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**The functional significance of duetting in the
canebrake wren (*Thryothorus modestus zeledoni*)**

by

Lorraine Marshall-Ball

Submitted for the degree of Doctor of Philosophy to the
University of St. Andrews



Thesis supervisor:
Professor P. J. B. Slater
School of Biology
University of St. Andrews

September 2003



Abstract

The phenomenon of duet singing has been described in a polyphyletic array of bird, primate and Orthopteran species, however, a surprising paucity of robust investigative studies means that we still have very little awareness of the roles duets play in the behaviour of mated pairs of animals. This study investigated the functions of duets in a sub-species of the plain wren, known as the canebrake wren (*Thryothorus modestus zeledoni*). This Neotropical species performs highly complex antiphonal duet songs, and the aim was to test, through both observations and experiments, several hypotheses for duet function, as well as to quantify the behaviour of this little-known species. Duet singing in the canebrake wren appears to function in both cooperative territorial defence by the pair, and in mate guarding, particularly by males. The duets involved a learning period in the ability to form a consistent duet repertoire, and this led to differences in the performances of different pairs. Despite predictions that duet learning would provide a signal of pair bond strength, the behaviour was such that it was unlikely to function in signalling to other pairs. The learning period may instead have served as an investment in the pair bond, and thus as an ongoing affirmation of commitment to the mate. Aside from defining functions that may have been the reason for the evolution of duetting, this study also demonstrates that duet songs are capable of carrying information on the breeding state of the pair, individual aggression levels, and individual characteristics. Females in particular, showed characteristic changes in duetting behaviour, appearing to use duet song to signal their fertility, and later reducing their duetting behaviour in response to a trade-off with parental care. Both males and females shared part of their phrase repertoires with conspecifics, and this repertoire overlap allowed the use of phrase type matching as an aggressive signal during counter-singing. Interestingly, although pairs appeared to work very cooperatively in response to intrusions, with the duets themselves as a tightly coordinated performance; aggressive behaviour and aggressive signals were distinctly intra-sexual. Thus within the cooperative behaviour of duet singing, each member of the pair was able to transmit independent signals about both breeding state and aggressive levels.

Declaration

- i) I, Lorraine Marshall-Ball, hereby certify that this thesis, which is approximately 45,000 words in length, has been written by me, that it is a record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

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- ii) I was admitted as a research student and candidate for the degree of Doctor of Philosophy in November 2000; the higher study for which this is a record was carried out in the University of St. Andrews between 2000 and 2003.

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- iii) I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Doctor of Philosophy in the University of St. Andrews and that the candidate is suitably qualified to submit this thesis in application for this degree.

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Acknowledgements

There are very many people who deserve my thanks for helping me to survive my PhD. The first person to thank is, of course, my advisor, Peter Slater. He has been the perfect combination of a fount of wisdom, eternal optimist, and non-meddler! He has never appeared disappointed in my many bouts of cluelessness, and always averted my looming crises without even seeming to try. The sound communication group has been a constant source of inspirational advice, support and computer-fights. Nigel Mann particularly, has been generous with his time in the few moments when we were on the same continent, and he, Vincent Janik, and Jared Wilson were kind enough to slog through several of my chapters without a word of complaint. Murray Coutts saw me through my complete and blissful ignorance of anything involving electricity; from replacing a hard-drive that had taken to eating itself after being invaded by termites, to building me a wonderful playback system with which I deafened several areas of rainforest. Many of my statistics-induced blind panics were solved by an array of the wonderful 'grown-ups' in Biology, especially Mike Ritchie, Jeff Graves and Joe Tompkins. They were all very good at making me feel better, although still often bewildered.

All of the 'kids' in Biology are, it has to be said, pretty fab. It has been wonderful to be part of a community of friends who are always there when beer therapy is needed, and who made me feel instantly as if I belonged, even when I was spending more than half my time several thousand miles (and a few worlds) away. They have been very good at rebuilding my alcohol tolerance after months with Americans, and giving me a good appreciation of decent BBQ food, awful night clubs, kite flying, Cranium evenings, and the odd dodgy night with flaming Drambuies or red bull and vodkas. Big fat thanks to you all.

Before I forget, I must thank my two field assistants, Claire Brenner and Carol Christie, who worked for me voluntarily for part of their holidays. I made them get up at 4.30am, work in the silliest tropical rainstorms, measure 1000s of bits of grass, and nearly got them killed in a rather drunken horse race. But they still seemed to enjoy it, and I am both grateful and slightly bemused by their spirit and hard work. There were also the utterly wonderful staff at both La Suerte and El Zota. All of the staff at both stations (nasty, evil builders very definitely excluded!) were welcoming and helpful. They were also keen assistants whenever I needed them, and became, after some persuasion, distinctly pro-snakes, night walks and all things wren-like. Israel Mesen, in particular, was a friend, a rock and a little brother proxy. Kim Dingess found me, after several months of solitude, slightly loopy and lacking in several social skills. Despite this, she took Nigel's word that I was a nice person, and set about returning me to sanity, and teaching me how to not hide from the influx of students! But I think she may, just about, have had enough of wrens for this lifetime.

A quick mention of my escapism friends, the ex-Aberdeen contingent, whose willingness to go camping at daft times of year, and at very short notice, provided the bolthole I sometimes needed. The nights of whisky, talk, whisky, Guinness, philosophical talk, and more whisky; have led me to realise that Resolve really is the best invention ever. And that old friends are the best relaxation ever.

