THE FUNCTION OF DUETTING IN THE CRIMSON-BREASTED SHRIKE (*LANIARIUS ATROCOCCINEUS*)

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Dipl.-Biol. Irene M. van den Heuvel
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in Itzehoe

Gutachter: Prof. Dr. Georg M. Klump

Zweitgutachter: Prof. Dr. Peter H. Becker

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Summary

Avian vocal duets are complex acoustic displays produced by mated pairs with a striking degree of temporal precision. Duets have evolved independently several times; nonetheless is it a rare phenomenon and occurs in less than 5% of all described species. Duetting species are extremely diverse and include both non-passerine and passerine species. Both songs and calls are integrated into duets and the behaviour occurs worldwide, although the majority of duetters belong to tropical and subtropical species.

Hypotheses that have been brought forward to explain the function of duets include the mate guarding, paternity guarding, territorial defence, mutual recognition, pair bond maintenance, and signalling commitment hypothesis. Chapter 1 describes these hypotheses in more detail. Despite the existence of these hypotheses, the functional significance of these elaborate displays remains unclear. Their function can differ between the different species, and even within one species duets can be multi-functional. The aim of this thesis is to contribute to the general understanding of avian duetting and its ecological and social significance. For this purpose a free living population of Crimson-breasted Shrikes (*Laniarius atrococcineus*) has been studied over three consecutive years and the above mentioned major hypotheses for the function of their duets have been investigated.

Chapter 2 describes the singing behaviour and song repertoire of the Crimson-breasted Shrike and assesses four main hypotheses proposed for the function of duets. These involve the acoustic mate guarding, mutual recognition, pair bond maintenance, and joint territory defence hypothesis. The results show that duetting is most likely a cooperative endeavour in this species serving as mutual recognition but probably not used for pair bond maintenance. Observations suggest that duetting is not essential for territorial defence. Crimson-breasted Shrikes have a sex-specific song repertoire which is a precondition for duets to function in acoustic mate guarding.

The experiments described in chapter 3 were conducted to investigate the territorial defence and the acoustic mate guarding hypothesis in more detail. To discriminate between these two explanations for duetting, playback experiments were conducted on a wild Crimson-breasted Shrike population. Results indicate that females may use duets for acoustic mate guarding as well as to assist their males in territorial defence against intruding pairs. Males also practise acoustic mate guarding using solo songs. Territorial defence is probably achieved by singing in duet with the female and through elevated jamming rates. This chapter provides evidence that both sexes practise acoustic mate guarding, suggesting an underlying promiscuous mating system in this species.

The mating system of the Crimson-breasted Shrike and whether it is related to certain duet characteristics is the focus of chapter 4. This genetic approach investigates the existence of extra pair paternity in this species and examines the two hypotheses concerning the function of duets related to extra pair paternity. These are the paternity guarding and signalling of commitment to genetic monogamy hypothesis. This chapter provides evidence that extra pair paternity occurs at a surprisingly high level and contrary to predictions is unrelated to male duet characteristics. The findings suggest a relationship between female duet characteristics and extra pair paternity, possibly describing a female tactic to avoid the costs of their extra pair activities.

Zusammenfassung

Vogelduette sind komplizierte akustische Signale, die von Männchen und Weibchen eines Paares gemeinsam und mit bemerkenswerter zeitlicher Präzision vorgetragen werden. Dieses Verhalten hat sich mehrere Male unabhängig voneinander entwickelt, dennoch ist es ein seltenes Phänomen und kommt in weniger als 5% aller beschriebenen Arten vor. Duettierende Arten schließen Singvögel und Nichtsingvögel ein, welche sowohl Gesänge als auch Rufe in Duetten integrieren. Diese Arten kommen weltweit vor, der Großteil konzentriert sich jedoch auf tropische und subtropische Gebiete. Aufgestellte Hypothesen zur Funktion von Duetten sind unter anderem die Hypothese der Partnerbewachung, der Vaterschaftsbewachung, der Revierverteidigung, der gegenseitigen Erkennung, der Paarbunderhaltung und der Signalisierung der Bereitschaft zur Monogamie. Diese werden in Kapitel 1 detailliert beschrieben. Trotz dieser bestehenden Hypothesen bleibt die funktionelle Bedeutung dieser komplizierten Signale unklar. Duettfunktionen können sich je nach Art oder sogar innerhalb einer Art unterscheiden. Das Ziel dieser Dissertation ist es, einen Beitrag zum generellen Verständnis des Duettierens bei Vögeln und seiner ökologischen und sozialen Bedeutung zu leisten. Hierzu wurde eine Population frei lebender Rotbauchwürger (*Laniarius atrococcineus*) in drei aufeinanderfolgenden Jahren beobachtet und die oben genannten Hypothesen zur Funktion der Duette untersucht.

In Kapitel 2 wird das Gesangsverhalten und Gesangsrepertoire des Rotbauchwürgers beschrieben und im Hinblick auf die vier zentralen Hypothesen zur Funktion von Duetten untersucht. Diese sind die Hypothese der akustischen Partnerbewachung, der gegenseitigen Erkennung, der Paarbund-Erhaltung und der gemeinsamen Revierverteidigung. Die Ergebnisse zeigen, dass das Duettieren vermutlich ein kooperatives Verhalten innerhalb dieser Art darstellt, welches der gegenseitigen Erkennung, aber wahrscheinlich nicht der Paarbund-Erhaltung dient. Die durchgeführten Beobachtungen lassen auch vermuten, dass das Duettieren für die

Revierverteidigung nicht zwangsweise notwendig ist. Es konnte gezeigt werden, dass Rotbauchwürger ein geschlechtsspezifisches Gesangsrepertoire besitzen, welches eine Prämisse für die akustische Partnerbewachung darstellt.

Die in Kapitel 3 beschriebenen Experimente dienen der genaueren Untersuchung der Hypothesen zur gemeinsamen Revierverteidigung und zur akustischen Partnerbewachung. Um zwischen diesen zwei Hypothesen differenzieren zu können, wurden Playback-Experimente durchgeführt. Die Ergebnisse legen nahe, dass Weibchen die Duette sowohl zur akustischen Partnerbewachung als auch zur Unterstützung des Männchens bei der Revierverteidigung nutzen. Die Männchen betreiben vermutlich auch akustische Partnerbewachung, verwenden hierzu jedoch Sologesang. Revierverteidigung erreichen die Männchen durch duettieren mit dem Weibchen sowie durch vermehrte Rufe, die der Signalstörung der Eindringlinge dient. Dieses Kapitel liefert Hinweise auf die akustische Partnerbewachung durch beide Geschlechter, was auf ein promiskes Paarungssystem dieser Art hindeuten könnte.

Der Fokus des vierten Kapitels liegt auf dem Paarungssystem des Rotbauchwürgers und der Frage, ob dieses mit bestimmten Duett-Eigenschaften zusammenhängt. Dieser genetische Versuchsansatz testet, ob bei der untersuchten Art außerpaarliche Vaterschaften existieren. Die zwei damit verbundenen Hypothesen, bezüglich der Funktion von Duetten, sind die Vaterschaftbewachungs-Hypothese und die des Signals der Bereitschaft zur genetischen Monogamie-Hypothese. Die Ergebnisse zeigen, dass eine überraschend hohe Anzahl außerpaarlicher Vaterschaften vorkommt, welche nicht im Zusammenhang mit den Duett-Eigenschaften der Männchen stehen. Zudem deutet sich eine Beziehung zwischen außerpaarlichen Vaterschaften und der Duett-Eigenschaften der Weibchen an, welche eine Taktik der Weibchen darstellen könnte, um eventuelle Kosten ihrer außerpaarlichen Aktivitäten zu vermeiden.

Contents

1 Introduction

1.1. General Introduction

Most social interactions between individuals involve communication, which can be defined as “the process in which actors use specially designed signals or displays to modify the behaviour of reactors” (Krebs and Davies 1993). Communication therefore involves signals or displays that transfer information and are used by one individual to influence the actions of another individual in some way. Both individuals, actor and reactor, have to benefit from the transfer of information to ensure that the particular form of communication sustains. There are a variety of signals used by animals such as olfactory, acoustic, visual or tactile cues. The form of communication varies greatly between the different species and often a combination of two or three forms is used (Krebs and Davies 1993). Birds, for example, communicate with each other through visual displays but primarily and most obvious through acoustic signals. Birdsong is best developed in the order Passeriformes, in which many species have evolved complex acoustic displays. Most song is emitted by male rather than female birds, at least in temperate regions (e.g. Catchpole and Slater 2008). Although female song is less common than male song, it is not nearly as rare and functionless as previously thought (Langmore 1998). Female birds may sing for very much the same reasons than males do, for example mate attraction, territorial defence, mate defence, or coordination of breeding activities (Price 2009). In duet song, the male and the female of a mated pair combine their vocalisations with an astonishing degree of temporal precision. Duets are not only limited to the avian taxa, but they are also produced by insects, frogs, and primates (Tobias et al. 1998, Geissmann 2002, Bailey 2003). Avian duets received by far the most attention (Farabaugh 1982, Hall 2009) but it has been shown that the structure, the function and the factors that influence duets are similar between the taxa (Geissmann 2000, Bailey 2003). The definition of duetting has been and still is being debated (Langmore 2002), as it depends on the type of acoustic display (song, bill clattering, drumming) and the identity of the participants. Duets can be produced by the members of a mated pair, extended family groups, or male-male pairs of lekking birds (Hall 2009, Trainer et al. 2002) and hence the functions of the signals produced differ considerably. Here I will focus on avian vocal duets produced by mated pairs.

Generally, duets characterise coordinated singing by two individuals, one that initiates and one that answers, in such a way that their vocalisations either overlap or alternate (Farabaugh 1982). Duetting has been reported in a little over 420 different species (Hall 2009), approximately less than 5% of the world's bird species. As female song is imperative for creating duets, the phenomenon occurs more frequently in the tropics, where a higher proportion of female birds sing. Female song is connected to life-history traits such as small clutch sizes, many nesting attempts, slow development with extended parental care, and high adult survival, traits which are typical of the tropics (Price et al. 2009, Price 2009). Despite being a mostly tropical phenomenon, duetting is taxonomically widespread and has evolved several times independently (Farabaugh 1982), demonstrating that the coordination of vocalizations into duets must be beneficial and selected for. Comparative studies have examined the ecological and evolutionary factors that distinguish duetting species from their non-duetting kin. Above all, long-term monogamy and year-round territoriality are identified as being connected with duetting and mentioned as key characteristics (for example: Farabaugh 1982, Benedict 2008). Prolonged monogamous pair bonds are often associated with year round territoriality. As high annual survival is favoured by a non-migratory lifestyle and enables the maintenance of a territory throughout the year (Kunkel 1974), female song and duetting are more common in the southern than in the northern hemisphere. Migratory species have to re-form partnerships each year and as mortality rates increases, so does new formation of pairs. There are some duetting species, however, such as the Anseriformes, which form lifelong pair bonds without defending a territory all year round.

In addition, similar sex roles (Slater and Mann 2004) and monomorphism in plumage has been proposed as being associated with duetting (Thorpe 1972, Malacarne et al. 1991). That monomorphism is more common among duetting species than nonduetting species has been disproved by other studies (Farabaugh 1982, Hall 2009). Nevertheless, some differences between duets of monomorphic and dimorphic species do exist, as duets of the latter group appear to be less elaborate (Farabaugh 1982), probably because sexual distinctiveness is already visually apparent. Sex role convergence is more common in tropical birds and female song is just one manifestation of this (Morton 1996). Slater and Mann (2004) argued that weaker sexual selection caused by higher fidelity leads to sex role convergence in the tropics. Whether similarities of sex roles or genetic monogamy are directly related to duetting has not yet been elucidated.

As the precisely coordinated presentation of male and female vocalisations in form of duets appears to be beneficial under certain ecological conditions, the factors that influenced the evolution of this unusual display and the functions it might serve have been studied intensely. The following paragraphs will provide an overview of duet structure and the proposed functions of duets. I will describe the genus *Laniarius* including the study species *Laniarius atrococcineus* and the aim and scope of this thesis are outlined in detail at the end of this chapter.

1.2. The structure of duets

The duet structure varies considerably between species as well as within species (reviewed in Hall 2004, 2009). There are differences in how often duets are produced, the relative participation of each sex towards the duets, and how precisely timed these vocalisations are. Furthermore, the song types used for duet creation may vary as well as the sequence in which these song types or elements are presented. Some species exhibit distinct male and female solo repertoires, with each sex using songs of their solo repertoire to contribute to the duet creation (e.g. Wright and Dahlin 2007). Other species use the same vocalisation for both sexes (Hall 2006) or do not even posses solo repertoires, like the Red-crowned Crane (*Grus* *japonensis*) which never vocalises solo but presents almost all vocalisations as duets (Klenova et al. 2008). Depending on the number of different song types a species or individuals have, the duet repertoire of pairs can vary greatly. The presentation of the element patterns can range from antiphonally without any overlap (Hall 2006) gradually shifting to complete overlap of male and female vocal contributions (Maurer et al. 2008). Another variable involves the sequential ordering of male (M) and female (F) elements within the duet. The order of sequences describes the rate of alternation between elements, for example the sequence MMMFMFMMMMMFMMMFMFMMM has fewer alternations than the shorter sequence MFMFMFMFMFM (after Farabaugh 1982).

Duetting requires both participating individuals to be attentive to their mate (Smith 1994). Not only do they have to pay attention to the precise timing of their vocalisations but also to their partner's song type and how to answer it. By randomly combining their individual repertoires, duetting pairs could produce a much larger repertoire of duet types than by nonrandomly associating their vocalisations. To date, random combination has never been observed and the mechanism thought responsible for nonrandom song type associations has been coined 'duet code adherence' (Logue 2006). Pairs can either have their own specific duet repertoire as some species do (e.g. Levin 1996a) or repertoires can be identical for an entire population (e.g. Grafe et al. 2004, Rogers et al. 2006).

The individual responsible for duet creation is the second bird singing. If it did not respond to its partner's song, the first bird to sing would just be giving solo song. Which sex initiates a duet, and which sex answers, differs between species. Steere's Liocichlas (*Liocichla steerii*) and Eastern Whipbirds (*Psophodes olivaceus*), for example, almost always sing male initiated duets (Rogers et al. 2007, Weng et al. 2012) whereas Subdesert Mesites (*Monias benschi)* and Bay Wrens (*Thryothorus nigricapillus*) generally create duets initiated by the female (Levin 1996b, Seddon et al. 2002). In other species, such as Tropical Boubous (*Laniarius aethiopicus*), Magie-larks (*Grallina cyanoleuca*) or Black-bellied Wrens (*Thryothorus fasciatoventris*) males and females initiate an equal number of duets (Hall 2000, Grafe and Bitz 2004, Logue 2007a).

The precision of timing is the second aspect of duet coordination and also requires high levels of attentiveness (Smith 1994). Thorpe (1972) suggested that only true duets are precisely timed. Precision of timing may vary in some degree (Table 1.1) between species (Farabaugh 1982) but also within species as in Magpie-larks (*G. cyanoleuca*) where the duration of the pair bond affects temporal coordination (Hall and Magrath 2007). Temporal precision of duets can be described by calculating the coefficient of variation (Farabaugh 1982) of the sexes' response intervals (reaction times). A response interval is defined as the time elapsed between the beginning of one bird's element and the beginning of the other bird's next element. Low coefficients of variation indicate a high precision of timing; therefore variability in reaction times does provide a simple measure of duet coordination that can be quantified.

Table 1.1: Temporal relationships between the schematically presented duet elements. The bars symbolize duration of elements of initiator, respectively responder. Time proceeds from left to right. The pattern can be continued arbitrarily long. Modified after Farabaugh (1982).

	No overlap	Overlap caused by responder at first motif	Overlap caused by initiator at second motif	Overlap caused by both duetters
initiator				
responder				

1.3. Proposed functions of duets

A series of different hypotheses (Table 1.2) for the functional significance of duets have been proposed by previous authors (reviewed in Farabaugh 1982, Hall 2004, 2009). Some that have been argued apply to intrapair communication, such as keeping mates in contact in dense habitat, reaching reproductive synchrony, coordinating nets relief, enabling mutual recognition and localisation, or signalling commitment to the mate as a form of pair bond maintenance.

