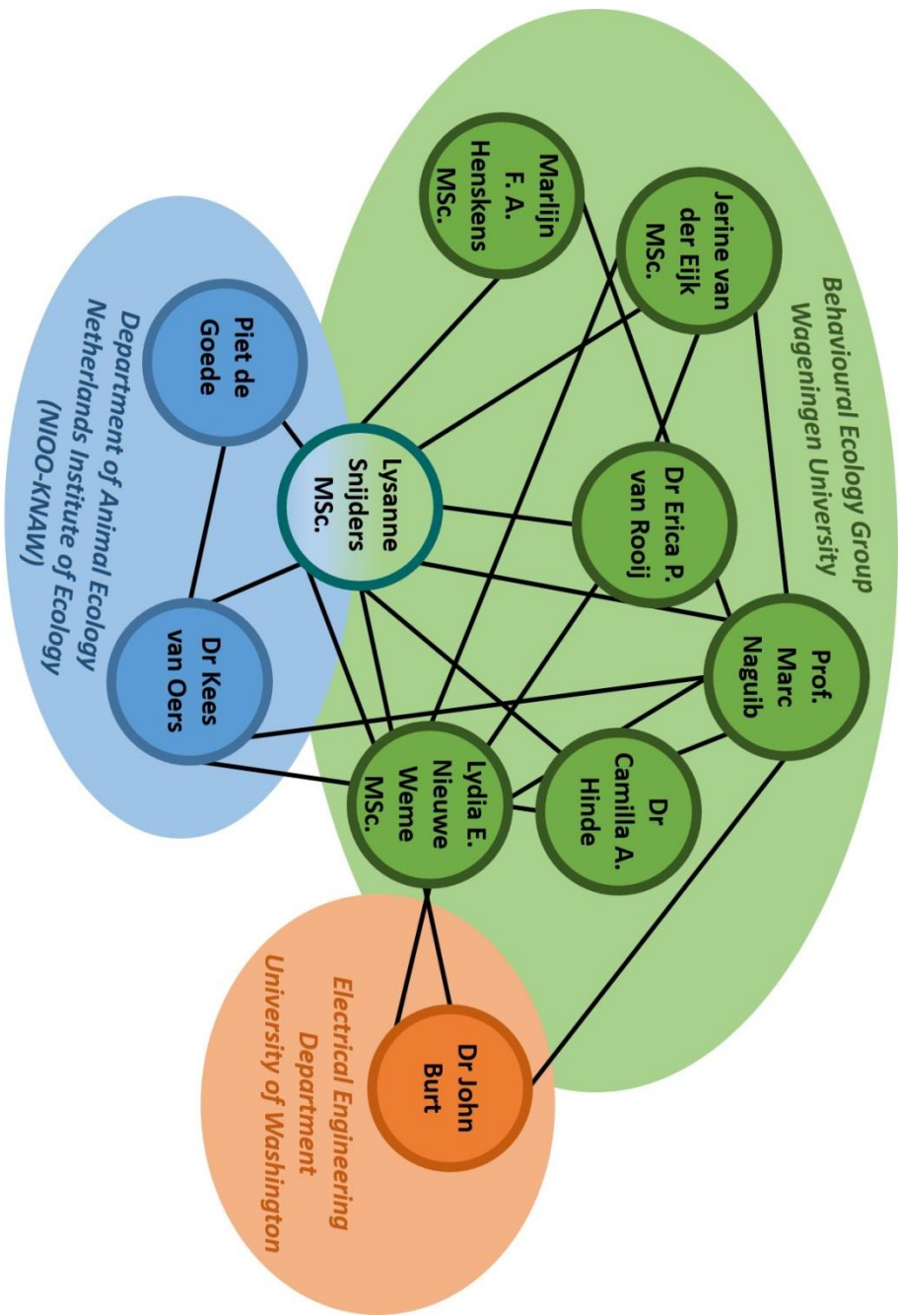
vooral belangrijk om te realiseren dat verstoringen in het communicatie netwerk, bijvoorbeeld door geluidsoverlast veroorzaakt door machines, aanzienlijke gevolgen kan hebben voor de sociale verbondenheid binnen populaties.