And now onto the sappy stuff. My family, what can I say? They are, to say the very least, a unique bunch (all new step-bits included). They are also the only reason I am where I am today. The assuredness of their love, and their unfailing belief in me, combined with the grace with which they accept my slightly unconventional life, has given me the strength to do what I love most. My mother, who doubles as my closest friend, has loved, counselled, supported, joked, forgiven and bullied me, all with perfect timing. She is my safety net and my foundation. I apologise to them all for the worry they live with while I am having a fab time in remote corners of the world, and promise that someday I might get a job in the UK. I also promise to try to cut down on the near-death experiences in future. I have, after all, not got any more appendixes to burst! And lastly, Jared, who only appeared in my life at the beginning of the year, but who is already the centre of it. Thank-you; for being my cornerstone and my sunshine.

Dedication

I would like to finish with a wee dedication. My little (or not so little) brother, Stuart, has been through some dark and lonely times over the last three years, and before. There have been times when I have feared for him with all my soul. But he has fought, and is still fighting, his way back; and it is not easy or quick. I am so proud of everything he is, and of the integrity and love and humour that he never lost along the way. His successes in his life are greater than anything that I have achieved on paper, and I dedicate this thesis to him as a token of the oceans of respect and love I have for him.

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Chapter 1: Introduction

This chapter aims to provide a general introduction to duet singing, specifically as performed by some species of birds. To discuss duetting, and the functions of duetting, we must first be familiar with the functions of solo song by both males and females. Thus the first section of this chapter provides a brief summary of the functions of solo song in birds. This is an immense subject, but for the purposes of providing an introduction to duetting, I present here a précis of the predominant patterns to emerge from the vast array of literature. From this background, I go on to consider the occurrence of duets, and the form that they take in different species. The first review of duet singing (Helversen, 1980) outlined nine potential functions for duets, and discussed which of these functions were likely to have driven the evolution of the phenomenon. In this chapter, the nine original theories are discussed in turn, with respect to more recent research and hypotheses. The state of our current understanding of duetting is discussed and the aims of this thesis outlined.

1.1 *Solo singing*

In the majority of animal species that produce song, it is the male who is responsible for the performance. Males of animals as diverse as crickets, frogs, whales and flycatchers will use a form of song for one or both of two fundamental functions: mate attraction and resource defence. Passerine birds, in particular, demonstrate wonderfully ornate acoustic performances that have inspired a fascination in naturalists and biologists for many years. The more recent development of methods for laboratory studies and equipment for sound analysis, along with a greater emphasis on robust experimental design has permitted more and more quantitative examination of precisely how male song is used to attract females or repel rivals (e.g. Becker, 1982; Catchpole, 1982; Kroodsma, 1991). Aspects such as repertoire size (e.g. Kroodsma and Verner, 1987; Balsby and Dabelsteen, 2001), use of specific song types (e.g. Burt *et al.*, 2001; Naguib *et al.*, 2002), and effects of fitness on song (e.g. Galeotti *et al.*, 1997; Buchanan *et al.*, 1999; Forstmeier *et al.*, 2002; Buchanan *et al.*, 2003) have all received considerable attention in recent years. Many of these studies have demonstrated how female choice may have acted as a driving force for

the song variety seen in some species (e.g. Collins, 1999; Martin-Vivaldi *et al.*, 2000; Balsby and Dabelsteen, 2001). It seems that in many avian species, female preferences and energetic costs of singing (including retaliation costs from rivals) have led to the evolution of honest or 'handicap' signals of individual quality (Zahavi, 1975; Grafen, 1990). Recent studies have demonstrated that male song can serve as such a signal (Gil and Gahr, 2002).

Although the general view is of males singing primarily to attract mates and to defend resources, there are an increasing number of bird species in which females have been reported to sing. In the same way that male song ranges from the extremely simplistic (e.g. house sparrow *Passer domesticus*) to highly elaborate (e.g. nightingale *Luscinia megarhynchos*), so female song occurs in various forms (Eens and Pinxten, 1998; Langmore, 1998). Originally considered by some to be a functionless by-product of hormonal fluctuations (see Catchpole and Slater, 1995), there is a growing body of evidence that females sing for much the same reasons that males do: to attract or defend mates and to defend territories (Yasukawa *et al.*, 1987; Arcese *et al.*, 1988; Baptista *et al.*, 1993; Langmore *et al.*, 1996; Langmore, 1998). The first thorough study of female song was on the song sparrow (*Melospiza melodia*), where females appeared to use song in exclusively intra-sexual territorial competition (Arcese *et al.*, 1988). Females of the red-winged blackbird (*Agelaius phoeniceus*) suffer reduced breeding success if their mate is polygynous, and they use solo songs both to exclude other females and in pair bond maintenance with the male (Beletsky, 1983; Yasukawa *et al.*, 1987; Whittingham *et al.*, 1997). In contrast, Langmore *et al.* (1996) found that female song in the alpine accentor (*Prunella collaris*) was used solely to attract males and not in any defensive role. The authors proposed that female song in this context served to increase the numbers of males available to the female, and thus, through either male-male competition or direct female choice, enabled mating with better quality males. The study of female song as a behavioural phenomenon in its own right is still in its infancy (Langmore, 1998). Further research is needed to build up detailed mechanistic and functional information equivalent to that available for male song. As yet, there is very little understanding of the natural hormonal control of song in females (Schwabl and Sonnenschein, 1992; Elekonich and Wingfield, 2000), nor whether the evolution of

female song has been through either intra- or inter-sexual selection to form an indicator of quality as has been demonstrated for some male songs.