Birds might use duets cooperatively to stay in contact or reveal their identity and location to each other (hypothesis 1, see Table 1.2) when visibility is limited. Support for this thesis comes from Black-bellied Wrens (*T. fasciatoventris*) and Rufous-and-white Wrens (*Thryothorus rufalbus*) (Logue 2007b, Mennill and Vehrencamp 2008). Both studies showed that the first bird to sing approached the answering bird directly after duet creation, suggesting that the latter individual revealed its identity and location and enabled the approach. Duets might be better suited as solo songs in so far as solos could easily be learned and copied by another bird, whereas precisely timed answers or unique ‘duet codes’ are much more reliable for identification (Logue 2007a). It has been shown that both male and female song can stimulate ovarian development and nesting activity (Catchpole and Slater 2008), and it has been suggested that duetting could help mates to ensure reproductive synchrony (hypothesis 2) (Dilger 1953). Tropical species would have to use cues other than external ones, as seasonality is less pronounced and breeding cycles are extended so one mechanism to reach a mutually attuned breeding stage could be duetting (Kunkel 1974). Because many species produce duets outside the breeding season (e.g. Harcus 1977, Morton 1996), it seems unlikely that ensuring reproductive synchrony is the sole function of duetting. As proposed by Hall (2004), more insight into the relationship between duetting and hormones (e.g. Schwabl and Sonnenschein 1992) may help determine whether duetting stimulates and synchronizes reproduction. In the Slate-coloured Boubou (*Laniarius funebris*) pair members use duets to coordinate nest relief (hypothesis 3) as both sexes incubate (Sonnenschein and Reyer 1983). The same function has been reported for Tropical Boubous (*L. aethiopicus*) (Thorpe and North 1965). The hypothesis that participating in a duet signals commitment to the mate (hypothesis 4) was proposed by Wickler (1980) and refers to the partner-directed adjustment required to learn duet repertoires and produce consistent reaction times. This “coyness” hypothesis asserts that both pair members invest by learning to duet with one another, leading to a reduced propensity to desert

Table 1.2: The major hypotheses proposed to explain the functions of duets and their predictions regarding the form and context of duets including available evidence from other duetting species. See text for detailed explanation and discussion of individual hypotheses.

	Functions (proposed by)	Predictions	Characteristics	Existing evidence
1	Mutual recognition and location, maintaining contact (Thorpe and North 1966)	All year, visually occluded habitat	Locatable elements, must carry info about identity	(Logue 2007b, Mennill and Vehrencamp 2008)
2	Ensure reproductive synchrony (Dilger 1953)	Onset of breeding season, just before nesting	-	-
3	Nest relief (Sonnenschein and Reyer 1983)	During breeding season, when incubating	-	(Sonnenschein and Reyer 1983)
4	Signalling commitment (coyness hypothesis) (Wickler 1980)	Same-sex solo song is more threatening than opposite-sex or duet song	Responsiveness and precision is related to signal quality	-
5	Joint territorial defence (Seibt and Wickler 1977)	Duets more threatening than solo song	Loud, locatable, responsiveness and precision is related to signal quality	(Hall 2000)
6	Sex-specific territorial defence (Marshall-Ball and Slater 2004)	Similar sex roles, duets more threatening than solo song, during intrusion the same-sex intruder is targeted	Loud, locatable, weakest response to opposite-sex solo song	(Levin, 1996b)
7	Mate guarding (Stokes and Williams)	Same-sex solo song is more threatening than opposite-sex or duet song	Loud, locatable, sex-specific elements, responsiveness and precision is related to signal quality, sex-recognition is required	(Rogers et al. 2007, Seddon and Tobias 2006)
8	Paternity guarding (Sonnenschein and Reyer 1983)	During fertile period of female	Female initiates	-

their mate. Duetting needs to be learned during ontogeny (Wickler and Sonnenschein 1989) but so far, studies failed to demonstrate that adult birds have to practise duets with a new partner (Levin 1996a, Seddon 2002, Grafe et al. 2004, Brumm and Slater 2007).

Other functions for duetting that have been brought forward involve outsiders, for instance the territorial defence hypothesis and the mate guarding hypothesis. Paternity guards by males that involve duets are also directed at individuals outside the pair bond. Two forms of territorial defence have been debated for duetting species, joint and sex-specific territorial defence. Joint territory defence (hypothesis 5) assumes that duets are used because two birds are more successful in defending a territory, so the joint song signals the presence of both members of the pair that claim ownership of the territory acoustically. Additionally, the degree of coordination of duets might signal the coalition strength of the pair to the intruders, as coordination could depend on pair bond strength (Hall and Magrath 2007). Evidence for duets being used as joint territorial defence was, among others, found in Magpie-larks (*G. cyanoleuca*), Purple-crowned Fairy-wrens (*Malurus coronatus*), Rufous-and-White Wrens (*T. rufalbus*) and Yellow-naped Amazons (*Amazona auropalliata*) (Rogers at al. 2004, Hall and Peters 2008, Mennill and Vehrencamp 2008, Dahlin and Wright 2012).

Sex-specific territory defence (hypothesis 6) presumes that the sexes concentrate on repelling same-sex rivals and implies that each sex reacts more aggressively in response to a same-sex intruder. This function is been supported by findings in Plain Wrens (*Thryothorus modestus zeledoni*), Stripe-breasted Sparrows (*Aimophila r. ruficauda*), and Happy Wrens (*Pheugopedius felix*) (Marshall-Ball and Slater 2004, Illes and Yunes-Jimenez 2009, Templeton et al. 2011).

The mate guarding hypothesis (hypothesis 7) claims that by answering its partner's song in duet, an individual can advertise the mated status of its partner to outsiders and potential rivals (Stokes and Williams). For example the females of the Warbling Antbird *(Hypocnemis cantator*) and the Steere's liocichla (*L. steerii*) join their partner in duets to deter other female rivals through display of their pair bond status (Seddon and Tobias 2006, Rogers et al. 2006, Weng et al. 2012). In Rufous-and-white Wrens (*T. rufalbus*) it is the males that guard their mates by creating duets (Mennill 2006), whereas in Subdesert Mesites (*M. benschi*) and Tropical Boubous (*L. aethiopicus*) both sexes practice mutual mate guarding (Seddon et al. 2002, Grafe and Bitz 2004).

The hypothesis that males guard paternity (hypothesis 8) via duets suggests that males join their fertile females in duets to repel rivals that might be attracted by the female song. So far this hypothesis has been refuted by several studies (Hall and Magrath 2000, Gill et al. 2005, Hall and Peters 2009) and as most species display duets throughout the year this hypothesis seems unlikely to solely explain the evolution and maintenance of such an elaborate display (Hall 2009).

These hypotheses (reviewed in Hall 2004, 2009) are not mutually exclusive and there is evidence that duets serve multiple functions, even within a single species (Grafe and Bitz 2004, Marshall-Ball et al. 2006, Mennill and Vehrencamp 2008, Sonnenschein and Reyer 1983). It has been proposed to categorize these hypotheses with regard to whether they base on conflict of interests or on cooperation between pair partners. This is not always explicit, for example mate guarding can base on either conflict of interest (Johnsen et al. 2008) or on cooperation (Low 2008), depending on the circumstances. Therefore, the functions of duets and whether they are a cooperative display or not, may depend on the context they are used in.

To date, most experimental evidence on duetting behaviour has been provided by studies on Central and South American as well as Australian families such as Antbirds (Thamnophilidae), Wrens (Troglodytidae) and Australian Wrens (Maluridae). Although singled out by Thorpe (1972) as one of the nine families in which antiphonal song at its highest development is produced, the family of the Bushshrikes (formerly included in Laniidae but the ones Thorpe referred to are today grouped separately as Malaconotidae), especially the genus *Laniarius* which performs the most elaborate duets of this family (Thorpe 1972), has received very little attention in recent years.

1.4. The genus Laniarius

The genus *Laniarius* comprises 16 species and 34 subspecies of which all sing in duets (Nguembock et al. 2008). Sexes are, with few exceptions, alike and tend to be either strikingly colourful or largely black. These carnivorous passerine birds are commonly known as boubous, shrikes and gonoleks. This African group of species is mostly found in scrub or open woodland, or marshes and swamps. They are similar in habits to true shrikes (*Laniidae*), hunting insects and other small prey either from a perch on a bush or hopping on the ground and gleaning inside dense vegetation (Harris and Arnot 1988). So far, all *Laniarius* species are described as socially monogamous breeders with long-term pair bonds and no helpers at the nest (Ridley and van den Heuvel 2012). There is no information available on occurrence of extra-pair copulations or resulting extra-pair young in this genus. Duets in *Laniarius* species comprise ringing whistles combined with harsh croaking or snoring and harsh tearing and clicking sounds (Harris and Arnot 1988). Interspecific and intraspecific repertoire variations have been reported (Thorpe 1972). Earlier studies described the singing behaviour of five *Laniarius* species, *L. atrococcineus* (Tarboton 1971, Merkle 2006), *L. funebris* (Sonnenschein and Reyer 1984, Seibt and Wickler 2000), *L. barbarus* (Grimes 1965, Slater et al. 2002), *L. ferrugineus* (Merkle 2010) and *L. aethiopicus* (Grafe and Bitz 2004, Grafe et al. 2004), but only the last-mentioned work included experimental evidence for duet functions. Tarboton and Merkle both described *L. atrococcineus* as “not as shy as other species of this genus” and its distribution is confined to dryer areas which favours observations. These traits make *L. atrococcineus*, the Crimson-breasted Shrike or Gonolek, an ideal study species to test general hypotheses on duetting behaviour. This widespread, medium-sized species (42-58 g) is a typical dry country species inhabiting the dryer Acacia thorn-bush or dense thorn jungle with a range centred on the Kalahari Desert region of southern Africa (Hockey et al. 2005). Sexes are monomorphic with black upperparts to below the eye, black tail and wings showing a white wing stripe. The entire underparts are brilliant crimson (Figure 1.1) except for a yellow morph that occurs occasionally which has lost the ability to transform the yellow food carotinoids into red Astaxanthin based on a gene defect (Völker 1964). Both sexes vocalize frequently solo as well as in precisely coordinated, antiphonal duets with their mates (Figure 1.2). They are socially monogamous and remain paired throughout the year in their established territories which they defend against conspecifics (Tarboton 1971, Merkle 2006).

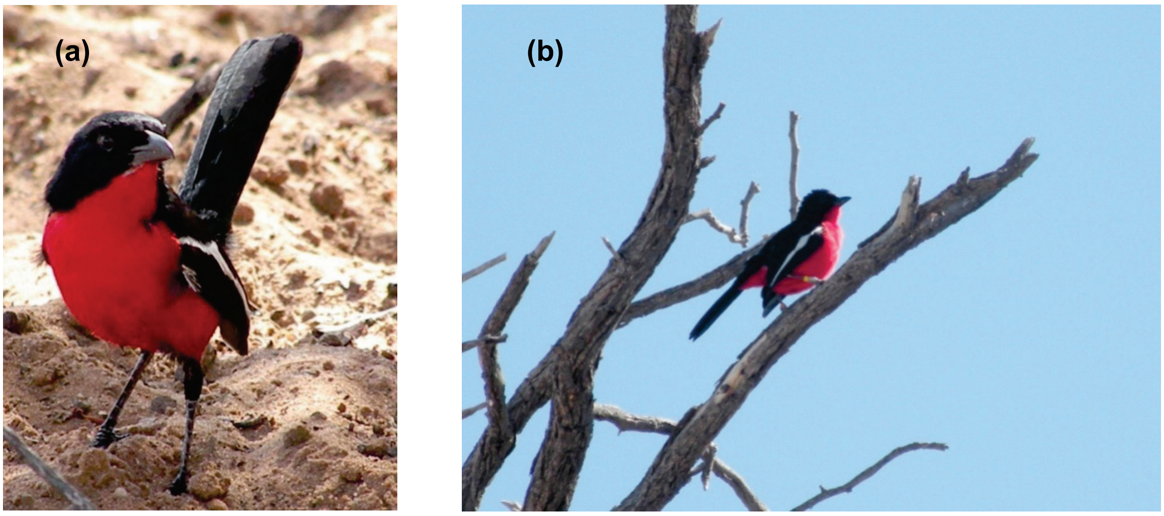

Figure 1.1: Crimson-breasted Shrike male (a) and female (b) of the study population at the Kuruman River Reserve, South Africa. Photographed by the author.

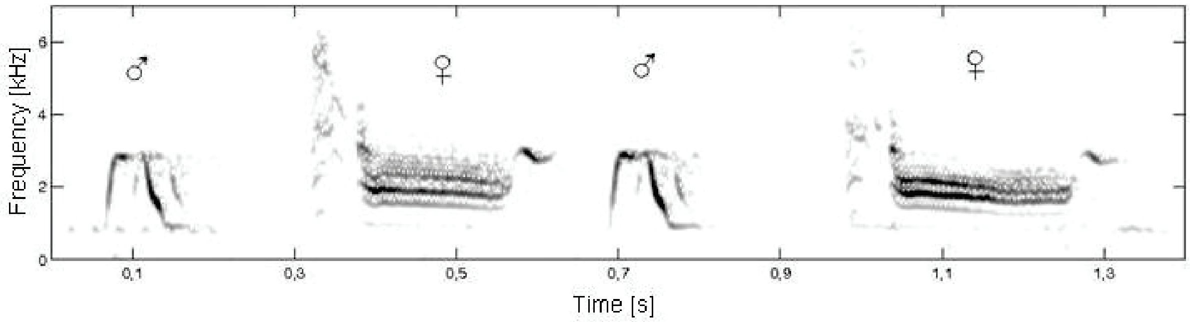

Figure 1.2: Sonogram of a Crimson-breasted Shrike duet from the study population at the Kuruman River Reserve, South Africa.

1.5. Aim and scope of this thesis

The aim of my thesis is to contribute to the understanding of the functional significance of avian duetting. The investigations were divided into three major parts: first I established a study population and observed and recorded singing behaviour of individuals and pairs. By identifying the song repertoire of individual birds and pairs, measuring initiation and answer rates of duets, and determining the precision of coordination between the two duet participants, general conclusions can be drawn about the function of duets, e.g. if they are a cooperative effort by a pair or if they are driven by intrapair conflict and serve sex-specific individual interests. Additionally, it can be determined if possible preconditions for certain functions (see Table 1.2) are given and whether duetting behaviour changes over time or under certain conditions. These are important facts which are necessary to know before conducting manipulative experiments to interpret possible results correctly and in the respective context. The study presented in chapter 2 was directed at establishing an overview over natural occurring song and duet repertoires and rates, song combinations, and continuity or changes in duetting behaviour of the study species *L. atrococcineus* over several seasons.

Chapter 3 represents the second part of this investigation and examines two major functions proposed to explain duetting, the territorial defence and the mate guarding hypothesis. The territorial defence theory predicts that duets present a greater threat to territory-holding birds than solo song. Partners should defend the resource jointly rather than independently, for example by approaching intruders together. They also should be more likely to sing in duets than to sing alone when faced with outsiders. By coordinating their signals, partners should be more effective than single territory-holders at excluding potential intruders and maintaining territories. Additionally, a bird should be less likely to maintain a territory after its mate disappears. In contrast, the mate guarding hypothesis argues that same-sex song should always elicit a stronger response from resident birds as compared to opposite-sex song, and that paired individuals of the guarding sex should answer the song of their mates more frequently when a same-sex intruder is present. In addition, unmated birds should respond less aggressive towards same-sex rivals than mated birds if mate guarding is the primary function of duetting. The aim of this chapter was to determine whether Crimson-breasted Shrike duets used in aggressive encounters with other individuals and pairs serve to defend a territory together or serve as mate guarding, which one or even both sexes might practise.