Ter samenvatting, in mijn thesis heb ik een tipje van de sluier opgelicht over de rol van persoonlijkheid in de sociale netwerken van koolmezen. Daarnaast heb ik de relevantie van communicatie in sociale netwerken onderzocht. Veel dieren zijn sociaal verbonden met elkaar. Mijn bevindingen suggereren dat er rekening gehouden moet worden met de persoonlijkheid van individuele dieren wanneer hun sociale dynamiek wordt onderzocht of wordt veranderd, zowel in het wild als in gevangenschap. Er kunnen namelijk gevolgen zijn voor het sociale netwerk in het geheel wanneer de kleinste componenten worden veranderd.

*De natuur is in haar geheel nergens beter te vinden dan
in haar kleinste deeltjes.*

Plinius

Affiliation coauthors



Curriculum Vitae of Lysanne Snijders

Already from a young age, Lysanne knew she wanted to do something with animal behaviour. She started her academic path with the Bachelor Biology at the University of Utrecht (2005), including a Minor in Organization and Governance. In the second year of her Bachelor she became fascinated with the field of Behavioural Ecology and decided that this was the direction she wanted to further develop herself in.



Consequently, she continued with a Master Biology (2009), specialization Ecology, at Wageningen University. Lysanne started a Master thesis with the Resource Ecology Group studying parental care in wild barnacle geese (*Branta leucopsis*), supervised by Dr Rudy Jonker. For this thesis she was awarded the Alfred Russel Wallace award for the best Master Thesis of the Resource Ecology Group. During her Master she gained some working experience by working part-time for a couple of months with De Natuurkalender, supervised by Dr Arnold van Vliet, writing and editing small news articles about current phenological events in nature (2009-2010). Additionally she spent three months coordinating a nature education project in the Phillipines with the Centre for Sustainability, supervised by Kyra Hoevenaars MSc (2010). Lysanne finalized her Master Biology with a second Master Thesis with the Centre for Wildlife Ecology of the Simon Frasier University in Vancouver, Canada, supervised by Dr Holly Middleton and Prof. Ron Ydenberg (2010-2011). Here she studied the behavioural responses of dabbling ducks to anthropogenic disturbances, including space use and vigilance behaviour.

Shortly after finishing her Master Biology she started her PhD project with the Behavioural Ecology Group of Wageningen University and the Department of Animal Ecology of the Netherlands Institute of Ecology (NIOO-KNAW), supervised by Prof. Marc Naguib and Dr Kees van Oers (2012-2016). While studying the role of personality in the social networks of great tits, she spent one month at the University of Wyoming, USA, supervised by Prof. David McDonald (2015).

Additionally, Lysanne cooperated in a research project studying collective decision making in human groups and a study researching social networks and social foraging in Trinidadian guppies with the department Ecology of Fishes (Prof. Jens Krause) of the Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin, Germany. For the year 2016, Lysanne, together with Prof. Marc Naguib and Dr James Savage, was awarded a Wageningen University Innovation Grant to develop knowledge clips and online assessment tools for the Animal Behaviour Bachelor course of the Behavioural Ecology Group. She will spent the spring and summer of 2016 putting this grant into practice.

Lysanne now wishes to continue her research career, moving into the direction of wildlife conservation and is currently writing a personal grant with PD Dr Christian C. Voigt of the Department of Evolutionary Ecology at the Leibniz Institute for Zoo and Wildlife Research (IZW) in Berlin, Germany. Finally, she aims to further develop her skills in popular science writing and video-making and so share the wonders of nature with the general public.

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A project can never run well without reliable technical support. **Lydia**, thank you for all the help, orders, student supervisions and more. Together we must have seen more batteries than one wishes to see in a life time. I started in our chairgroup a little bit before you, but I can not imagine how it would be without you anymore. I love hearing your stories about Fay, Bullit and your clicker training experiences. You are the glue that makes our chairgroup a group.