1.2 *The occurrence and form of duets*

A step beyond the production of solo song by both sexes is the coordination of these songs into a joint performance. Duet singing is a joint performance of song from the two members of a pair. Definitions vary and, according to some, a duet may also be any visual or acoustic display by any group of two or more individuals (Serpell, 1981a). However, terms such as *pas de deux* and chorus singing respectively cover joint performances based on movement, and singing performances by social groups; and so, in this study, the term duet is used to refer exclusively to a mated pair producing coordinated song.

Duet song is reported in three groups of animals: Orthopteran insects (e.g. Heller *et al.*, 1997; Tauber *et al.*, 2001), primates (e.g. Thalmann *et al.*, 1993; Geissmann, 2002; Muller and Anzenberger, 2002), and birds (e.g. Payne, 1971; Seibt and Wickler, 1977; Farabaugh, 1982). As with solo songs, duets have reached levels of greatest complexity in passerine birds and, again, different species demonstrate an array of forms (Payne, 1971; Helversen, 1980). There are two basic categories for duet structure: they can be formed by the totally overlapping or 'synchronous' songs of two birds, or they can be formed through call and response or 'antiphonal' singing with one bird answering the other, one or many times.

Synchronous duets can be simpler to produce than antiphonal duets, as the temporal coordination required is only to sing at the same time as one another. In some species, however, this synchrony is almost perfect, for which a great deal of inter-individual coordination is required (Payne and Skinner, 1970). Antiphonal duets, by contrast, involve timing of singing so that each individual's song follows on from that of the partner, sometimes forming repeating cycles of call and answer. Both categories can arise from songs that, for each bird, are either very simple or very ornate. Thus in a non-passerine Gruiforme, the Aldabra white-throated rail (*Dryolimnas cuiveri aldabranus*), both sexes produce very simple one-syllable phrases in approximate synchrony (Huxley and Wilkinson, 1979). Yet in black-

throated wrens (*Thryothorus atrogularis*) which again have overlapping song, both sexes have highly complex, slightly sexually differentiated song phrases (Skutch and Stiles, 1989 and this thesis, Chapter 5). Antiphonal duets of the utmost simplicity are demonstrated by several non-passerine and passerine species such as owls (Klatt and Ritchison, 1993; Appleby *et al.*, 1999) and some *Laniarius* shrikes (Payne and Skinner, 1970), where only one call and response of structurally simple calls is produced. Remaining within the African shrikes, fairly simple but impressively well coordinated antiphonal duets, come from certain African barbets (Payne and Skinner, 1970). Pairs of the black-collared barbet (*Lybius torquatus*), for example, sing long and rapid cycles of antiphonal song, with each bird having a simple short syllable at slightly differing frequencies (Payne and Skinner, 1970). At the other end of the scale for antiphonal duets, several species of *Thryothorus* wrens show large sexually differentiated repertoires of complex phrases, that they again combine in precise prolonged cycles to form duets (Farabaugh, 1983; Levin, 1985; Mann *et al.*, 2003).

Most of the examples given above are of species where the contribution to the duet is roughly equal for both sexes, and thus where female song is as highly developed as male song. This is, however, not always the case. In many duetting species, the duet is formed when females produce simple calls or short song phrases in combination with much more fully developed male song. In two more examples from *Thryothorus* wrens, female Carolina wrens (*T. ludovicianus*) sometimes produce a rough 'churr' call overlapping the male song; and female striped-breasted wrens (*T. thoracicus*) have a single note that is sung antiphonally between long and complex male phrases (Farabaugh, 1982; Skutch and Stiles, 1989 and pers. obs.).

1.3 *Functions of duets*

We would expect duetting to incur a greater investment in song development than solo song. However simple the duet, singing even approximately at the same time as a mate requires remaining attentive to their song and some degree of behavioural coordination with them. This greater investment involved in duetting has begged the question of what evolutionary advantages over solo song led to its evolution. The fact that duetting has arisen in over 200 species from a wide polyphyletic array

strongly suggests that a selective advantage to the behaviour must be present (Farabaugh, 1982).

Duetting species often fit a set of general characteristics such as sexual monomorphy, social monogamy, year-round territoriality, and a tropical distribution (Seibt and Wickler, 1977; Wickler and Seibt, 1980; Short and Horne, 1983; Malacarne *et al.*, 1991, Langmore, pers. comm.). These very general patterns have been cited as evidence for various functions such as formation and maintenance of long-term pair bonds and territory defence (Seibt and Wickler, 1977; Wickler and Seibt, 1982). The first review that pulled together and defined the various proposed functions for duet singing was by von Helversen (1980). She outlined nine functions as follows (see refs in paper):

- a) Acoustic contact in dense vegetation
- b) Ritualised appeasement of aggression
- c) Transfer of information
- d) Species isolation mechanism
- e) Protection from predation (confusing directionality of the song)
- f) Recognition of sexes (vocal sexual dimorphism)
- g) Synchronisation of reproductive behaviour
- h) Pair bond strengthening
- i) Territorial defence

In this early review, von Helversen proposed that a role in territory defence could be the only initial function for duetting as all other functions act primarily within the pair and 'duets are much too loud to be of significance solely to the mate'. With duetting as a precondition, other functions may later have come into play. I will outline and discuss the nine functions above in the order listed, with reference to more recent work and views.