The third part of this thesis, reported in chapter 4, focuses on a genetic approach to examine the function of Crimson-breasted Shrike duets. Several reasons may contribute to the likelihood that a female is unable to choose the optimal male as her social partner and a common behaviour in socially monogamous birds to secondarily adjust their mate choice is to engage in extra-pair matings (EPM) (reviewed in Griffith et al. 2002). Duetting species represent an exception to socially monogamous species with promiscuous mating strategies as the vast majority has been found to be genetically faithful. This fact has led to the common belief that duetting and genetic monogamy are related and suggested theories include the paternity guarding (Sonnenschein and Reyer 1983) and the signalling commitment to reinforce pair bond hypothesis (Wickler 1980, Hall 2004). Aspects of duets that may function as paternity guards are male responsiveness to female song or male duet initiation rates. Both features increase the duet rates which in turn should discourage any male rival to mate with the female. If participation in a duet is a signal of commitment to genetic monogamy and reinforces the pair bond, duet characteristics such as the likelihood of answering and the speed of the responses to the mates' calls should indicate the degree of commitment of each pair member. By determining paternity rates of individual males of the study population and linking them to the mentioned duet traits I can evaluate (a) if Crimson-breasted Shrikes follow a promiscuous or monogamous mating strategy and (b) which duet characteristics might be typical for the respective strategy.

Ultimately, the goal of the above mentioned studies was to determine the function of duets in the Crimson-breasted Shrike. The examined hypotheses and their presented results are discussed in comparison to established comparable investigations and may contribute to the general understanding of the functional significance of duetting in birds.

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In the context of this dissertation, the following peer-reviewed publications have been published:

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2 Individual identity, song repertoire and duet function in the Crimson-breasted Shrike (Laniarius atrococcineus)[1]

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2.1. Abstract

Avian vocal duets are joint displays where paired birds produce temporally and structurally coordinated vocalisations. Duets show great variety in form that can reflect different functions such as mate guarding, mutual recognition, pair bond maintenance or territory defence. By describing the structure of duets and singing behaviour, we can investigate whether these signals are based on cooperation or conflict and which functions they might have. Here we describe the singing behaviour and song repertoire of the Crimson-breasted Shrike in the Kalahari Desert, and assess four main hypotheses proposed for the function of duets. We found that Crimson-breasted Shrikes have a sex-specific repertoire and individuality of males, females and pairs is expressed acoustically. Differences in vocal strategies between the sexes indicate male mate guarding as one possible function. Temporal coordination is high and duetters follow strict codes, suggesting that duetting could be a cooperative endeavour in this species. The observed duetting behaviour is also consistent with the predictions for mutual recognition because each bird has its individual vocal characteristics which are consistent over time. Our results provide no support for the hypothesis that duets serve pair bond maintenance in this species as no partner-directed adjustment of temporal coordination took place.

2.2. Introduction

Often socially monogamous bird and primate species coordinate their vocal displays in time, producing highly synchronized duets (Langmore 1998, Wachtmeister 2001, Müller and Anzenberger 2002). Duetting in birds has been reported for over 360 species from 50 families (Hall 2009), and is considered to have evolved phylogenetically independent (Hall 2004). Four widely discussed hypotheses concerning the primary functions of duets are mate guarding, mutual recognition, pair bond maintenance and territorial defence - although these hypotheses are not mutually exclusive (reviewed in Hall 2004, Marshall-Ball et al. 2006). Researchers have found evidence in favour of all four hypotheses, but because some studies support and others reject each hypothesis, an overall theory remains elusive (Templeton et al. 2011). Levin (1996) found that male Bay Wrens (*Thryothorus nigricapillus)* participate in duets as a means of acoustically guarding their mates from other males. Acoustic mate guarding requires at least sex recognition, which could be facilitated by sexual dimorphism in song repertoire (Hall 2006) and individual differences in vocal displays. Logue and Gammon (2004) proposed that Black-bellied Wrens (*Thryothorus fasciatoventris*) use duets to identify their mates, which points to individual identification based on vocal cues. Individuals could also use duetting to maintain the pair bond by signalling commitment to their partner, as high rates of song answering and consistent reaction times with coordination that improves over time requires partner-directed adjustment (Wickler 1980). This “coyness” hypothesis asserts that both pair members invest by learning to duet with one another through adjustment in singing behaviour which leads to a reduced incentive to desert their mate (Grafe et al. 2004, Brumm and Slater 2007). Conspecifics could then use this information to assess the pair bond strength of rivals and estimate the relative threat a pair poses to them (Hall and Magrath 2007), in which case duets would additionally serve in territorial defence. Related to the function of duets is the issue of whether duetting is a cooperative endeavour, or whether it based on a conflict of interests between the sexes. Here the term “cooperative” acknowledges the separate interests of the individuals participating in a duet, with the implication that the cooperative display will break down if the interests of both individuals are not met (Hall 2009). These underlying motivations do not exclude each other within one species but are context dependent as shown by Grafe and Bitz (2004) where Tropical Boubous (*Laniarius aethiopicus*) use duets to cooperatively defend a territory but males also practice mate guarding, which bases on a conflict of interest between the sexes. Analysing parameters such as adherence to answering rules (meaning the non-random combination of male and female song types) and the temporal precision of produced duets could indicate whether duetting arises from an underlying conflict or cooperation between the sexes.

In this study we investigate whether the duetting behaviour of the Crimson-breasted Shrike (*Laniarius atrococcineus*) is cooperative or conflict based, and which functions it may serve. Both males and females vocalise frequently with independent solos as well as precisely coordinated, antiphonal duets. The repertoire of two different populations has been described previously (Tarboton 1971, Merkle 2006), revealing local differences in repertoire, but no study has investigated the existence of a sex-specific repertoire or duet code adherence. As the Crimson-breasted Shrike is monomorphic, molecular sexing is required to identify sex-specificity in vocal repertoires. Also known as the Crimson-breasted Gonolek, it is a medium-sized insectivorous passerine (42-58 g) with a range centred on the Kalahari Desert region of southern Africa (Hockey et al. 2005). Crimson-breasted Shrikes are socially monogamous and remain paired throughout the year in their established territories which they defend against conspecifics (Tarboton 1971, Merkle 2006). Independent offspring leave their parents’ territories on average three months after fledging in search of mates and own territories (I.M. van den Heuvel, personal observation). In our study population 60% of the early pair bonds ended in divorce when breeding attempts failed, so observations of duet development of newly formed pairs as well as of established long-term bonded pairs were possible.

Thus the Crimson-breasted Shrike is an excellent study species in which to investigate duetting behaviour and examine the following hypotheses:

1. If duetting serves acoustic mate guarding in this species, sex and/or individual recognition is a prerequisite and must be facilitated acoustically. Individual answer rates should indicate which sex practices mate guarding. A biased sex-ratio in solitary birds could support this indication as the rarer sex should be the guarded one.
2. If duets serve as mutual recognition between the partners, individuals of each sex as well as pairs should differ in their vocal signals from each other to enable individual identification.
3. If duets are used to maintain and strengthen the pair bond through partner-directed adjustment, we predict that temporal coordination of each pair should improve over time and should generally be lower in newly formed than in established pairs.
4. If duetting is based on cooperation, duet code adherence should be abided and temporal coordination of duets overall should be high.
5. If two birds are necessary to defend a territory by singing in duets, lone birds that have lost their mate through desertion or death, and thus do not present duets to intruders, will be unable to successfully maintain their territory.

2.3. Materials and Methods

2.3.1. Study site and population

Fieldwork was conducted at the Kuruman River Reserve in the southern Kalahari Desert, South Africa (26°58'S, 21°49'E, 913 m) between September 2008 and March 2011. The study area is semi-arid grassland and acacia savanna, with an average annual rainfall of 217 mm (a detailed description of the climate and vegetation is given in Clutton-Brock et al. 1999). We studied 20 individually colour banded breeding pairs of Crimson-breasted Shrikes occupying territories with an average size of 14.1 ha (± 1.1 ha SE, ranging from 10.4 – 20.2 ha) (I.M. van den Heuvel, unpublished data) in a study area of approximately 3300 ha. These findings are similar to home range sizes Merkle (2006) found in a population of Crimson-breasted Shrikes but twice as large as the territories described by Tarboton (1971). The latter study took place in the Gauteng province, where vegetation conditions are rather different to our and Merkle's study site, which are more arid. It is likely that in this species, as in many insectivorous territorial bird species, territory size is related to resource availability (e.g. Both and Visser 2003).

The Crimson-breasted Shrikes are sexually monomorphic in plumage but wing and tarsus length in adults showed significant differences between the sexes [Wing: males (M = 102.23 mm ± 0.5 mm SE), females (M = 98.43 mm ± 0.6 mm SE); $t_{(37.16)} = 4.96$, $p = 0.000$; Tarsus: males (M = 33.60 mm ± 0.2 mm SE), females (M = 32.73 mm ± 0.2 mm SE); $t_{(43.9)} = 3.09$, $p = 0.003$]. As substantial overlap in the range of males and females makes clear distinctions in the field very difficult, a DNA test (Griffiths et al. 1998) was used to sex individuals. Birds were caught in mealworm-baited flap-traps or, later in the season, in mist-nets. All birds were removed within five minutes of capture, banded with a numbered metal ring (issued by the South African Bird Ringing Unit SAFRING) and two individually combined plastic colour rings. A blood sample of ca. 60 µl was obtained by brachial venipuncture (under SAFRING license no. 1510). Birds were blindfolded to calm them and the ringing process rarely took longer than five minutes. No adverse effects of the procedure were observed. This study was conducted with clearance from the Ethical Committee of Stellenbosch University (2008B01006).

2.3.2. Sound recordings and behavioural observations

We observed and recorded breeding pairs for 30 to 90 min periods, in the mornings approximately one to three hours after sunrise and in the afternoons starting two hours before sundown when birds were most active. These observations and recordings of songs were done over three consecutive breeding seasons using a Marantz PMD660 digital recorder (44 100 Hz, 16-bit resolution, Mono) and a Sennheiser ME67 directional microphone with a windshield. All audio data were obtained inside the birds' territories from a distance between 2-40 m away from the birds. Whenever possible, vocalisations were recorded on windless days to gain the best possible signal to background noise ratio. To determine the natural Sound Pressure Level (SPL) of the different vocalizations we used a Radioshack digital sound level meter (33-2055) and measured the SPL (Lr) of different individuals at known distances (r, in metres) and calculated the SPL at 1m (L1) according to the following formula: $L1 = Lr + 20 * \log_{10}(r)$. The distance of the individual to the microphone was measured by two investigators using a tape measure after the individual had left.

To identify individual male and female song repertoires, recordings of colour banded birds were linked to results of the molecular sexing. All identified solo songs and duets were then described and a vocal repertoire of the observed population was established. To investigate whether lone birds that lost their mates either through desertion or death would be able to keep their territories, daily behavioural observations were conducted on nine males and one female that remained solitary in their territories after such "divorce" events and re-mating or eviction events were recorded. Observations ended once individuals were remated or lost the territory which took a maximum of 20 days.

2.3.3. Acoustic analysis

To determine whether there are individual differences between birds of the same sex we focussed on the most common solo of each sex; male solo type 1 (1299 of 2314 recorded solos = 56%) and female solo type 1 (59 of 115 recorded solos = 51%). To detect differences between duets of different pairs we chose the most common duet (type 1 in the combination M-F-M, 1991 of 3327 recorded duets = 60%) as the frequently used vocalisations are most likely to reveal such differences between pairs. For the duet analysis we used only acoustic parameters not involved in detecting individual differences. This was to ensure that the same parameters were not used in two different analyses and that the analysis of duets was unaffected by variation based on individual differences. If differences were to be detected they should now base on duet-related features alone. As under natural circumstances the listening birds have all parameters available, duet related as well as individual ones, we subsequently analysed the duets with all parameters included.

To investigate whether the same males differ between seasons, the vocalisations of individuals sampled over two consecutive seasons were compared. The same was done for individual female songs and duets produced by individual pairs. All recordings were band pass filtered (cutoff frequencies: 300 Hz and 11 kHz) prior to analysis to remove background noise, re-sampled to 22 050 Hz, and normalised (using AviSoft SAS-Lab Pro 4.53, R. Specht, Berlin, Germany). Only duet recordings where the two pair mates were very close together (< 6 m) and recordings showed similar amplitudes for male and female were analysed to ensure accurate measures of temporal coordination and avoid effects from spatial differences between the two duetters. This ensured that observed variation would not result from geometrical effects nor from atmospheric conditions, but only from variation of temporal coordination within a pair.

The following song parameters were measured with AviSoft SAS-Lab Pro 4.53 for male and female solo songs: (1) total duration of the song, (2) peak fundamental frequency, (3) lowest frequency, (4) highest frequency, (5) bandwidth, (6) peak frequency at song centre, (7) peak frequency at song beginning, (8) peak frequency at one-third song length, (9) peak frequency at song end (10) transition onset (difference in frequency at the beginning of the song and at one-third of the song) and (11) transition offset (difference in frequency at two-third of the song and at the end of the song). The parameters measured for duet analysis were: (12) total duration of the duet, (13) duration of the first motif (male), (14) pause between first and second motif, (15) duration of the second motif (female), (16) pause between second and third motif and (17) duration of the third motif (male). Measurements of vocalisations were taken from flat-top-window spectrograms with a FFT size of 256 samples, 96.87 % time overlap and 0.3628 ms resolution. Duet analysis with all parameters included: parameters 2-7 and parameter 9 for both sexes, plus parameters 12-17.

2.3.4. Molecular sexing

Blood samples were preserved in about 0.5 ml of a standard non-lytic PBS buffer and stored at 4°C until DNA extraction and further processing. Total DNA was extracted following the methods described in Friedl and Klump (1999). Sex of the sampled birds was determined by PCR amplification of a larger fragment of the CHD gene including introns that are longer in the W-linked CHD gene (unique to females) compared with the Z-linked CHD gene (which occurs in both sexes) using the primers H1272 and L1237 (Kahn et al. 1998). Sequences were analysed by electrophoresis on 3% agarose gels (Griffiths et al. 1998). This method results in one PCR product of around 250 bp corresponding to the amplified fragment of the Z-linked CHD gene in both males and females, and an additional female-specific PCR product of around 300 bp which corresponds to the longer fragment of the W-linked CHD gene. The results confirmed all preliminary classifications based on behavioural observations and body measurements.

2.3.5. Statistical analysis

Continuous high-quality recordings of 30 min were made from eight pairs in 2010/2011 and the following parameters were extracted: individual initiation, termination, and answer rates of each sex as well as the percentage of song sung as song answers, (the terms initiation and termination denominates here the first bird to sing and the last bird to sing respectively). Answer rates were calculated as follows: female answer rate = 100 x total male initiated duets/(total male initiated duets + total male solo), and vice versa for male answer rates (Rogers et al. 2007). The percentage of own songs sung as song answers was calculated as: female song sung as answers = 100 x male initiated duets/(female solos + female initiated duets + male initiated duets), and vice versa for males (Hall 2009). Differences in means of these parameters were tested using a paired-samples t-test.

We characterised temporal precision of duets of type 1 for each pair for each season. To quantify the precision of a given duet we generated a mean Coefficient of Variation (CV= standard deviation/mean x 100) for male and female Response Intervals (RIs) (Levin 1996) from 10 measured duets per pair. A RI was defined as the time elapsed between onset of one bird's motif and the onset of the other bird's next motif. We then used exact Wilcoxon-signed ranks test to compare mean male and female RI CVs within each pair for two successive seasons, as a decrease in mean CV was predicted with increasing pair bond length. Additionally, we compared eight newly formed pairs (first breeding season in this pair formation, min. three weeks and max. nine months together) with eight established pairs (two or more breeding seasons in this pair formation) to test for differences in temporal precision comparing mean CVs using an exact Mann-Whitney-U-test. To control for variation that might arise from merging data obtained under different conditions, we created CVs for males and females within one bout with identical positions of birds to each other and to the microphone. These were compared to the same individuals CVs created from recordings with different conditions using a Wilcoxon signed-ranks test.