Many, many people have helped me in the field and especially the third year I was lucky enough to have two fun and reliable guys to work with. **Rick**, your enthusiasm and drive for efficiency as a goal on its own made you the perfect field assistant. Research stations don't know what they are missing not having you as

their field technician. **Davide**, you have been a great support during my last field year. I hope that your PhD project with Encounternet will work out for the best. With your determination I have no doubt you will make your project a success.

Most people who helped me out in the field and/or analysing the first data were master students. Thank you: **Kim, Marlijn, Diego, Thomas, Ellis, Ramona, Joris, Jerine and Lusanne**. Sharing this project with all of you, working in a team, was a wonderful experience for me. Good, bad, funny and sad field experiences only really mean something when you can share them with others.

When I started my PhD journey I did not start alone. **Maaïke**, time goes sooo fast. I am grateful that we could share our PhD experiences together. The likes and the dislikes, all the different PhD-rooms and most importantly, the parties! You are an open and loving person and I love that about you. I wish I had your courage to take risks and follow the heart. That is why I am superhappy with you as my paranimph.

Stijn, I am very grateful to have you as my friend. Whenever I am negative about something, you show me, without judgement, the positive sides of things. You can be caring and objective at the same time, which makes you a great scientist, journalist and most of all, a great friend. Knowing what you do not know, being uncertain, is a valuable thing and thanks to you I am starting to see that now. I hope we can keep discussing all of our unknowns still many times in the future. Thank you for being my paranimph and my friend.

During my time as PhD-student with the Behavioural Ecology Group, the group has grown in a fun and enthusiastic bunch of people. Watching movies, quizing having drinks and dinner and being locked up in a prison cell together all have been great fun. **Lies**, it was great to have another PhD-student at the Behavioural Ecology Group to discuss BHE, great tit and PhD things with. I wish I had some of your calmness, determination and thoroughness. **James**, thank you so much for all your wonderfull copy-editting of my manuscripts and of course for your enthusiasm for science-education. I am looking forward to making many more knowledge clips with you. There is always at least one light shining in our group and that is **Nienke**. Thank you for being such a positive and supportive secretary. Also, I would like to thank **Jerine and Nina** for all the fun activities and the amusing coffee breaks. Being able to relax and have fun are essential parts of a good working environment. Thanks to **Bas, Bonne, Joanne and Jutta** for making our groups such an interesting

mix of fundamental and applied science. And lastly, thank you **Camilla**, for helping me make my first manuscript on social networks a success.

I was lucky enough to work at two inspiring places at the same time, the Wageningen University and the Netherlands Institute for Ecology. This way I got to meet many wonderful people and have some fantastic opportunities. Thanks to all the AnE PhDs for making the NIOO such a fun place to work: **Lucia, Barbara, Thomas, Davide, Jip, Irene, Rascha, Els**. And of course thanks to all the other AnE colleagues, especially Marcel for letting me hang around. Thanks to the **Beleef de Lente team**: Lies and **Peter** and the **Dokter Ecologie team**: Stijn, **Sven, Mandy, Rascha** and of course **Froukje**! These were great ways for me to practice science communication.

Mandy and Sven, thanks for all the ferret (Simon) sitting when I was away for work or on holidays. I also very much enjoyed our beers, animal talks and the fun games of squash together. Lots of fun times at the NIOO were made possible by the **Party Planners**, such as the colloquium disco, bbq, Christmas dinner and the Prosecco party. It was fantastic to be a part of all that! Finally, I want to thank **Marylou** for being the most inspiring person I know. Your love for all things great and small and your endless positivity are truly amazing!!

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Lieve **Ralf**, dank je voor al je steun, geduld en liefde. Jij maakt dat ik een betere versie van mijzelf kan en wil zijn. Je mocht me niet vaak helpen omdat ik het zelf wilde doen. Maar simpelweg door mijn voorbeeld te zijn heb je me meer geholpen dan je ooit zult weten.

Kunst

Wat we willen:

Momenten

Van helderheid

Of beter nog: van grote

Klaarheid

Schaars zijn die momenten

En ook nog goed verborgen

Zoeken heeft dus

Nauwelijks zin, maar

Vinden wel

De kunst is zo te leven

Dat het je overkomt

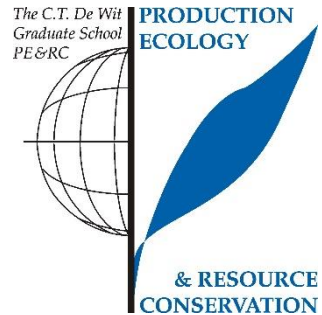
Die klaarheid, af en toe.