1.3.a Acoustic contact: The use of duets to maintain acoustic contact has been much refuted by many observations of pairs flying together to duet, or duetting whilst sitting side by side (e.g. Hall, 2000 and this thesis, Chapter 3). Such observations strongly contradict predictions that duets are primarily used as a means of maintaining contact when separated. Duets may, however, serve to maintain contact in specific circumstances, such as during mate-guarding behaviour by males,

and this use of duets in mate-guarding will be discussed later. Duetting may secondarily serve to localise mates during any separation, but the strong trends seen for duets to be preferentially performed when mates are together mean that this function cannot play a primary role. The tight temporal coordination in many duets means that increased distance between mates can reduce their ability to continue a song performance. This occurs when the lag between one bird singing and the other hearing the song becomes significant enough to cause the duet to disintegrate (pers. obs.). This effect of separation distance on duet performance may enforce proximity to a mate if well-performed duets are important in some other function.

1.3.b Appeasement: Ritualised appeasement of the mate may be important where both birds defend a territory and thus need to ensure that aggressive behaviours are not directed at one another. There is little evidence supporting this theory (Serpell, 1981b), but it is also a difficult hypothesis to test. However, if duetting served to appease partners, we would expect duets to occur almost exclusively during and after aggressive encounters, as there should be no need to duet when aggression levels are low. Although many studies cite increases in duet rates in response to real or simulated intrusions, duets are also, in many species, frequently performed during unstimulated broadcast song (Sonnenschein and Reyer, 1983; Levin, 1985; Hall and Magrath, 2000; Mann *et al.*, 2003). Of course, the original objection that duets, especially in response to intrusions, do not seem to be directed at the mate, applies to this theory perhaps even more strongly than to some of the others.

1.3.c Information transfer: This proposed role for duets is more motivational than it is functional, thus we are looking at what information might duets transfer rather than whether or not they evolved to transfer information. Information transfer implies that the signalling by one or both birds refers to some sort of context, and is an idea that has been difficult to test in solo song, let alone in duets. It differs from the role of song in honest signalling, where the bird has not selected to pass on information, but where costs of the behaviour enforce changes that are indicative of either fitness or motivation levels. Solo song has been shown to function as an honest signal of individual fitness (e.g. Buchanan and Catchpole, 1997; Galeotti *et al.*, 1997; Doutrelant *et al.*, 2000). Through the same principles of retaliation costs that apply to honest fitness signals, aspects of song such as song type matching and overlapping

the opponent's song, can serve as honest signals of aggression during agonistic interactions (Naguib, 1999; Vehrencamp, 2001; Burt *et al.*, 2002). The difference between this sort of enforced signalling, and information transfer, is that the latter predicts precise contexts in which certain song types are used, where such changes in song types do not represent simply a graded signal of motivation. Although Sonnenschein and Reyer (1983) proposed that the slate-coloured boubou *Laniarius funebris* used four different duets in three distinct contexts, their data were mostly from only three pairs, and thus conclusions about whether this indicated context dependence or graded motivation, are very preliminary. No other studies have explored how use of different types of duet song varies with motivation or context, and so the suggestion that duetting arose to allow information transfer remains untested.

1.3.d Species isolation: The idea that duets evolved to act as a species isolation mechanism seems inadequate considering the fact that the majority of avian species arose without the need for duet song. There is an apparent correlation in the tropics between greater species diversity and higher occurrence of duetting, and the greater speciation may have led to a need for much greater innovation and breadth in species recognition cues such as song. There still seems however, no specific advantage to evolving duetting during species divergence over simply modifying solo songs. One case where duetting may serve as an isolation mechanism may be after duet evolution for another function, where further speciation may then lead to selective pressures acting on the duet in the same way that they can act on male solo song. Phylogenetic studies in duetting species have not yet been completed, but these are hoped to shed light on how duet song evolved within a genus, and whether selection may have acted on the duets of sister species to drive greater divergence.

1.3.e Predator protection: Duets may serve to confuse predators by not providing a clear directional signal, and thus preventing accurate location of either individual. This possibility has not been tested formally, however it is unlikely to act as a major function for several reasons. First, as already stated, pairs tend to sing whilst very close together, and so there would still effectively be a single source of the sound. Second, several studies also reported that pairs tend to perch conspicuously at the top of vegetation when duetting in response to intrusions, a behaviour that would render

any acoustic ambiguity irrelevant to a predator (Tingay, 1974; Farabaugh, 1983; Sonnenschein and Reyer, 1983). Thus, at the very most, this function to duetting would serve in limited circumstances as an additional benefit on top of more fundamental roles.

Another angle was taken on this potential function by Hasson (2000), where he considered that the duetting alarm calls of some ungulates may be a way of signalling to a predator that both members of the pair are aware of the predator and are near to one another. He did not consider the duet songs of other animals in this theory, nor the fact that duet songs, as opposed to 'duetted' alarm calls, are not exclusively associated with threatening contexts. Thus although this idea may have some, as yet untested, merit within the particular case of alarm call duets, it is highly unlikely to apply also to true duet singing.

1.3.f Sex recognition: In species with sexual dimorphism of song, this could obviously play a role in recognition of sex, especially where the birds are sexually monomorphic in plumage (Langmore, pers. comm.). However, as a reason for the evolution of duetting, this does not appear to fulfil the most fundamental requirement: that this function cannot be attained through solo song. Any sexually differentiated song would serve as a vocal sex recognition cue, without the songs needing to be combined as a duet. It seems that, again, sex recognition cannot be a primary function to duets; however, the very marked sexual dimorphism in duet songs in some species suggests that it does perform some role. The need to identify sex may come into play in conjunction with duets being used in another way, such as in joint territory defence or mate guarding, which are discussed below.