To assess whether acoustic structure could be assigned to identity of pairs of birds and individuals, we applied a Discriminant Function Analysis (DFA). The data for male and female solo songs was log-transformed before applying the DFA and we performed checks for colinearity amongst the parameters using the Variance Inflation Factor (VIF). The VIF detects strong linear relationships between variables which indicate high correlations between them (Field 2009). We found that for the duet measurements with only duet related parameters "duration" had an unacceptable VIF and was therefore excluded from the DFA. The analysis including all parameters, duet and individual related ones, showed an unacceptable VIF for "duration, male bandwidth" and "female highest frequency" which were then excluded. The other parameters were all in acceptable range (VIF < 7.0; range: 1.16 - 2.34). For the solo songs of males and females the parameter bandwidth had an unacceptable VIF and was therefore removed from both analyses; all other parameters were in acceptable ranges (VIF < 7.0; range: 1.02 - 1.48). The low values for VIF indicate that our parameters did not suffer from colinearity (Field 2009, Townsend et al. 2010). For external validation we used a leave-one-out cross-validation procedure and to estimate the significance of the classification with DFAs we used two-tailed binomial tests with a corrected level of chance corresponding to the number of categories discriminated between (Mundry and Sommer 2007). To investigate if the birds are constant in their vocal displays over time and if this classification can be used over a longer time period to reliably identify individuals and pairs, we used a permutated DFA (pDFA) with each individual and pair over two successive seasons. A pDFA differs from a normal DFA that the distribution of test statistics is not predefined. Instead, for each data set to be tested this distribution is newly generated from exactly this data set by repeatedly randomizing (or permutating) it, and obtaining the expected distribution (Mundry and Sommer 2007). We also performed checks for colinearity amongst the parameters and excluded the ones with unacceptable VIFs. All analyses were conducted in SPSS version 18.0 (SPSS Inc., Chicago, IL, U.S.A.) except for the pDFA which was performed using R version 2.8.1 (R Core Development Team, 2008), with the software package ‘MASS’ (Venables and Ripley 2002). Tests were two-tailed and significance levels were set at $\alpha = 0.05$.

2.4. Results

2.4.1. Vocal repertoire

Molecular sexing and observations of colour-banded birds revealed a sex-specific vocal repertoire with five distinct male and four distinct female solo songs, as well as three shared calls. The duets consist of a combination of one male and one female song type with variable alternating repeats but always in a nonrandom combination. Male type 1 was always answered with female type 1, male type 2 was always answered with female type 2 and so forth (Table 2.1), indicating that the birds followed strict answering rules. Observed duet combinations and their frequencies of occurrence in 30min recordings are presented in Table 2.1. Duets were initiated and terminated by either sex but male initiated duets were more common (24.6 vs 2.8) ($t_{(7)} = 3.669$, $p > 0.008$), as were male terminated duets (26 vs 1.4) ($t_{(7)} = 3.994$, $p = 0.006$).

Table 2.1: Duet combinations and their relative frequencies of occurrence in 30min recordings of eight pairs of Crimson-breasted Shrikes.

	contributing songtype		
duet type	male	female	relative frequency (%)
1	1	1	93.6
2	2	2	4.9
3	3	3	1
4	4	4	0.5
5	5	1	0

All distinct sex specific solo songs were used to form duets. The calls from the shared repertoire were not used to produce duets. There was no difference in solo repertoires of same sex individuals, nor in duet repertoires of pairs. In other words, all males of the population shared the same repertoire of solo notes, as did females and pairs.

Solo song

The males in the population sang five solo songs (Figure 2.1). Types 1 - 4 consist of tonal notes and type 5 of a broadband note. The most frequent solo heard was type 1a/b. The females sang four solo songs (Figure 2.1). All female specific solos were broadband notes. Types 1 and 3 were the most frequently sung solos. Solo song occurred when there was no response by the pair bonded individual or when sung by single, unpaired birds. Males were more vocal than females (47.5 vs 1.4 solos per 30min), initiating more songs than females (24.6 vs 2.8 per 30min), and responding to a higher percentage of their partner's song initiations (76.56 % vs 37.71 %). Males also sang the last notes in more duets than females (81.58 % vs 18.42 %). Most female songs were given in response to their partner (69.79 % vs 6.65 % for males). The average SPL of these vocalizations was 78.4 dB (± 2.3 dB SE, at 1m).

Shared vocalizations

The shared repertoire consisted of three vocalizations. Type 1 can be heard when birds are threatened by predators but not mobbing them. Type 2 is a soft contact call, mostly uttered when the birds are less than 10 metres apart and when feeding nestlings and fledglings. The mobbing or distress call (type 3) was heard when birds were either caught in mist-nets or flap-traps as well as when they were mobbing a predator.

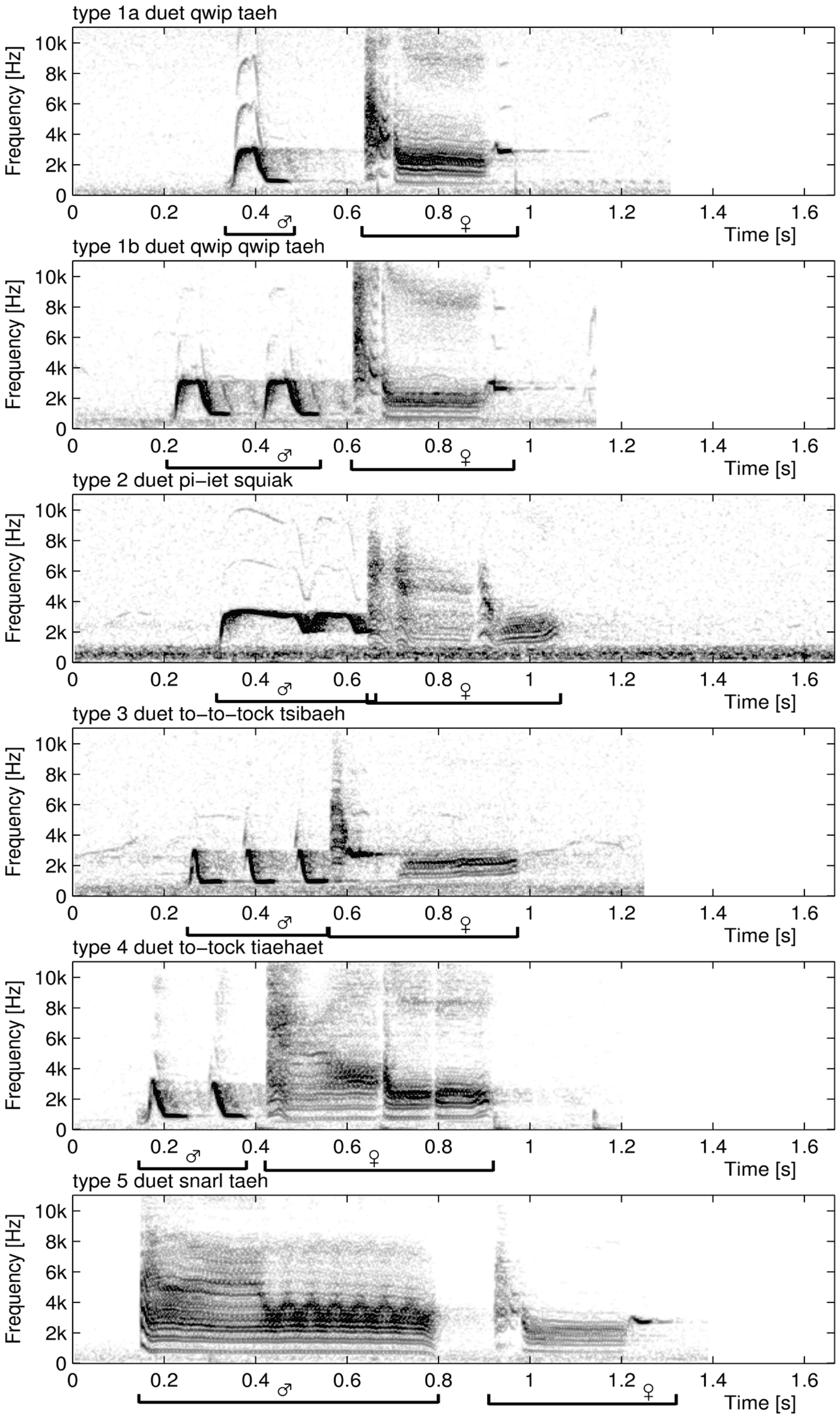
Duet song

Five duet types were produced by the shrikes in our study area (Figure 2.1). Female type 1 is given in duet with both male type 1(a + b) and type 5. Duets varied in length according to the number of alternating repeats produced. The most frequent duet type was type 1a in the combination male-female-male.

2.4.2. Temporal precision of duets

Duets were very precise; the mean CVs for male RIs were 11.2 % (± 2.1 SE) in season 2 and 9.3 % (± 2.4 SE) in season 3. For female RIs the mean CVs were 14.9 % (± 0.9 SE) in season 2 and 15.8 % (± 2.2 SE) in season 3. RIs for males were on average 0.16 (± 0.01 SE) sec and for females 0.13 (±0.009 SE) sec long.

Comparing RI CVs of the sexes from within one bout and from different bouts revealed no differences (males: N = 9, Z = -0.652, p = 0.57; females: N = 9, Z = -1.599, p = 0.13). Differences between means of RIs CVs of birds singing with the same mate in two successive seasons were not significant (males: N = 9, Z = -296.0, p = 0.820; females: N = 9, Z = -415.0, p = 0.734; Figure 2.2), implying that there is little or no variation of the intervals between the motifs and hence no change in temporal precision over time. The comparison of male and female means of RI CVs of eight newly formed and eight established pairs also showed no difference (males: Z = -0.42, p = 0.721; females: Z = -0.105, p = 0.959).


type 1a duet qwip taeh

type 1b duet qwip qwip taeh

type 2 duet pi–iet squiak

type 3 duet to–to–tock tsibaeh

type 4 duet to–tock tiaehaet

type 5 duet snarl taeh

Figure 2.1: Sonograms of the five identified duets of Crimson-breasted Shrikes from the Kuruman River Reserve. Presented duets are all male-initiated. Male song type 1a and 1b consist of the same note but are either presented once or twice but never mixed and are always answered with the same female song type. Male song type 3 and 4 are different notes and are answered with different song types by the female. Female song type 1 is given in duet with any one of the two different male types 1a/b and 5.

2.4.3. Differences in vocal distinctiveness

Individual/pair identity

DFA of 150 male solo songs (N = 15) revealed that songs could statistically be distinguished based on the chosen parameters which is one of the requirements for individual identification [Wilks' lambda = 0.47, χ^2 (df = 98) = 421.885, $p < 0.005$]. Classification probability was 40.7 % (cross-validated) [binomial (0.07), $p < 0.005$, 2-tailed]. Functions 1 and 2 explained 77.2 % of the observed variance (Figure 2.4) and the variables that contributed most to the differentiation were total duration of song, highest frequency and centre frequency (Table 2.2).

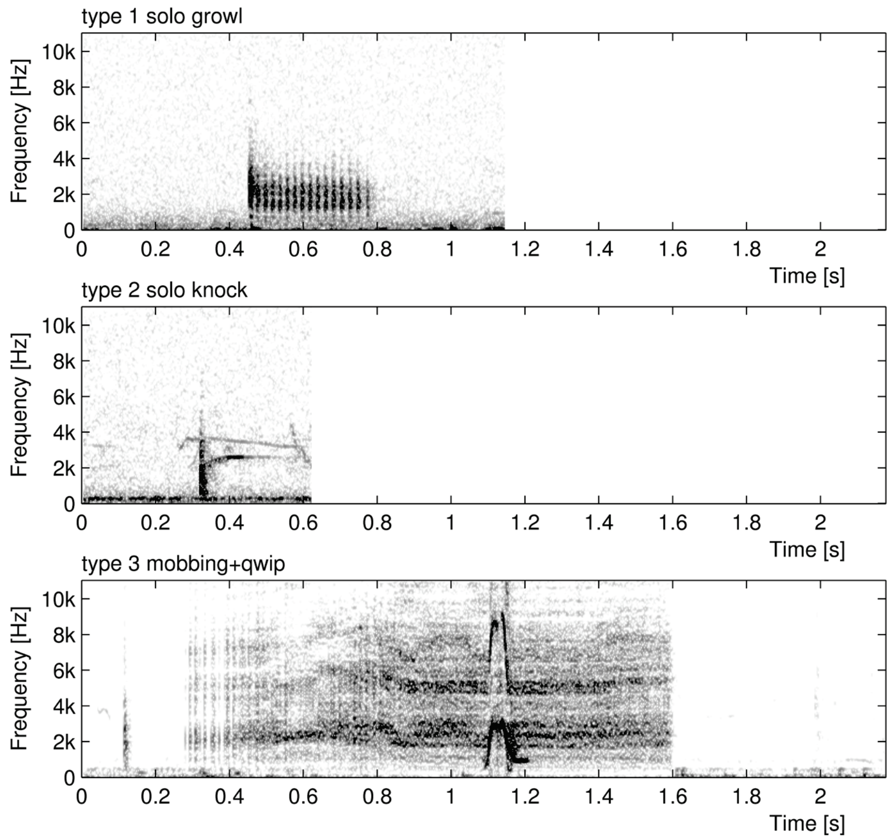


Figure 2.2: Sonograms of the three identified shared calls of Crimson-breasted Shrikes from the Kuruman River Reserve.

The DFA for the 160 female songs (n = 16) also showed differences between individuals [Wilks' lambda = 0.75, χ^2 (df = 105) = 381.654, $p < 0.005$]. Classification probability was 29.4 % (cross-validated) [binomial (0.06), $p < 0.005$, 2-tailed]. Function 1 and 2 (Figure 2.4) explained 81.1 % of the observed variance and the most important variables here were total duration of song, lowest frequency and start frequency (Table 2.2).

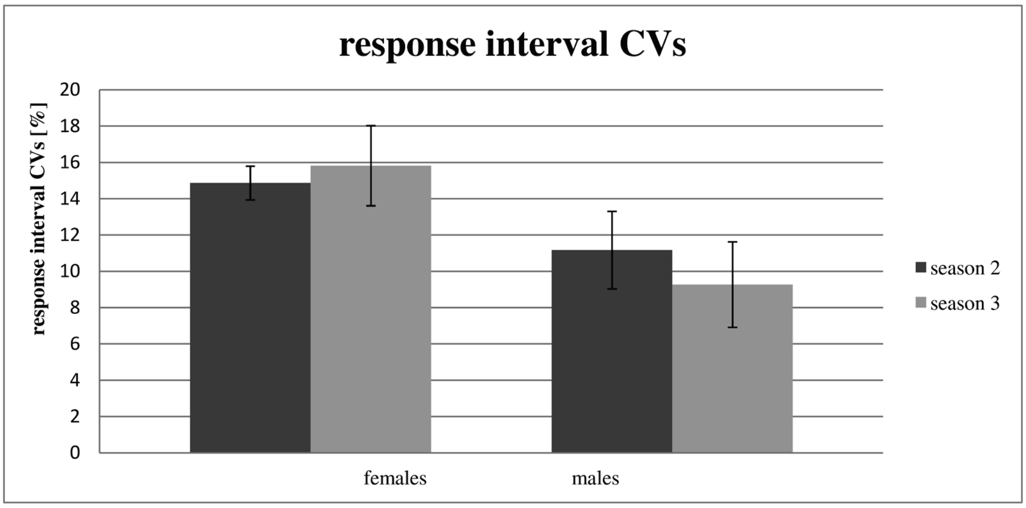


Figure 2.3: RI CVs of 9 male and 9 female Crimson-breasted Shrikes from the Kuruman River Reserve in two successive seasons. Individuals showed no significant differences in performance between the seasons. Mean values are shown ± SE.

For the 140 type 1a duets from 14 pairs analysed using only duet related parameters, results showed a significant difference among pairs [Wilks' lambda = 0.17, χ^2 (df = 65) = 524.072, $p < 0.005$] with 60.0 % classification accuracy (cross-validated) [binomial: (0.07), $p < 0.005$, 2-tailed]. The most important variables allowing the duets of different pairs to be distinguished were the duration of the second motif (female), and the duration of the first pause (Table 2.2). This difference was even more distinct when we included all parameters in the DFA [Wilks' lambda = 0.000, χ^2 (df = 221) = 1055.523, $p < 0.000$], showing 69.3% classification accuracy (cross-validated), [binomial: (0.07), $p < 0.000$, 2-tailed]. The additional variable contributing to the stronger distinction was female peak frequency at song end.