Martin Bril

Als laatste dan toch een beestje. Simon, wanneer ik mezelf te veel liet meeslependoor de vele ups en downs van dit project kon ik altijd mezelf terugvinden bij jou. Nu zal jij altijd terug te vinden zijn in mij.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



Review of literature (4.5 ECTS)

- The social network signals (2012-2013)

Post-graduate courses (3.5 ECTS)

- Social network workshop; ZIFF (2012)
- Introduction to R; PE&RC (2012)
- Animal movement analysis; CSE-UVA / IBED (2015)

Laboratory training and work visits (6.0 ECTS)

- Personality and social networks; IGB, Germany (2013)
- Animal tracking; University of Colorado, USA (2015)
- Social network theory and analysis; University of Wyoming, USA (2015)
- Personality and spatial tracking in bats and birds; IZW, Germany (2015)

Invited review of (unpublished) journal manuscript (11 ECTS)

- Behaviour: aggressive signalling in songbirds (2012)
- Ethology: personality in zebra finches (2013)
- Animal Behaviour: social networks in water dragons (2014)
- Animal Behaviour: familiarity in cooperative bird species (2014)
- Journal of Ornithology: information in alarm calls barn swallows (2015)
- PLoS ONE: calibration of proximity tags (2015)
- Journal of Ornithology: bird song at night (2015)
- Animal Behaviour: personality in squirrels (2015)
- Behavioral Ecology and Sociobiology: hissing as personality in great tits (2015)
- Animal Behaviour: social context and boldness sticklebacks (2015)
- Behaviour: familiarity in Iberian magpies (2015)

Competence strengthening / skills courses (10.3 ECTS)

- Animal ethics; KNAW (2012)
- PhD Competence assessment; WGS (2012)
- High impact writing; WIAS (2012)
- Stress identification and management; WGS (2014)

- Presenting with impact; WGS (2015)
- Writing grant proposals; WGS (2015)
- PhD Carrousel; PE&RC and other graduate schools (2014, 2015)
- Masterclass personality; IBL/NVG (2015)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.4 ECTS)

- PE&RC Introduction weekend (2012)
- PE&RC Mid-term weekend (2014)
- PE&RC Final weekend (2015)
- PE&RC Day (2015)

Discussion groups / local seminars / other scientific meetings (23.1 ECTS)

- NAEM; 1 oral presentation (2012-2014)
- NVG Meeting; poster and 2 oral presentations (2012-2015)
- WEES seminars (2012-2015)
- AnE and NIOO seminars; 1 oral presentation (2012-2015)
- WEES; organising member (2012-2016)
- NIOO-AnE Journal club (2012-2016)
- NIOO Days; 1 poster presentation (2013, 2015)
- REG PhD Discussion group (2014)
- BHE and CAWA seminars; 2 oral presentations (2015)
- Wageningen PhD day; oral presentation (2015)
- Local seminar; oral presentation; University Colorado (2015)
- Local seminar IZW; oral presentation; Berlin (2015)

International symposia, workshops and conferences (12.2. ECTS)

- Behaviour; 2 oral presentations; New castle & Cairns (2013,2015)
- ISBE; oral presentation; New York (2014)
- ASAB Winter meeting; poster presentation; London (2014)
- Meeting of the Ethologische Gesellschaft; poster presentation and oral presentation; Bielefeld & Hamburg (2013, 2015)
- Behaviour, Physiology and Genetics of Wildlife; oral presentation; Berlin (2015)

Lecturing / supervision of practical's / tutorials (2.4 ECTS)

- Ecology of animal life histories (2012, 2014)
- Behavioural ecology (2012, 2013, 2015)
- Animal behaviour (2015)

Supervision of MSc students

- Social networks (4 students)
- Territorial defense (3 students)
- Dawn song traits (2 students)
- Extra pair paternity (1 student)

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