1.3.g Reproductive synchrony: Although many duetting species perform duets throughout the year, changes in duet use during the breeding season may provide information within the pair about one another's reproductive state, or allow coordination of parental care. There is some evidence for song answering (very basic antiphonal duets) being used in the northern cardinal (*Cardinalis cardinalis*), with different types of song by the female being associated with increasing and decreasing likelihood of the male approaching the nest (Halkin, 1997). Studies on the duetting slate-coloured boubou showed that female song behaviour changes during the

breeding season, whereas male song does not (Sonnenschein and Reyer, 1983; Schwabl and Sonnenschein, 1992). The authors suggested that changes in female song might allow the male to synchronise his reproductive behaviour with that of his mate. This ability might be specifically selected for in the tropics where there are no environmental cues such as day length to serve as cues for reproduction. Many tropical populations show no real temporal synchrony of breeding, thus it might be necessary to have some means of synchronising behaviour within each pair. Although large time scale studies such as on the slate-coloured boubou, cannot tell us about fine detail such as the nest song-exchange behaviour in cardinals, it does lead us into more general theories of duet use: specifically that duets are used in pair bond formation and maintenance.

1.3.h Pair-bonding: In her review, von Helversen (1980) mentioned ‘strengthening of the pair bond’ as the eighth of his proposed functions. This is a very general term, and more recent reviews have referred to two specific means by which duets may be involved in pair bonding: they may have a role in the formation and maintenance of a pair bond, or they may be used to mate guard and thus to defend the pair bond (Farabaugh, 1982; Hall, In press). The first theory assumes that duets are used as a signal or mechanism within the pair, whereas the second predicts that duet use is directed to birds outside the pair who may represent a threat as a usurper or cuckolder.

That duets are used to form or protect a pair bond is the first theory that truly offers a role that could not be as well fulfilled by solo song. Given the nature of coordination and apparent cooperation involved in producing duets, it is intuitively attractive that this could be a way of signalling commitment to a partner, and of the pair signalling their pair bond strength to other birds. Wickler (1980) put forward the first mechanistic theory for how duets may be used in pair bond formation. His ‘coyness model’ proposed that the coordination of songs into the duet involved a learning period that served as an investment into the pair bond. This investment would be non-transferrable, i.e. re-mating would involve another learning period, and thus simply the process of learning to sing with a new mate incurs a cost to divorce and an advantage to remaining with the same partner. There has been some debate over the validity of the assumption that such a pre-breeding investment should be considered

in decisions about whether to divorce (Serpell, 1982a; Serpell, 1982b; Wickler and Seibt, 1982). Serpell (1982a) stated that such decisions should be based on future reproductive benefits rather than past investment, and therefore that the coyness model committed the 'concorde fallacy': continuing to invest in a bad cause because of past investment. As yet, the problem with the coyness model is not just whether or not it commits the 'concorde fallacy', but whether there is any evidence for it. Two studies on birds have attempted to detect a period of learning in newly mated duetting birds, but so far no such behaviour pattern has been proven (Farabaugh, 1983; Levin, 1996a). The only study on any group of animals that has demonstrated pair-specific adjustments of duetting was in gibbons (Geissmann, 1999) where changes in duetting behaviour occurred in two pairs after forced remating.

If duets were used to form or maintain pair bonds, whether through the coyness model or some other mechanism, the precision of the performance in different pairs would be expected to contain information on the duration or strength of that pair bond. This is something that applies uniquely to duetting species, and it is a reflection on the relative lack of research in this field that there are almost no data on differences in duetting ability between pairs. Again, the only information we have in this area comes from a study of gibbons (Geissmann and Orgeldinger, 2000), where duetting activity in different pairs was found to be correlated with behaviour associated with pair bonding such as grooming and staying close together.

The use of duets to mate-guard is the most recently proposed theory for duet function (Sonnenschein and Reyer, 1983), and is interesting in that it is the only theory that suggests that duets are a non-cooperative behaviour. There are two mechanisms by which mate-guarding through duetting may come about: first, that the first bird sings to advertise their availability and their mate responds to thwart the signal by labelling them as mated; and second, that the first bird sings to try to elicit a response from their mate and thereby monitor the mate's movements. Both mechanisms are intertwined: in the second, why would the second bird respond unless the first bird's solo song would otherwise attract rivals? In a study of magpie-larks (*Grallina cyanoleuca*, Hall and Magrath, 2000), although males appeared to mate-guard by staying much closer to the female during pre-breeding (fertile) stages, their duetting behaviour did not change. This suggests that duets in this species were not used to

mate-guard. However, mate-guarding may manifest itself in other ways, such as in the greater intra-sexual territorial aggression which was demonstrated in magpie-larks in later studies (Hall, 2000). The problem with testing for mate guarding is that there are few clear hypotheses and experimental predictions, even as far as solo song or non-song behaviour is concerned (Whittingham *et al.*, 1995; Hall, In press). Thus we have no clear consensus on how to interpret behaviours that have been reported in the field. Does high responsiveness to the mate, for example, necessarily imply mate guarding as stated by Levin (1996b), or simply a commitment signal to the mate? And can we be sure that an unanswered song is a signal of availability without proof that solo songs will attract potential mates? Such issues, and the fact that only three or four studies have ever tried to examine duet use in mate guarding, means that at present this theory remains largely untested.

1.3.i Territory defence: The coordination of a pair's song into a duet for use in defence of a shared territory was the theory that von Helversen (1980) favoured as the most likely initial trigger for the evolution of duets. As many duetting species are tropical and territorial, greater environmental stability and higher adult survival would be expected to lead to high levels of competition for territory ownership. In this situation, two birds would be better able to defend a territory than one, and both birds singing would signal the presence of this stronger defence. The combination of the pair's songs into a duet could provide several signals: that the pair are together and thus both birds will attack, that motivation levels of both birds are high, or that the pair bond is strong and thus the birds will be well coordinated and unified in their defence.