Seasonal differences in vocal identity

The pDFA detected no difference between songs of male individuals (N = 8), female individuals (N = 9) and pairs (N = 9) from different seasons (males: originally included elements: 160/1000 permutations, cross-classified elements: 0/1000, p = 1; females: 180/1000, 0/1000, p = 1; pairs: 180/1000, 0/1000, p = 1).

Table 2.2: Percentages of explained variations of observed discrimination and the corresponding variables of the DFA in Crimson-breasted Shrikes: ^{a}function 1, ^{b}function 2 and ^{c}function 3.

	% variation	cumulative %	most contributing variables*
male solos	62.9^{a} / 14.3^{b} / 11.1^{c}	62.9^{a} / 77.2^{b} / 88.2^{c}	duration^{a} / highest freq. ^{b} / centre freq.^{c}
female solos	72.1^{a} / 9.7^{b} / 8.3^{c}	72.1^{a} / 81.1^{b} / 90.1^{c}	duration^{a} / lowest freq.^{b} / start freq.^{c}
duets	57.6^{a} / 20.7^{b} / 15.0^{c}	57.6^{a} / 78.3^{b} / 93.3^{c}	motif 2^{a} / pause 1^{b} / motif 1^{c}

*the variables that contributed most to the discrimination and therefore explain the highest proportion of the observed variation.

2.4.4. Divorce events

In season 1 we observed six pairs of which one divorced (17 %); in season 2 eight of 23 pairs divorced (35 %); and in season 3 four of 18 pairs divorced (22 %). We were able to monitor ten (nine males, one female) of these birds that remained solitary in their territory thereafter. All succeeded in maintaining the territory and obtaining new mates within a maximum of 20 days after a divorce event.

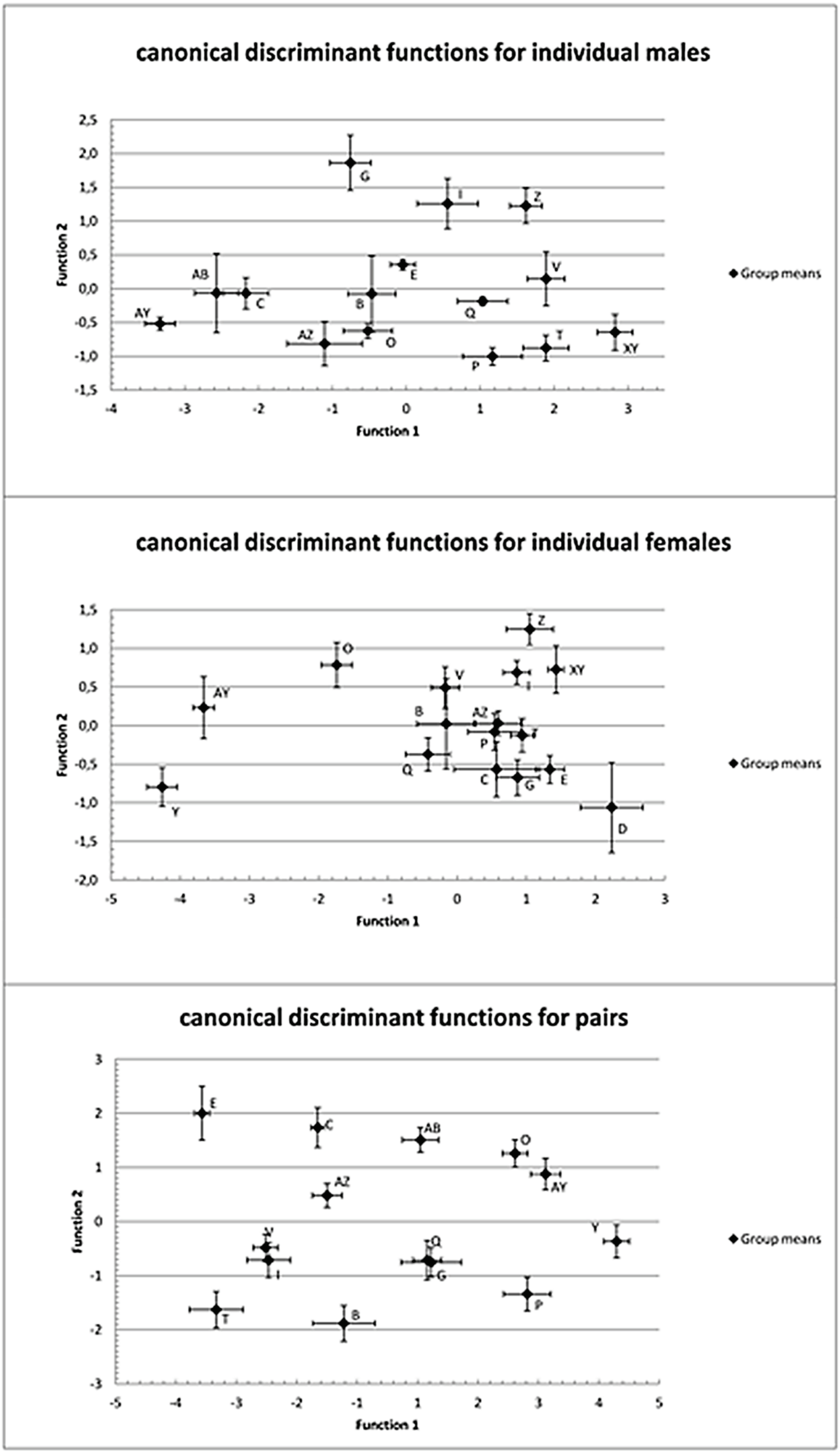


Figure 2.4: Canonical DFA means ± SE of 14 pairs, 15 male and 16 female Crimson-breasted Shrikes at the Kuruman River Reserve with function 1 and 2.

2.5. Discussion

Of the three studies conducted on Crimson-breasted Shrikes, this is the first to use molecular sexing methods to investigate sex specificity in song (Tarboton 1971, Merkle 2006). Sex recognition is a prerequisite for mate guarding in monomorphic bird species (although it does not necessarily imply that it takes place as sex recognition could have evolved for other reasons). Sex specific vocalizations facilitate sex recognition and hence enable mate guarding. The guarding sex can be identified by having higher answer rates, and our findings showed significantly higher answer rates for males than for females, suggesting that males suffer higher costs when letting their mates sing alone than vice versa. Given the male biased ratio of birds that became solitary after the partner disappeared or left, mate guarding practiced by males is suggested as a function of duetting in this species. Sex differences in vocal repertoire has been found in other duetting species such as the Bay Wren (Levin 1996), the Subdesert Mesite (Seddon 2002), the Eastern Whipbird (Rogers et al. 2006), the closely related Slatecoloured Boubou (Sonnenschein and Reyer 1983) and Tropical Boubou (Grafe et al. 2004), but not in duetting Australian Magpie-Larks (Hall and Magrath 2000). Consistent with these findings is the evidence of acoustic mate guarding in the species above which have sex differences in vocal repertoire (Sonnenschein and Reyer 1983, Rogers et al. 2007, Seddon 2002, Grafe et al. 2004), whereas Magpie-Larks show no such function for their duets (Hall and Magrath 2000). Future playback experiments should confirm this suggested function of duets in the Crimson-breasted Shrike.

As each individual and each pair has its own vocal identity consistent over successive seasons, mutual recognition is another possible function of Crimson-breasted Shrike duets and solo songs. When listening to a duet, birds have not only duet related characteristics available but also those arising from individual characteristics, so it is not surprising that duets are the most easily distinguished category of vocalizations (see Table 2.2). All duets measured were male initiated which suggests that it is females that make duets distinctive as they determine the duration of the first pause (i.e. by how quickly they reply) and the second motif. Individual acoustic identity has been shown in several other species (e.g. Gilbert et al. 2002, Fitzsimmons et al. 2008, Kirschel et al. 2011, Berg et al. 2011, Koren and Geffen 2011), mostly with stable social structures where individual identity is imperative, such as the Crimson-breasted Shrikes with their long-term pair bonds and resulting stable neighbouring territorial structures (Tarboton 1971, 1998, I.M. van den Heuvel, personal observation). Duets could therefore serve mutual recognition within a pair, as well as help discriminate neighbouring pairs from intruding strangers.

For duets to function in pair bond maintenance, the conventional wisdom is that partner directed adjustment is required (Wickler 1980). Our study shows no differences in temporal precision of pairs over time, which is consistent with findings in other passerines (Levin 1996, Grafe et al. 2004). Also, we found no difference in temporal coordination of newly formed versus established pairs, which is contrary to the idea that duets require partner-directed adjustment, as this would result in an increase of temporal coordination over time. Crimson-breasted Shrike duets are not very complex, consisting of one male and one female song type combined to duets of various lengths and it is unlikely that these require a lot of learning and temporal adjustment. Hence we suggest that duets in this species do not represent a time-consuming pair-specific investment by either sex as proposed in the “coyness model” by Wickler (1980).

To investigate the cooperation or conflict based nature of duets we examined duet structures. Crimson-breasted Shrikes combine their individual song types non-randomly into duets (“duet code”) as in many duetting species (Logue 2006). Compliance with duet codes and the resulting high degree of partner directed attention, also reflected in the generally low CVs of RIs, leads us to believe that in this species duets represent a collaborative display. But as mate guarding is another possible function, we believe that duets could be either cooperative or conflict based in this species depending on the context in which they are used.

The majority of Crimson-breasted Shrikes in our study were found to live in stable long term pair bonds as stated in the literature. Out of 47 observed pairs over 3 seasons only 12 dissolved (just over 25 %). The fact that none of the 10 observed temporarily solitary birds lost its territory to neighbouring or intruding pairs suggests that duetting is not essential for territorial defence, at least not on a short term basis. Based on observations alone it would be premature to dismiss this hypothesis, especially as cooperation between partners when creating duets has been found to take place. Whether duets are required for initial establishment of territory boundaries or whether they enhance territorial defence against rival pairs could not be measured in this study. Again, we recommend experimental playbacks for further analysis of the territory defence hypothesis.

2.6. Acknowledgements

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3 Land or Lover: territorial defence and mutual mate guarding in the Crimson-breasted Shrike

3.1. Abstract

Crimson-breasted Shrikes produce duets which are used in interactions with neighbours and intruders. We examined two major hypotheses explaining duetting, the territorial defence and the mate guarding hypothesis, using playback experiments. The responses of 12 pairs towards solo male, solo female and stereo duet playback were analysed using principal component analysis and ANOVA. Females reacted most aggressively towards unpaired same-sex intruders by increasing answer rates, and least strongly towards unpaired opposite-sex intruders. During duet playback, females directed their aggression equally at both intruding pair members. Males also reacted least strongly towards opposite-sex intruders, paired or unpaired. In contrast to females, they reacted equally aggressively towards unpaired same-sex intruders and intruding pairs, but in the latter case directed their aggression at the male pair member. Furthermore, to repel unpaired same-sex intruders, males used solo song, but to repel intruding pairs they increased jamming rates and duetted with their mates. These results indicate that females use duets for mate guarding against unpaired females, whereas males engage in mate guarding as well as territorial defence, with the former being achieved by singing solo songs; and the latter by singing solo songs and duets.

3.2. Introduction

The function of avian duetting remains widely debated and unresolved (Templeton et al. 2011): although several hypotheses have been proposed to explain it (reviewed in Farabaugh 1982, Hall 2009, Douglas and Mennill 2010), the evidence is contradictory. Two major hypotheses, in particular, the territorial defence hypothesis and the mate guarding hypothesis, are difficult to discriminate as predictions for both are very similar (although Table 3.1 lists some fine distinctions between them). Both hypotheses predict duets to be loud, conspicuous and used in interactions with neighbours and intruders, unpaired or paired (Seddon and Tobias 2006).

Territorial defence can take two forms. In joint territorial defence, two birds represent a united front against conspecifics using duet song to repel them (Sonnenschein and Reyer 1983, Logue and Gammon 2004, Grafe and Bitz 2004, Mennill and Vehrencamp 2008) and to communicate information about the strength of the pair bond (Hall and Magrath 2007). Alternatively, duets can facilitate a division of labour in defence of the territory by allowing each sex to target its aggression at same-sex intruders (Levin 1996, Marshall-Ball and Slater 2004). But both forms assume that duets are based on cooperation between the sexes. In contrast, the mate guarding hypothesis suggests that members of a mated pair have divergent agendas when performing duets and the second bird to sing (thus being responsible for creating the duet) does so to advertise the mated status of its partner. This signal functions to discourage rivals and to prevent its mate from attracting potential extra-pair partners (Levin 1996, Rogers et al. 2007, Tobias and Seddon 2009, Illes and Yunes-Jimenez 2009). Females have a strong interest in not being replaced, but provided that a female does not suffer any other costs (e.g. reduced male investment, inadequate fertilisation of her eggs, or the male having a second female in the territory) associated with her partner fertilizing other females' eggs through extra-pair copulations (EPCs), females have no reason to guard their males. Males on the other hand, have a strong interest in preventing females from having EPCs, as they would suffer high costs when raising extra pair young (Birkhead 1979, Gladstone 1979, Birkhead et al. 1987). So the question revolves around whether the two participants in a duet are pursuing the common goal of territory defence, or simply attempting to guard their mates against potential competitors of the opposite sex?

The purpose of this study was to use playback experiments to discriminate between these two explanations for duetting. Much contradictory evidence for duetting comes from studies using single speaker experiments: to interpret responses of both sexes it is important to use the more appropriate approach of simulating the intrusion of pairs using the stereo duet playback technique (Logue and Gammon 2004, Douglas and Mennill 2010), which allows intrasexual comparisons of responses to male and female duet components crucial for the distinction between hypotheses (Table 3.1). Some studies have revealed that duetting birds can distinguish between single speaker and multi speaker designs, to which they exhibit different responses (Rogers at al. 2004, Molles and Waas 2006). We presented three types of playback stimuli to territorial pairs of Crimson-breasted Shrikes: male solos, female solos and duets. A more aggressive response to duetting and equal male and female answer rates would support the territorial defence hypothesis, as the perceived threat of two mated intruders is higher than of a solitary intruder. By contrast, a weaker response to duetting than to same-sex solos and differences between male and female answer rates would support the mate guarding hypothesis (Table 3.1). The Crimson-breasted Shrike is a widespread and common songbird inhabiting the dry acacia dominated bushveld of southern Africa (Hockey et al. 2005), which produces antiphonal duets, initiated by either sex (van den Heuvel et al. 2013). This species is sexually monomorphic and forms long-term socially monogamous pair bonds (Tarboton 1971). Both sexes actively engage in interactions with neighbours and intruders, occupying exclusive territories all year round. Male and female Crimson-breasted Shrikes contribute equally to incubation, brooding, provisioning young and nest defence during each reproductive attempt (Ridley and van den Heuvel 2012). Genetic analysis indicates that 20% of offspring, in 30% of clutches, are not sired by the social father (I.M. van den Heuvel, unpublished results).

Table 3.1: Key hypotheses proposed to explain why individuals may respond to the song of their mate to form a duet and their main predictions, including examples with supporting evidence from other dueting species

Hypotheses	Territorial defence – joint (Hall 2000)	Territorial defence – sex-specific (Levin, 1996)	Mate guarding – paternity (male specific)	Mate guarding – exclusive status or prevent EPCs (Rogers et al. 2007, Seddon and Tobias 2006)	Mate guarding – preventing usurpation / injury of mate (Appleby et al. 1999)
Timing	All year	All year	Fertile period	All year	All year
Strongest response to	Duet song	Duet song	Male solo song	Same-sex solo song	Opposite-sex solo song
Weakest response to	Solo song	Opposite-sex solo song	Female solo song	Opposite-sex solo song	Same-sex solo song
Responses to duet song directed at	Both pair members	Same-sex pair member	Male	-	-

This makes the Crimson-breasted Shrike an ideal species in which to test whether males and females duet to defend a territory cooperatively or whether they are using duetting for mate guarding.