That duets function in some way in territory defence is qualitatively proven relatively easily, simply by the demonstration of an increase in the use of duets in response to intrusions. Such data are available from all species where researchers have simulated an intrusion: playbacks always lead to increases in duetting (for reviews see Farabaugh, 1982; Hall, In press). More precise predictions that distinguish territory defence from mate-guarding, are that duets will act as a greater threat than solo song and, as a basis to the theory, that solo birds will be less able to maintain a territory than a pair. The former prediction was tested by Hall (2000) on magpie-larks, where duets are used preferentially in territorial interactions, and where responses were

greater when exposed to duet playback than when exposed to solo songs. In contrast Levin (1996a; 1996b), studying the bay wren (*Thryothorus nigricapillus*), found that solo birds were able to maintain territories, but she did not compare responses to solo song or duets in either pairs or solo birds. Thus although joint territory defence is often assumed to be the principle function for duet singing, the two most robust studies carried out provide inconclusive results.

1.4 *Current knowledge*

Whilst listing and discussing the various potential functions for duet song, I have mentioned several times that evidence is lacking in certain areas. In fact, although there is an ongoing accumulation of knowledge about duetting species, there are still very few studies that have involved both observation and specific experimental tests of the different functions. Tropical, territorial species are by their very nature difficult to study. Their population densities are always low, making it hard to amass data on sufficient numbers, and the habitats they occupy can range from arduous to inaccessible. It is not surprising therefore that studies suffer from small sample sizes and a paucity of behavioural observations. With an understanding of the hazards of pseudoreplication and the need for increased robustness to playback experiments (Kroodsma, 1989; Kroodsma *et al.*, 2001), we must also treat many of the experimental studies with some caution. Thus, although we can largely dismiss some of the more unlikely initial theories, we are still left with no consensus about the relative importance of pair bond formation, mate guarding or joint territory defence to the evolution of duetting.

Aside from the question of why duetting evolved, there are also many other aspects to duet song that have not been the subject of study. Male solo song is known to vary both between individuals (see reviews Falls, 1982; Stoddard, 1996) and populations; the latter affected by learning patterns, dispersal behaviour, and the advantages of having similar songs to neighbours (see Kroodsma *et al.*, 1999; Nelson *et al.*, 2001; Slabbekoorn and Smith, 2002). This sharing of repertoires or parts of repertoires with neighbours, is thought to feature both in recognition of other individuals (Beecher *et al.*, 2000; Molles and Vehrencamp, 2001; Griessmann and Naguib, 2002), and as a basis for the application of song type matching in aggressive interactions

(Vehrencamp, 2001; Burt *et al.*, 2002; Naguib *et al.*, 2002). These are growing areas of research in male song, but almost nothing is known about such aspects of song in duetting species.

1.5 *Aims of this study*

This thesis focuses on one species, a subspecies of the plain wren (*Thryothorus modestus*) that demonstrates highly complex antiphonal duets. The intensive study of one species involved a combination of observational and experimental work, intended to fill some of the gaps in our knowledge of duetting that were highlighted above.

As there is very little known about the plain wren, the work was initially observational, with pairs being followed intensively through the first field season. Recordings made during this time allowed a detailed analysis of repertoires, phrase types and duet formation within each pair. The first part of the thesis will focus on describing the use of duets in this species, looking at the occurrence of duets relative to male solo song, the responsiveness of mates to one another's song, and the behaviour of pairs when they were duetting. By closely following the breeding behaviour of pairs, I also examined changes in duet use with different breeding states and linked these to functions of song behaviour such as mate attraction, mate guarding and coordination of parental care.

Populations were studied at two sites to increase the numbers of pairs available, and also to determine whether song types or duet use varied between different populations of the same subspecies (hereafter referred to as the canebrake wren: *T. m. zeledoni*). I investigated patterns of duet singing similar to those studied in solo singing species, including the extent of individual and geographic variation. Analysing repertoires from both populations enabled me to determine whether neighbours share repertoires, and to test experimentally whether song type matching is used in aggressive signalling.

Observational data, along with ecological studies provided the background from which relevant predictions about duet use were made. The hypotheses for the

evolution of duetting that have been outlined above were applied to the canebrake wren, providing predictions that were tested through a series of playback and removal experiments. Depending on the role that duetting plays in the canebrake wren, subjects were predicted to respond differently to, for example, duets and solo songs, or same-sex and opposite-sex playback. These experiments, in combination with conclusions drawn from observations, provide data that discriminate between the different theories for the development of duet singing.

Exact hypotheses being tested will be defined in more detail within each chapter, but the thesis as a whole is an attempt at completeness within one species. The study species has not been the subject of any detailed research, so basic information such as which sex sings which part of the duet, what the nest looks like, and what their repertoire sizes are, are all defined for the first time here or in the pilot study for this project (Mann *et al.*, 2003; Marshall-Ball and Slater, 2003). I aimed to provide a set of data that not only investigated the major hypotheses for the evolution of duet singing, but also examined the use of duet song in aspects of behaviour that have only previously been demonstrated in solo song.

Chapter 2: Study species, study sites and general methods

2.1 *Study species*

The *Thryothorus* genus of wrens (Family Troglodytidae) are archetypal wrens, being small and stocky (length 12-16cm) and with sexually monomorphic plumage (Brewer, 2001). They are territorial and socially monogamous, with long-term pair bonds that are maintained year-round. Behaviour is stereotypically wren-like: they favour cryptic movements, gleaning for invertebrates in dense undergrowth, but have loud and generally musical song (Skutch and Stiles, 1989). The genus contains about 25 species and is exclusive to the New World, extending from northern USA to as far south as Peru, Bolivia and southern Brazil (Brewer, 2001).

The plain wren, *Thryothorus modestus*, occurs through most of Central America, from southern Mexico to Panama. Pairs hold year round territories and tend to inhabit marshes, scrub and rough pasture. This habitat preference ensures that the species is not threatened by forest loss and fragmentation, its range even expanding within Costa Rica as they move into deforested pastures and regenerating scrub. The species has, as the name suggests, the least patterned or coloured plumage in the genus, being a mid brown over the wings and upperparts, with light banding on the tail. Underparts are a pale grey, legs dark grey and bill grey-black. There is a strong white supercilium and grey-brown eye stripe, with the eye itself brownish. One interesting feature is that the inside of the bill and mouth is entirely black (pers. obs. and N. Mann, pers. comm.). The birds have a stocky body shape, with broad rounded wings, and a slender pointed bill; according to Skutch and Stiles (1989) they weigh approximately 18g.

From Nicaragua to Panama, the plain wren is divided into two subspecies. The nominate species (*T. modestus modestus*) occupies the Pacific slope lowlands, and the canebrake wren (*T. modestus zeledoni*) occupies the Caribbean slope. The canebrake wren is reportedly larger than the plain wren: in my study, adults weighed between 15-28g (Skutch & Stiles, 1989, quote 23g). The plumage is grey as opposed to brown, with more marked barring on the tail and wings. My observations revealed

a wide range in eye colour, from pale grey to bright red-brown. The brightness and redness of the eye appeared to be sexually dimorphic, with the male of a pair usually having brighter eyes than the female (in only 2 pairs out of 26 did the female have brighter eyes than the male). This difference between members of a pair also extended to general morphological measurements, so that although the ranges of male and female sizes overlapped considerably (Fig. 2.1), males were consistently larger than their mates (Paired t-tests: Wing length: $T=5.89$, $p<0.0001$, $df=17$, Bill length: $T=2.57$, $p=0.019$, $df=17$; Tarsus length: $T=2.28$, $p=0.035$, $df=17$; Mass: 3.24, $p=0.005$, $df=17$).

The wrens in my study occupied lightly grazed or fallow pasture, and marshes both within the pastures and in forest gaps. Most territories were centred around a marsh; where these marshes were in pasture, the territories would extend into this, but where marshes were bordered by forest, the birds remained exclusively on the marsh. Pairs occupied these territories year round, and appeared to maintain long-term pair bonds, although divorce and rapid re-mating after female predation were both observed. This species breeds during the first half of the wet season, which in the Caribbean lowlands extends from May to December. The breeding season commences in late March and finishes in August, with pairs making several attempts during this time (see Appendix 1). Females carry out all nest building and incubation but males provision chicks and fledged young almost equally with the female. Males also assist females in the construction of dormitory nests for fledged young (see Appendix 1 for further details).

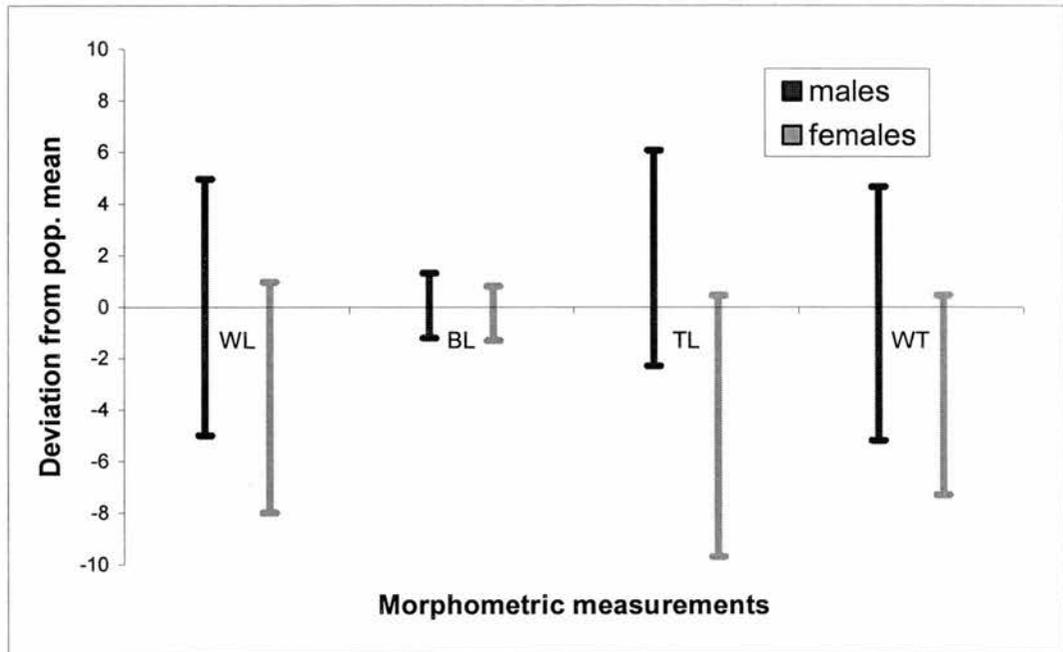


Fig. 2.1: The body size ranges for males ($n=32$) and females ($n=20$) are expressed here as the deviation of the maximum and minimum values from population means (male and female data combined). Wing length (WL), bill length (BL) and tarsus length (TL) are all expressed in mm, and weight (WT) is in grams.