3.3. Materials and Methods

3.3.1. Study site and subjects

We performed playback experiments on 12 pairs of wild Crimson-breasted Shrikes in the Kalahari Desert at the Kuruman River Reserve, South Africa (26°58'S, 21°49'E) between August 2009 and February 2011. The study site consists of approximately 33km^2 of semi-arid bushveld (for a full description of the site see Clutton-Brock et al. 1999), inhabited by a population of about 20 individually colour banded pairs of shrikes. Trapping and ringing procedures as well as the vocal repertoire of this population are described in detail in van den Heuvel et al. (2013).

3.3.2. Playback stimuli and protocol

Playback stimuli were created from high quality recordings of territorial pairs situated a minimum of three kilometres away from the subject pair's territory to ensure unfamiliarity to the stimuli. All recordings were made under natural conditions from a distance between 2-20 m of the subjects using a Marantz PMD660 digital Recorder (44 100 Hz, 16-bit resolution, Mono) and a Sennheiser ME67 directional microphone with a windshield. Recordings were band pass filtered (cutoff frequencies: 300 Hz and 11 kHz), resampled to 22 050 Hz, and normalised so that each signal had the same maximum amplitude (using AviSoft SAS-Lab Pro 4.53, R. Specht, Berlin, Germany). Duets were converted into stereo files and we silenced all of the male contributions from one channel and all of the female contributions from the other channel. This was done to maintain the temporal relationship of the duet when playing the recordings through separate loudspeakers, a technique called stereo duet playback (Logue and Gammon 2004). To create solo male and female playback stimuli we digitally removed the partner's duet contribution. Each stimulus comprised five solo or duet songs of the same type from the same individual or pair followed by a 10 second pause at the end (Figure 3.1). This block was looped to form a 5 min file. The experiments were conducted by two investigators during two consecutive breeding seasons (September 2009 to February 2010 and September 2010 to February 2011) just after sunrise between 0530 and 0600 hours. To avoid any effects of breeding status on playback response we tested only birds not currently involved in a breeding attempt or accompanied by fledglings. Each territorial pair of birds was presented with three playback treatments: (1) male solo, (2) female solo, and (3) duet. The order of stimulus presentation was randomized for each pair and experiments were spaced at least two days apart to prevent habituation to the playback. We broadcasted playback stimuli (16-bit WAV files) from an mp3-player (Philips SA1922) connected to a MiniVox Lite loudspeaker (Anchor Audio, Torrance, CA, USA) from inside the subject pairs' territory to simulate intrusion. When presenting solo playback one loudspeaker was used; when presenting duet playback we used the stereo duet playback technique with the loudspeakers presenting the male and female part of the duet spaced 10 metres apart to more closely resemble a natural intrusion. We held the volume of each loudspeaker constant across all presentations at a level of 78 dB Sound Pressure Level (SPL) at 1 metre horizontal distance from the upward-oriented loudspeaker (measured with a RadioShack digital sound level meter 33-2055). This resembles the natural SPL measured in this species (van den Heuvel et al. 2013). Each loudspeaker was mounted on a pole 1.2 m above the ground positioned next to or in a shrub or tree and investigators retired to cover under self-made bird hides nearby. Subjects showed no response to the presence of hides or investigators during the playback experiments. To determine the focal birds' positions in relation to the loudspeakers, 5 m intervals around the experimental setup centre were marked with aluminium poles resulting in an experimental radius of 25 m. Between each playback treatment, the experimental setup was moved to another location inside the territory to avoid habituation to any particular site. Focal subjects were in a maximum distance of 50 m to the experiment centre before each experiment began. Playbacks were broadcast for five minutes and experiments ended when both pair members had left the 25 m experimental radius which could take up to 20 min post-playback. We recorded the positions of the focal pair during the playback period every

15 seconds and recorded its vocalizations over the entire duration of the experiment with the same equipment used for making stimulus recordings.

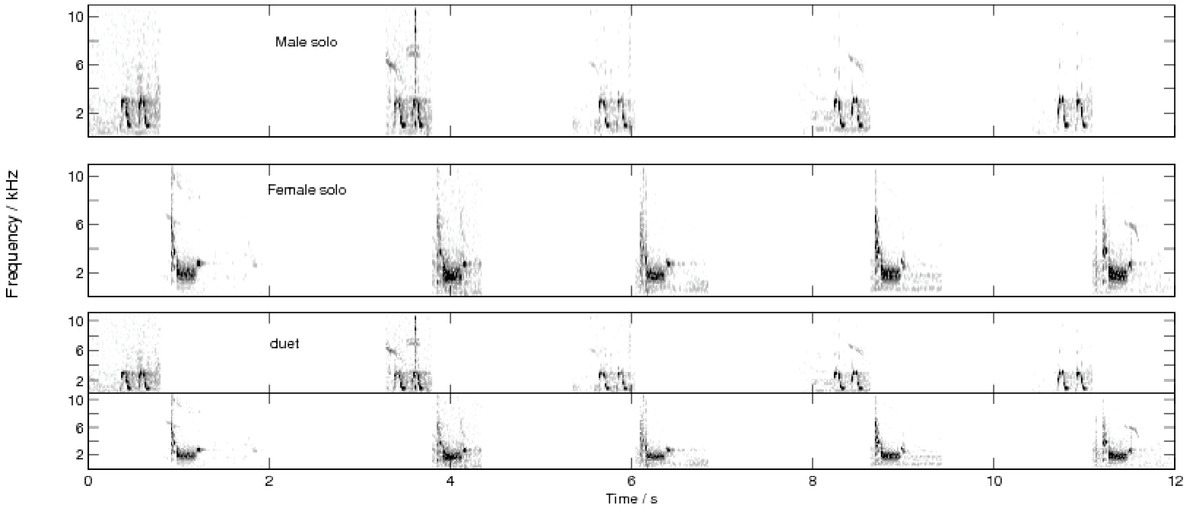

Figure 3.1: Spectrograms of male solo, female solo and stereo duet playback stimuli of Crimson-breasted Shrikes.

3.3.3. Response measures and statistical analysis

Playback recordings were viewed as sonograms (using Audacity 1.3 beta) and all responses of the focal pairs were marked and categorised. To assess level of aggression towards the playback, we extracted the following vocal and physical response variables for all three playback treatments: (1) latency of first response; (2) latency of second birds first response; (3) closest approach to loudspeaker (in metres); (4) time spent within 10 m of loudspeaker (in seconds); (5) answer rate (calculated as: female answer rate = 100 x total male initiated duets/(total male initiated duets + total male solo), and vice versa for male answer rates, after Rogers et al. 2007); (6) number of jammed playbacks; (7) number of total vocalizations; (8) number of solo vocalizations; (9) number of loudspeaker approaches; (10) time vocalizations continued after playback stopped (in seconds); and (11) number of total duets. Variables 3 - 9 were calculated for each sex.

Table 3.2: Factor loadings from principal component analysis of 12 pairs of Crimson-breasted Shrikes' responses to playback. Factor loadings contributing most to respective PCs are shown in bold. Separate comparisons of each response under the three playback conditions are indicated by (a) significant difference between playback treatment male solo and female solo; (b) significant difference between playback treatment male solo and duet; and (c) significant difference between playback treatment female solo and duet.

Response variable	PC1	PC2	PC3	PC4	PC5
Time of first response	-0.14	-0.12	-0.16	**0.96**	0.07
Time of 2nd birds first response	-0.14	-0.12	-0.16	**0.96**	0.07
Closest speaker approach male	**-0.82**	-0.26	0.10	0.11	-0.11
Closest speaker approach female	**-0.88**	-0.05	-0.02	0.04	0.15
Time spent within 10m male [c]	**0.74**	0.18	0.20	-0.13	0.15
Time spent within 10m female	**0.82**	-0.02	0.01	-0.12	-0.30
Male answer rate	0.22	0.31	-0.14	-0.001	**0.79**
Female answer rate [a]	-0.11	0.26	**-0.81**	0.32	0.11
Male jamming Playback [a, c]	0.34	0.51	**0.61**	0.05	-0.01
Female jamming Playback [a, b]	0.14	0.40	-0.21	0.01	**-0.59**
Total vocalisations male [a, c]	0.05	**0.84**	0.42	-0.06	0.21
Total vocalisations female	0.30	**0.83**	-0.21	-0.10	-0.32
Number of solos male [a, c]	0.05	0.25	**0.86**	-0.16	0.23
Number of solos female [a, b]	0.23	0.01	-0.13	-0.15	**-0.73**
Total duets	0.15	**0.95**	-0.01	-0.02	0.03
Time of vocalisation after Playback end [c]	0.17	**0.82**	0.22	-0.22	0.12
Number of speaker approaches male [b, c]	**0.50**	0.30	0.44	-0.09	0.25
Number of speaker approaches female	**0.79**	0.19	0.32	-0.04	-0.09
Eigenvalue	3.92	3.88	2.51	2.10	1.96
% variance explained	21.78	21.6	14.0	11.7	10.9

As these measures are unlikely to be statistically independent, we performed a Principal Component Analysis (PCA) to reduce the above mentioned variables to a smaller number of orthogonal components (McGregor 1992). As some of the variables had larger values than others based on the units of measurement (seconds versus metres versus count), all values were z-transformed before performing the PCA (McGregor 1992). We applied the Varimax rotation and generated five Principal Components (PCs) with an eigenvalue greater than 1, which cumulatively explained 79.9 % of the variation in responses (Table 3.2).

We evaluated variation in responses to the different playback treatments using repeated measures ANOVA where playback treatment was the within subject factor with three different levels, and the PC response scores were the dependent variables. Bonferroni correction was used to compare main effects and to adjust for multiple comparisons. To compare each response measure during the different playback treatments separately, we additionally performed Wilcoxon signed rank tests for each of the variables 1-11. To examine the responses of each individual within a pair towards duet playback in more detail, we extracted the following variables for each sex from the duet playback experiment recordings: (12 + 13) closest approach to loudspeaker presenting the male/female part of duet (in metres); (14 + 15) time spent within 10 m of loudspeaker presenting the male/female part of duet (in seconds); (16 + 17) number of approaches to loudspeaker presenting the male/female part of duet. As these variables were not normally distributed we chose a non-parametric test, the Wilcoxon-signed rank test, to compare responses of each sex towards the loudspeakers presenting the male and female part of duet during duet playback. All analyses were conducted in SPSS version 18.0 (SPSS Inc., Chicago, IL, U.S.A.) with a significance threshold of 0.05, and results are presented as means ± SE.

3.4. Results

Male and female Crimson-breasted Shrikes responded to all three playback treatments with vocalizations, and in many cases additionally approached or passed over the loudspeakers. In terms of physical responses during duet playback, females directed their aggression equally at both loudspeakers. Males, however, approached the loudspeaker presenting male duet components more closely than the loudspeaker presenting female components ($T = 4$, $z = -1.96$, $p = 0.05$, $r = -0.62$).

The PCA generated five PCs and their respective values and loadings are presented in Table 3.2. In terms of response variables, we refer to PC1 as “physical responses of pair”, to PC2 as “vocal responses of pair”, to PC3 as “male led vocal responses”, to PC4 as “latency of pair response”, and to PC5 as “female led vocal responses”. These five response scores were then tested for differences between the three playback treatments using repeated measures ANOVA. Of the five PCs tested only PC3 (male led vocal responses) showed a significant effect of playback treatment ($F_2 = 4.6$, $p = 0.02$). PC3 was influenced heavily by number of playbacks jammed by the male (variable 6), male solo song (variable 8), and female answer rate (variable 5). The last response variable had negative loadings, indicating that female answer rate correlates negatively with the other two variables, which had positive values (Table 3.2). A pairwise comparison within PC3 showed that playback treatment male solo elicited significantly stronger responses from focal pairs than the treatment female solo ($p < 0.01$). Focal pairs also showed a non-significant trend to respond less aggressively towards female solo song than towards duet song ($p = 0.14$). The responses to male solo song and duet song showed no significant differences (Figure 3.2).

In terms of individual variables influencing PC3, we observed that males jammed the duet playback more often than they did the male solo playback, whereas they produced more solo song in response to solo male playback than to duet playback. Female solo elicited only weak responses from focal males. Females showed the highest answer rates during female solo song, and answered less of their mates' calls during male solo playback than during duet playback (Figure 3.3). In other words, the female response was most aggressive towards the female solo song, intermediate towards the duet song and least aggressive towards the male solo song. Male responses were least aggressive towards the female solo song, and equally aggressive towards male solo and duet song.

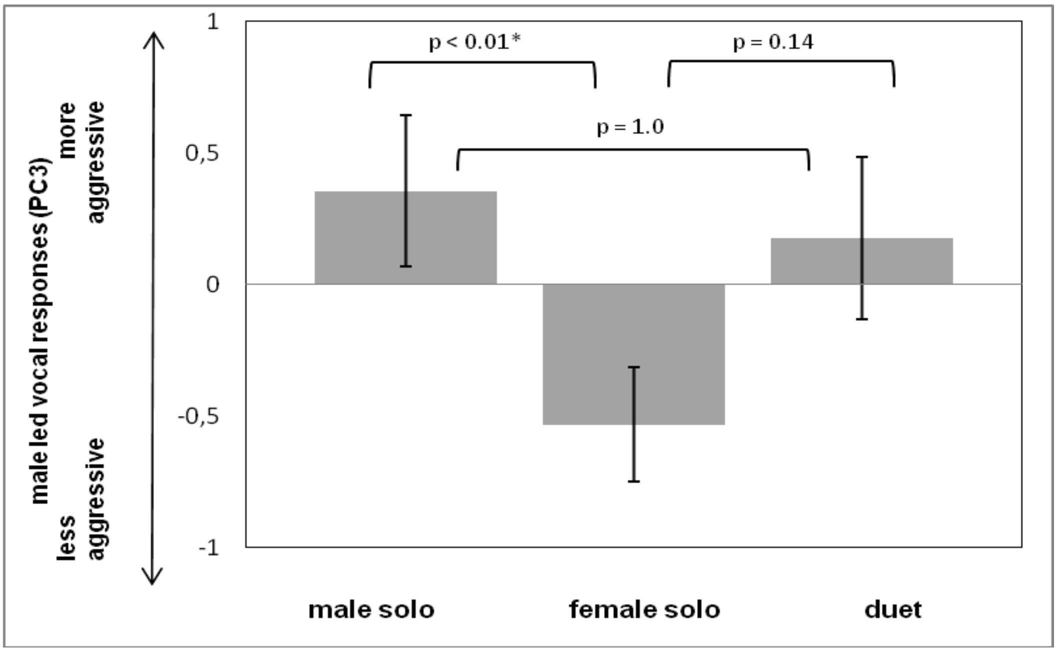

Figure 3.2: Variation in male led vocal responses (PC3) of paired Crimson-breasted Shrikes to three different playback treatments. Significant comparisons are asterisked.

These two treatments differed, however, in the manner males showed their aggression, with male solo treatment eliciting mostly solo song and duet treatment eliciting mostly jamming by the focal males (Figure 3.3). Separate comparisons of each response measure under the three playback conditions are shown in Table 3.2.

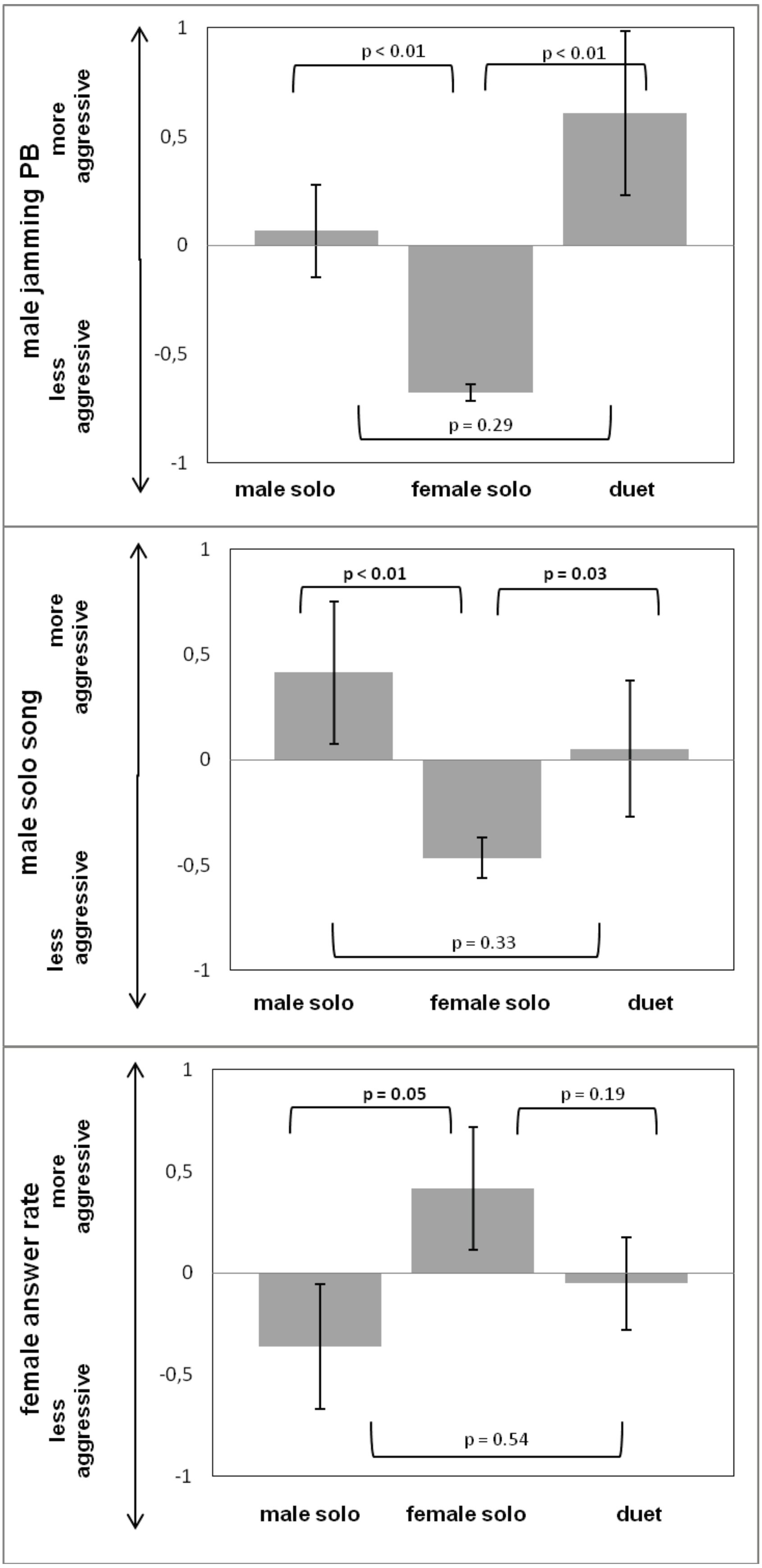

Figure 3.3: Variation of the individual response variables that influence the PC3 during three different playback (PB) treatments.

3.5. Discussion

Our study shows interesting differences between male and female Crimson-breasted Shrikes in response to stereo duet playback experiments. Female vocal responses were significantly stronger towards solo female song than to duets of paired intruders, but responded most weakly to solo male song. Males, by contrast, reacted equally strongly towards male solo song and duets of paired intruders, but significantly less so to female solo song. Males thus appear to use duetting to defend their territory and solo song to guard their mate, whereas females use duetting primarily to guard their mated status against unpaired females. Why should this be so?

Intruders present different risks to males and females. Males run a relatively high risk of being cuckolded, so are vigilant to male intruders, irrespective of whether they are paired or not. Females run the risk of divorce, which is particularly high (60%) among first-time breeders (van den Heuvel et al. 2013, chapter 2), and the associated loss of a territory (in 13 out of 15 cases of divorce males retained the territory, whereas in only two cases females did: van den Heuvel, unpublished results). So similarly to males, females react strongly to intruding females, but unlike males, they show far less concern about paired intruders, as paired females present less of a threat. This is confirmed by qualitative differences in the way the sexes responded to playbacks, with females showing no preference towards the loudspeaker presenting the male or female components of duetting intruders, while males approached the “male” speaker more closely. Neither sex reacts aggressively to solo intruders of the opposite sex, which may present opportunities for extra-pair copulation.

Other duetting birds in which females display a similar pattern of responding more closely to females are the closely related Tropical Boubous (*Laniarius aethiopicus*), Warbling Antbirds (*Hypocnemis cantator*), Eastern Whipbirds (*Psophodes olivaceus*), Barred Antshrikes (*Thamnophilus doliatus*) and Steere’s Liocichla (*Liocichla steerii*) (Grafe and Bitz 2004, Seddon and Tobias 2006, Rogers et al. 2007, Koloff and Mennill 2011, Weng et al. 2012). Logue (2005) concluded that intrasexual aggression in dueting birds may result from territoriality as well as mate guarding. However, if females defend the territory against other females, solo song would be sufficient to accomplish this (Marshall-Ball et al. 2006). We therefore conclude that female Crimson-breasted Shrikes use duets to guard their mates, respectively their exclusive status in the pairbond, against unpaired females.

Mate guarding practised by males using solo song has been reported in another duetting species, the Rufous-and-white Wrens (*Thryothorus rufalbus*) (Topp and Mennill 2008). The nature, as well as the strength, of responses to playbacks differed in male and female Crimson-breasted Shrikes. Females answered a significantly larger proportion of their partner's song, thus creating a duet, when confronted with female solo playback, whereas male solo song evoked more solo song from focal males. Duet song elicited an increased jamming rate from focal males, while focal females did not modify their answer rates, which did not differ significantly from those to male solo playback. If duets serve a function in territorial defence, we made two predictions: that duet stimuli would elicit more intense responses than solo stimuli (Table 3.1); and in addition, if the defence of the territory is a joint endeavour and not sex-specific, both males and females should display equally strong responses to solo song, regardless of the intruders' sex. Our results suggest that males perform mate guarding as well as defending the territory against intruders, with an equivalent emphasis on both functions but by different means. The increased rates of focal male solo song during male solo playback could relate to mate guarding, whereas increased jamming of the duet playback could serve in territorial defence as a disturbance signal. Signal jamming has been reported in a few other duetters (Grafe and Bitz 2004, Tobias and Seddon 2009) but not in the context of territorial defence. Grafe and Bitz (2004) described Tropical Boubou males jamming other males when their female joined intruder males in song, so concluded that jamming is used for mate guarding. Tobias and Seddon (2009) described female Warbling Antbirds jamming their own male in the presence of intruder females, to which males responded by adjusting their own song. Whether temporal coordination mediates coalition strength, which can be disrupted through jamming is an interesting idea but needs confirmation. As female answer rates and consequently duet rates are higher during duet playback than during male solo playback (Figure 3.2) it appears that duets as well as male solos are used to defend the territory, as in Magpie-larks (Rogers et al. 2004), Purple-crowned Fairy Wrens (Hall and Peters 2008) and Yellow-naped Amazones (Dahlin and Wright 2012).

Resource defence and mate guarding are not mutually exclusive functions and for most duetting species there is evidence for multiple duet functions (Hall 2004, Grafe and Bitz 2004, Marshall-Ball et al. 2006, Mennill and Vehrencamp 2008, Benedict 2010, Weng et al. 2012). However, the traditional idea of mate guarding should be reconsidered, especially when practised by females, as the reasons why males and females guard their mates can diverge. Female Crimson-breasted Shrikes use duets to defend their exclusive access to a breeding territory, and male parental investment, whereas males use solo song to reduce their chances of being cuckolded. Therefore we term the females' behaviour securing the exclusive status within the pair bond rather than mate guarding their males, by which it is achieved. While males defend both their land (using duets and solo song) and their lover (using solo song), females use duets to defend their mates, which in turn ensure them exclusive access to a territory. How effective these mechanisms are would be an interesting avenue of further research.

3.6. Acknowledgements

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4 Unfaithful females in the Crimson-breasted Shrike contribute more to duets

4.1. Abstract

Many species of socially monogamous birds follow a genetically promiscuous reproductive strategy. An exception within this group are the duetting birds as these generally exhibit very low levels of extra pair paternity which is thought to be connected to duetting. These duets are proposed to function either in paternity guarding, repelling rival males, or as a signal of commitment to the pair bond, reciprocally eliciting faithfulness between the sexes. In this study we used microsatellites to document extra pair paternity in the Crimson-breasted Shrike (*Laniarius atrococcineus*), a socially monogamous subtropical duetting species. The rate of extra pair paternity was surprisingly high, with extra pair males siring 20% of young in 30% of broods. Furthermore, we compared the levels of extra pair paternity of each pair to certain characteristics of their duets to test the paternity guard and the commitment hypotheses. Our results offered no support that duets function in paternity guarding. We also found no evidence for duets functioning as signals of commitment. Female vocal behaviour appeared to be the reverse of that predicted: levels of infidelity among social females were positively related to female answer rate of their mates' calls. These results indicate that females may use duets to manipulate their mates, and thereby avoid the costs of their extra pair activities.

4.2. Introduction

Vocal duets in birds describe mutual acoustic displays produced by mated pairs that coordinate their signals with some degree of temporal precision (Farabaugh 1982). Avian duetting is rare and occurs in less than 5% of all described species (Hall 2009). Several hypotheses have been suggested to explain duetting based on the idea that their purpose revolves around pair bond establishment and maintenance, in particular mate guarding, paternity guarding, signalling commitment, and reinforcing the pair bond (reviewed in Farabaugh 1982, Hall 2004, 2009). Recent studies have shown that these functions are not mutually exclusive and many species produce multi-functional duets (e.g. Marshall-Ball et al. 2006, Grafe and Bitz 2004, Benedict 2010). As duets can serve more than one function and functions can differ between species a universal theory of duetting remains elusive (Templeton et al. 2011).

We focus here on two hypotheses – the first of which states that duetting is used for paternity guarding (Sonnenschein and Reyer 1983, Levin 1996b). Males could do this in two ways: they can either be more responsive to the female's song to create more duets; or they can increase their initiation rates and thereby increase the duet rate (Sonnenschein and Reyer 1983). In both cases the duet rate is elevated and challengers to the paternity of the social male should be discouraged to mate with his female (e.g. Rogers et al. 2007; Tobias and Seddon 2009). The second hypothesis argues that participation in a duet is a signal of commitment to genetic monogamy and reinforces the pair bond between long-term pair members (Wickler 1980, Hall 2004). Hence, duetting species are commonly expected to have low levels of extra pair matings (EPM) and divorce (Hall 2004, Gill et al. 2005, Douglas et al. 2012). If this is the case, duets should occur at higher frequencies when both pair members are committed to their partner by investing in a monogamous bond. Aspects of duetting that should signal commitment include the likelihood that an individual will answer its partner's song in duet, and the speed or pair specificity of its response (Wickler 1980, Smith 1994, Levin 1996a). Some support for these hypotheses has come from studies investigating the existence of extra pair paternity (EPP) in socially monogamous duetting species with long term pair bonds. In contrast to non-duetting socially monogamous bird species which display on average 11% EPP in 19% of broods (Griffith et al. 2002), analyses of parentage in seven socially monogamous bi-parental duetters revealed high levels of fidelity with just one North Temperate Zone exception, the California Towhee (*Pipilo crissalis*), (Benedict 2008; Table 4.1). Few studies have investigated the genetic mating systems of duetting species, but it is nonetheless clear that EPP is consistently lower among subtropical and tropical duetting birds than among their nonduetting counterparts (Douglas et al. 2012). This may have contributed to the misconception that duetting is somehow associated with genetic monogamy (Farabaugh 1982, Hall 2009, Douglas 2012). More than 80% of the duetting species live in tropical or subtropical regions (Farabaugh 1982, Langmore 1998, Slater and Mann 2004) and possess most of the traits typically associated with low levels of EPP such as a non-migratory lifestyle, extended breeding seasons, low levels of divorce, high annual adult survival, long-term pair bonds and enhanced paternal care (Cramer et al. 2011).

In this study, we combine an analysis of the acoustic parameters of duetting with a genetic analysis using microsatellites to investigate the mating patterns of the Crimson-breasted Shrike (*Laniarius atrococcineus*). This species represents an ideal candidate for such a study as it is a subtropical passerine exhibiting the typical traits described above: pairs live in long-term socially monogamous bonds and occupy year-round territories which they defend against conspecifics over several years (van den Heuvel et al. 2013). Male parental care is high as they participate in nest building, incubation and provisioning of nestlings and fledglings (Tarboton 1971; 1998; Ridley and van den Heuvel 2012). If duets have a function in paternity guarding, we predicted that males which were more responsive to their females' songs or males that had higher duet initiation rates should have lower levels of extra pair offspring (EPO) than males that do not follow this strategy. Likewise, if duets are signals of commitment to monogamy which reinforce the pair bond, we expected that pairs with high answer rates and short response intervals for both sexes should produce low levels of EPO.

Table 4.1: The seven bi-parental socially monogamous duetting bird species on which paternity analysis have been performed including the identified levels of EPP and the species' distribution ranges.

Species	levels of EPP/ nests with EPP	distribution
Carolina Wrens (*Thryothorus ludovicianus*) (Haggerty et al. 2001)	0% / 0%	North Temperate
California Towhee (*Pipilo crissalis*) (Benedict 2008)	26% / 42%	North Temperate
Eastern Screech-Owls (*Otus asio*) (Lawless et al. 1997)	0% / 0%	North Temperate
Magpie-larks (*Grallina cyanoleuca*) (Hall and Magrath 2000)	3% / 6%	Subtropical
Dusky Antbirds (*Cercomacra tyrannina*) (Fleischer at al. 1997)	0% / 0%	Tropical
Rufous-and-white Wrens (*Thryothorus rufalbus*) (Douglas et al. 2012)	2% / 6%	Tropical
Buff-breasted Wrens (*Thryothorus leucotis*) (Gill et al. 2005)	4% / 3%	Tropical

4.3. Materials and Methods

4.3.1. Study species and field methods

A colour-banded population of Crimson-breasted Shrikes was studied at the Kuruman River Reserve (26°58’ S, 21°49’ E) in the southern Kalahari Desert, South Africa during three consecutive breeding seasons (2008/2009, 2009/2010 and 2010/2011), each lasting from September until March. The Crimson-breasted Shrike is a medium-sized passerine (42-58 g) and its range is centred on the Kalahari Desert region (Hockey et al. 2005). This species is monomorphic, socially monogamous and invests in bi-parental care of young (incubation and chick-rearing), forming long-term bonds and occupying territories that they defend year-round (Tarboton 1971; Merkle 2006; van den Heuvel et al. 2013). The reserve covers an area of ca 3300 ha and consists of semi arid bushveld and acacia savanna (a detailed description of the climate and vegetation is given in Clutton-Brock et al. 1999). This study includes 19 pairs and 83 nestlings from 44 broods sampled across three breeding seasons. All nesting attempts were monitored and success or failure of broods were recorded until the fledglings were independent and had left their natal territory (± 90 days after fledging) (van den Heuvel et al. 2013). Adult individuals were trapped either in spring traps baited with mealworms or in mist nets and banded with a unique combination of two coloured and one stainless steel band with an engraved number provided by the South African Bird Ringing Unit (SAFRING). Nestlings were banded the same way at 11 days of age when their ankle joints were big enough so the bands would not slide over them, which was usually one or two days after pin break. During banding a blood sample of ca. 60 µl was obtained by brachial venipuncture (under SAFRING license no. 1510) of each individual. Adult birds were blindfolded during the banding process to calm them and no adverse effects of the procedures were observed. Preservation of blood samples and subsequent DNA extraction followed the methods described in Friedl and Klump (1999). Each individual was sexed using a PCR-based analysis that amplifies dimorphic fragments on the Z and W sex chromosomes (Griffiths et al. 1998).

4.3.2. Paternity analysis

Using microsatellite genotyping we intended to confirm or negate paternity of the social father to each of the 83 sampled nestlings. Of each individual, 1 µl total genomic DNA was sent to GenoScreen, France (www.genoscreen.fr) for microsatellite library creation, identification of polymorphic microsatellite markers and subsequent genotyping. All individuals were genotyped at five variable nuclear microsatellite loci (CBS86992_28, CBS 86992_29, CBS 86992_31, CBS 86992_32 and, CBS 86992_35, Table 4.2). DNA was amplified with fluorescently labelled primers (NED, 6-FAM, VIC) using Polymerase Chain Reaction (PCR). Amplified PCR products were run on an ABI 3730 XL (Applied Biosystems) and alleles were scored using GeneMapper (Applied Biosystems).

We used a likelihood-based approach for the determination of paternity of the alleged fathers. For each combination of female, her offspring and the social male a confidence level for assignment based on the logarithm of odds (LOD) score of the candidate father was generated. Using allele frequencies from the study population, a simulation program generated criteria for LOD that permitted the assignment of paternity to the social male with a known level of statistical confidence. Significance was set at 0.05 meaning an alleged father was only confirmed or rejected with 95% or higher confidence. In this sense, distributions of LOD values were generated through 100000 simulations of paternity inference. The following parameters were taken into account in the simulation model: number of candidates' rates, proportion of candidate male samples, proportion of loci typed and error rate (1.5%). The statistical analysis was performed using the program CERVUS 3.0.5 (Kalinowski et al. 2007) including a test for null alleles, calculation of heterozygosity, calculation of exclusion probabilities, and to test that loci were in Hardy-Weinberg equilibrium for the entire population. This allowed us to estimate the level of cuckoldry for each alleged father (Table 4.3).

4.3.3. Duet-related characteristics

To investigate whether duets function as paternity guards by increasing responsiveness to female song and initiating duets at higher rates we calculated the following variables: (1) male answer rate and (2) male duet initiation rate. To test if duets are signals which communicate commitment by answering the partner's song more often and having short response intervals we used again the variable (1) male answer rate and additionally calculated (3) female answer rate; (4) male response interval and (5) female response interval. Initiation and answer rates were derived from continuous high-quality recordings of 10 - 30 minutes (the term 'initiation' denotes here the first bird to sing). Answer rates describe the proportion of calls that have been answered by the mate to create a duet and were calculated as follows: female answer rate = 100 x total male initiated duets/(total male initiated duets + total male solo), and vice versa for male answer rates (Rogers et al. 2007). A response interval was defined as the time elapsed between onset of one bird's motif and the onset of the other bird's next motif. Short response intervals indicate a high speed of the response.

We applied a generalized linear model (GzLM) with an ordinal logistic distribution and a logit link function to assess the effects of these variables on the proportion of cuckoldry experienced by each male over three seasons. The level of cuckoldry was the response variable (a level of 100% cuckoldry means that the tested male has not sired any of the offspring he and his social mate care for and 0% cuckoldry means a male has sired all of the offspring) and the five independent variables were entered as covariates. Additional post-hoc analysis was performed using a general linear model (GLM) which included the significant variables and provided an estimate of explained variance for the model. The tests were conducted in SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and significance levels were set at $\alpha = 0.05$.

4.4. Results

4.4.1. Genetic markers

For the loci shown in Table 4.2 the mean number of alleles per locus was 8.4 (range 4 - 13) and the mean percentage of typed individuals was 99%. Four of the five microsatellite loci were in Hardy-Weinberg equilibrium. As deviations at just one locus may occur because of natural selection on a nearby gene (T. Marshall pers. comm.) we decided against an exclusion of this locus. All five markers had an average expected heterozygosity of 0.7 (0.45 - 0.86, Table 4.2). The average probability that this set of markers will not exclude an unrelated candidate father from parentage of an arbitrary offspring when the genotype of the mother is known was 0.02.

Table 4.2: Characterization of 5 microsatellite loci for the Crimson-breasted Shrike population from the Kuruman River Reserve showing number of alleles (k), expected unbiased heterozygosities (Hexp), exclusion probability (NE-1P and NE-2P) and test for deviation from Hardy–Weinberg equilibrium (ns = non significant; *** p < 0.001).

Genetic marker	k	H_{exp}	NE-1P*	NE-2P**	HW
CBS866992_28	13	0.858	0.444	0.283	NS
CBS866992_29	11	0.799	0.565	0.387	***
CBS866992_31	5	0.604	0.789	0.610	NS
CBS866992_32	9	0.798	0.579	0.401	NS
CBS866992_35	4	0.448	0.899	0.779	NS

* Average non-exclusion probability for the candidate father.

** Average non-exclusion probability for the candidate father given the genotype of the known mother.

4.4.2. Paternity assignments

We genotyped 83 offspring in total, but for 19 individuals (23%) the paternity of the social father could not be confirmed or negated with 95% certainty. Therefore, these 19 unassigned offspring and consequently one tested pair and four tested broods were excluded from further analysis. For the remaining 64 offspring, we determined whether they were within pair offspring (WPO) sired by the social father or extra pair offspring (EPO) sired by another male (Table 4.3). This resulted in a total of 13 EPO out of 64 tested offspring (20%) reared in 12 out of 40 tested broods (30%). Of the remaining 18 tested males, eight (44.5%) were cuckolded by their females whilst the levels of cuckoldry varied considerably (Table 4.3). Females for which we sampled multiple broods showed varying levels of EPP between and within individuals. Six out of ten females with multiple nests had at least one brood containing EPO (ranging from 33 – 77% of broods with EPO); the remaining four females showed no extra pair activity.

4.4.3. Duet-related characteristics

Male song initiation rates were unrelated to their levels of cuckoldry (Wald statistic = 0.121, df = 1, p = 0.728, n = 12) as were male answer rates (Wald statistic = 1.227, df = 1, p = 0.268, n = 12). Female answered significantly more of their partners’ songs when the level of cuckoldry increased (Wald statistic = 6.068, df = 1, p = 0.014, n = 12): in other words faithful females that had no EPO were less likely to answer their mates’s calls than unfaithful females. The speed of the responses of males and females to their partners did not change with changing levels of cuckoldry (males: Wald statistic = 1.239, df = 1, p = 0.266, n = 12; females: Wald statistic = 0.527, df = 1, p = 0.468, n = 12). The post-hoc analysis including the significant variable female answer rate showed that 52% of the observed variance could be explained with this model (F = 10.818, df = 1, R^2 = .520, p = 0.008, n = 12).

Table 4.3: Descriptive statistics of the 19 tested pairs of Crimson-breasted Shrikes and their offspring regarding the occurrence of extra pair paternity and the degree to which males have been cuckolded between 2008 and 2011 in the Kuruman River Reserve, South Africa. Unassigned offspring where the social father could not be confirmed or negated with 95% confidence were removed from further analysis. The asterisks mark the pairs which entered the analysis including duet characteristics.

pair ID	observed broods	broods with EPP	total offspring sampled	WPO	EPO	unassigned offspring	level of cuckoldry of social father [%]
AW	1	1	2	0	2	0	100
AY*	2	0	5	5	0	0	0
AZ*	3	2	6	2	2	2	50
B*	8	1	16	10	1	5	6.25**
C*	4	2	7	2	2	3	28.57**
E*	2	1	3	2	1	0	33.33
FB	1	0	2	2	0	0	0
FX*	1	0	1	1	0	0	0
I*	3	0	6	6	0	0	0
L	1	0	3	1	0	2	0
O*	4	3	8	4	3	1	42.86
P	1	0	2	2	0	0	0
Q*	5	0	9	7	0	2	0
V	1	0	2	1	0	1	0
WX*	1	0	2	2	0	0	0
XY*	1	1	2	1	1	0	50
Y*	2	1	3	2	1	0	33.33
Z	2	0	3	1	0	2	0
MG	1	-	1	-	-	1	-
Total	44	12	83	51	13	19	

**These two values have been miscalculated and the corrected values are 9.09 for pair B and 50 for pair C. These changes result in a significant difference of the variable 2) male answer rate. The correct presentation of the results and the revised discussion is currently in preparation for publication as original article.

4.5. Discussion

The Crimson-breasted Shrikes in our study population have a surprisingly high level of EPP (20% offspring in 30% of broods), especially considering that they belong to a duetting, non-migrating, year-round territorial species with long-term pair bonds. Of the 18 tested females 44.5% engaged in extra pair activity, but their strategies varied for each brood. Some females stayed faithful for multiple broods, whereas others cuckolded their mates in up to 77% of their broods. Genetic studies of other socially monogamous duetting birds suggest that EPP levels are generally low, especially for tropical and subtropical species. Magpie-larks (*Grallina cyanoleuca*) were found to produce 3% EPO in 6% of broods (Hall and Magrath 2000); Buff-breasted Wrens (*Thryothorus leucotis*) show 4% EPO in 3% of broods (Gill et al. 2005); Rufous-and-white Wrens (*Thryothorus rufalbus*) have 2% EPO in 6% of their broods (Douglas et al. 2012); and Dusky Antbirds (*Cercomacra tyrannina*) have no EPP (Fleischer at al. 1997). Levels of EPP in Crimson-breasted Shrikes are slightly lower than in California Towhees (*P. crissalis*), another socially monogamous duetter with 26% EPP (Benedict 2008), which inhabits North Temperate regions. We know that duetting has evolved independently several times (Farabaugh 1982) but it is yet unclear, whether each time these signals might have evolved for the same reasons or if these reasons differ with climate region as considered by Douglas et al. (2012) who suggested that EPP might play a different role in north temperate than in tropical regions.

We expected that if duets are used as paternity guards in this species, males with higher answer rates and higher duet initiation rates would experience lower levels of cuckoldry. Our results do not support this hypothesis as male answer rates and duet initiation rates were unaffected by the levels of cuckoldry experienced by males. These results are consistent with findings from other duetting species which also rejected this idea, for example Purple-crowned Fairy-wrens (*Malurus coronatus*) (Hall and Peters 2009), Magpie-larks (*G. cyanoleuca*) (Hall and Magrath 2000) and Buff-breasted Wrens (*T. leucotis*) (Gill et al. 2005). In all three cases this hypothesis was rejected on the basis of no differences in call rates during fertile as opposed to non-fertile periods, and not on the basis of genetic analysis.

The predictions for the commitment hypothesis we tested were that pairs with higher answer rates and shorter response intervals for both sexes would produce fewer EPO than pairs with lower answer rates and longer response intervals. These duet characteristics could be used by either sex to communicate its commitment to genetic monogamy and elicit a reciprocal response. Male answer rates and male response intervals showed no association with the levels of cuckoldry experienced by males. This result can be interpreted in two ways. One possibility is that males don't use these characteristics to communicate their level of commitment to females, and females cannot react reciprocally with the same level of fidelity. Or, the males do communicate their commitment but cannot in return expect commitment to social monogamy from the females. Female response intervals also showed no relation to the level of cuckoldry experienced by males, implying that females don't use rapid answering to communicate their commitment. Female answer rates did show a relationship with the level of cuckoldry experienced by social males, but this relationship was opposite to that predicted. Unfaithful females paired to males with high levels of cuckoldry were significantly more likely to answer their mates' song than faithful females. The theory of manipulation implies that conflicts in pair bonds lead to exaggerated signals that manipulate the receiver to the advantage of the sender (Krebs and Dawkins 1978, Wachtmeister 2001). Unfaithful females answered their mate more often than faithful females and instead of being a signal of commitment, answer rates may represent a manipulative strategy which unfaithful females might have adopted to preclude the potential costs of EPM.

Generally, EPP is relatively uncommon in species with strong pair bonds (Segelbacher et al. 2005), but certain conditions might allow females to engage in EPM without being punished by their social mates (Benedict 2008). Potential costs of extra pair matings for females are reduced male parental care and divorce (reviewed in Griffith et al. 2002), which can exceed the benefits of such matings (Cramer et al. 2011). These costs might be avoidable and females do not have to limit EPM if, for example, inconsistent patterns of paternity among broods exists, as shown for our study species, which make it difficult for males to modify their behaviour to bias benefits towards their own offspring (Westneat and Sherman 1993). Even if males could assess their risk of cuckoldry, for example by measuring female absence during the fertile phase (Valera et al 2003), the positive association between female answer rates and levels of cuckoldry might indicate that unfaithful females are manipulating their social mates to avoid costs such as reduced investment, physical retaliation or divorce.

This study is the first to report a relationship between certain duet characteristics of pairs and the corresponding levels of EPP. Our results do not support the hypothesis that males use duets for paternity guarding or as a signal of commitment to genetic monogamy. This is consistent with previous findings refuting the commitment hypothesis on the basis of lacking differences in response intervals between pairs (Grafe et al. 2004), lower song rates in mated than unmated individuals (Levin 1996b), and the occurrence of high EPP levels despite duetting behaviour (Benedict 2008). Female Crimson-breasted Shrikes presumably use duets to guard their males against unpaired females, whereas males use solo song in response to rival males which can be interpreted as mate guarding (van den Heuvel et al. chapter 3), which was demonstrated by monitoring responses to different playback stimuli. But clearly this male mate guarding behaviour did not completely prevent female extra pair activity in this population: whether quality differences in the mate guarding behaviour of males exist which determine its effectiveness would be an interesting avenue of future research. Female Crimson-breasted Shrikes seem to profit much more from duets than males do, which would explain that usually the female is responsible for creating duets by answering her mate in this species (van den Heuvel et al. 2013). Gill et al (2005) and Douglas et al (2012) have both questioned whether males of duetting species need to guard their mates against challenges to their paternity, a question for which we can now provide an answer with the results of the present study. Crimson-breasted Shrikes, together with California Towhees, demonstrate that males of bi-parental, socially monogamous duetting species with long-term pair bonds and enhanced parental care do experience high levels of EPP, and thus have a need to guard their paternity.

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Lebenslauf

Dipl.-Biol. Irene M. van den Heuvel

geboren am 15. Oktober 1979

in Itzehoe

Staatsangehörigkeit: niederländisch

Seit April 2008	Promotion bei Prof. Dr. Georg Klump in der Arbeitsgruppe “Zoophysiologie und Verhalten” an der Carl-von-Ossietzky Universität Oldenburg, in Kooperation mit der Universität Stellenbosch.
September 2008 bis Januar 2011	Jahres-Stipendium mit Anschluß-Förderung für Doktoranden vom Deutschen Akademischen Austausch Dienst (DAAD).
April 2005 bis Oktober 2007	Hauptstudium der Biologie mit den Nebenfächern Naturschutz und allg. Botanik an der Universität Hamburg. Titel der Diplomarbeit: “Scatterhoarding in Cape Fynbos Rodents: The Acomys’ perspective”, in Kooperation mit der Universität Kapstadt.
Oktober 2002 bis Juni 2004	Grundstudium der Biologie an der Georg-August-Universität Göttingen.
Oktober 2004 bis Februar 2005	DAAD Reisekosten-Stipendium für ein Auslandspraktikum als Feld Assistent bei Okatumba Wildlife Research in Namibia.
August 1999 bis Januar 2002	Berufsausbildung zur Tierarzthelferin in Borsfleth.
Juli 1999	Abitur am Fachgymnasium Itzehoe - sozialwirtschaftl. Zweig.

Erklärung gemäß § 10 Absatz (2) der Promotionsordnung der Fakultät für Mathematik und Naturwissenschaften der Carl von Ossietzky Universität Oldenburg vom 11.12.2003.

Hiermit erkläre ich, daß ich die vorliegende Dissertation selbständig verfasst und nur die angegebenen Hilfsmittel verwendet habe. Die Dissertation hat weder in Teilen noch in ihrer Gesamtheit einer anderen wissenschaftlichen Hochschule zur Begutachtung in einem Promotionsverfahren vorgelegen. Die wesentlichen Teile der Dissertation wurden bereits veröffentlicht bzw. sind zur Veröffentlichung eingereicht, wie an den entsprechenden Stellen angegeben. Bei der Durchführung der Experimente sowie bei der Datenextraktion aus den Experimentaufnahmen wurde ich von wissenschaftlichen Hilfskräften unterstützt. Diese Arbeiten wurden alle unter meiner Überwachung übernommen.

Oldenburg, den 30. Oktober 2012

Irene van den Heuvel

Erklärung gemäß § 8 Absatz (3) der Promotionsordnung der Fakultät für Mathematik und Naturwissenschaften der Carl von Ossietzky Universität Oldenburg vom 11.12.2003.

Der Anteil der Mitautoren an den Veröffentlichungen bestand in der Betreuung der Arbeit und Korrektur der Manuskripte. Die Versuche wurden gemeinsam mit den Betreuern entwickelt, die Durchführung und Auswertung der Experimente lagen in meiner Hand.

Oldenburg, den 30. Oktober 2012

Irene van den Heuvel