The song of the plain wren is one of the most complex antiphonal duets so far described (Mann *et al.*, 2003). A duet consists of an introductory phrase (I) sung by the male, followed by cycles of a female duet phrase (F) and a male duet phrase (M) (sexes genetically determined, see Fig. 2.2), which is opposite to the previous descriptions in Skutch and Stiles (1989). Thus a basic duet has the structure $I(FM)_n$, although variations on this theme occur through missed phrases by either bird. Males may sing the introductory phrase repeatedly for extended lengths of time without the female responding. Both males and females have repertoires of their phrases, and thus the pair have a repertoire of different duets.

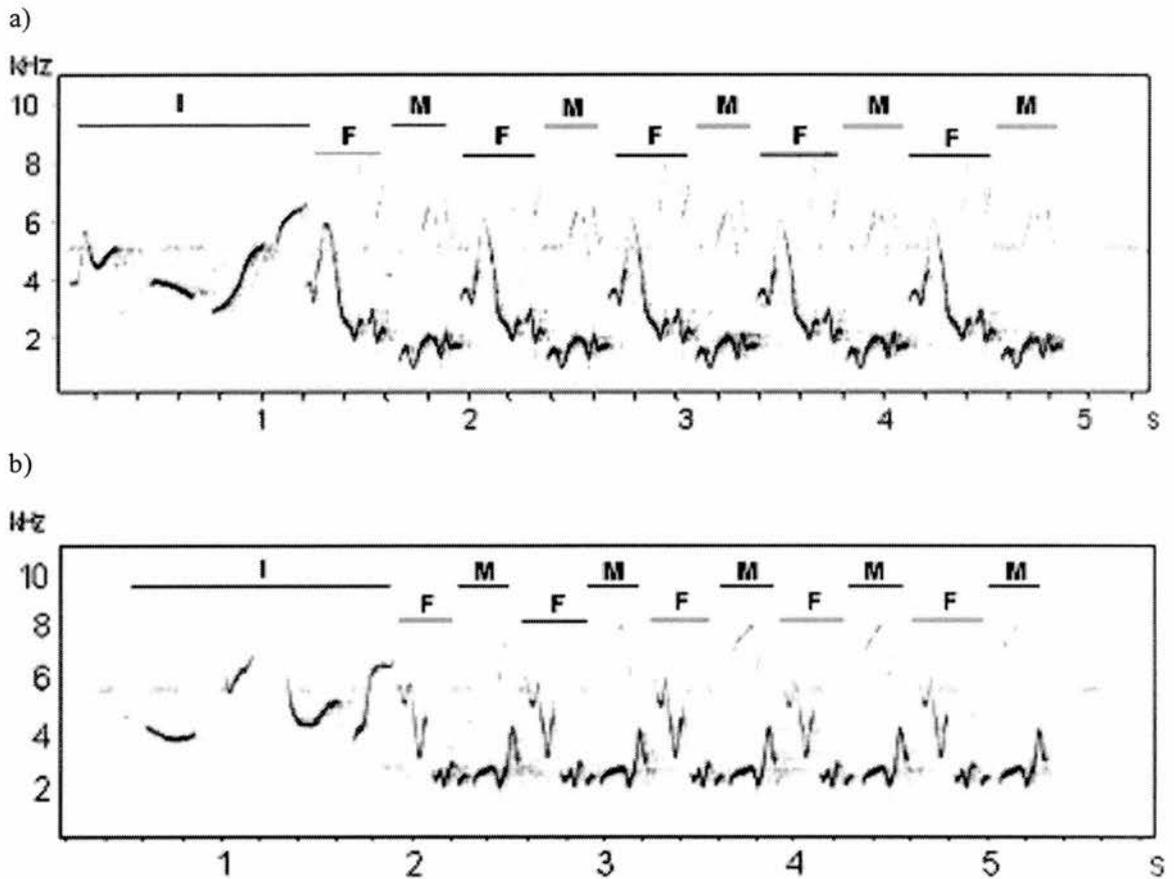


Fig. 2.2: Two examples of duets of the canebrake wren, recorded from pair 7 at La Suerte in 2001. The three song phrase types are shown as I: male introductory phrase, F: female duet phrase, and M: male duet phrase. In both duets, the I phrase contains 4 elements, although this can vary from 3 to 6 in different types. The F phrase in a) has two elements, and in b) has three. The M phrase always has only one element.

2.2 Study Sites.

The work for this project was carried out at two field sites in the Caribbean lowlands of Costa Rica: La Suerte and El Zota field stations. Both sites are in the province of Limón and are in the northeast of the country. These biological field stations are owned by Nicaraguan (La Suerte) and Costa Rican (El Zota) families, and their main use is as bases for undergraduate field courses from the United States, although they were both also visited by researchers and Costa Rican students.

La Suerte Biological Field Station is at 10°26'N, 83°47'W with 150 hectares of land. Much of the site was originally cleared for cattle ranching, with three patches of selectively logged forest remaining. The cattle farming was reduced in intensity when the field station was established (1995), and during the course of my research the cattle were limited to one area of pasture, allowing considerable succession to take place in the other areas. The pastures contain a network of marshes, and I was able to work on neighbouring land to gain access to further marshes and pairs of wrens. The area was densely populated with canebrake wrens, with 21 territories occupied at the start of the study and 18 at the end. The maps (Figs 2.3 and 2.4) show the distribution of territories occupied during the fieldwork. Territories were very heterogeneous in the habitat types covered, and Fig. 2.5 shows the areas of 5 basic habitat types in 8 territories that were mapped in 2001. This site was visited 4 times: March to August in 2001, April to June, August, and October to December in 2002.

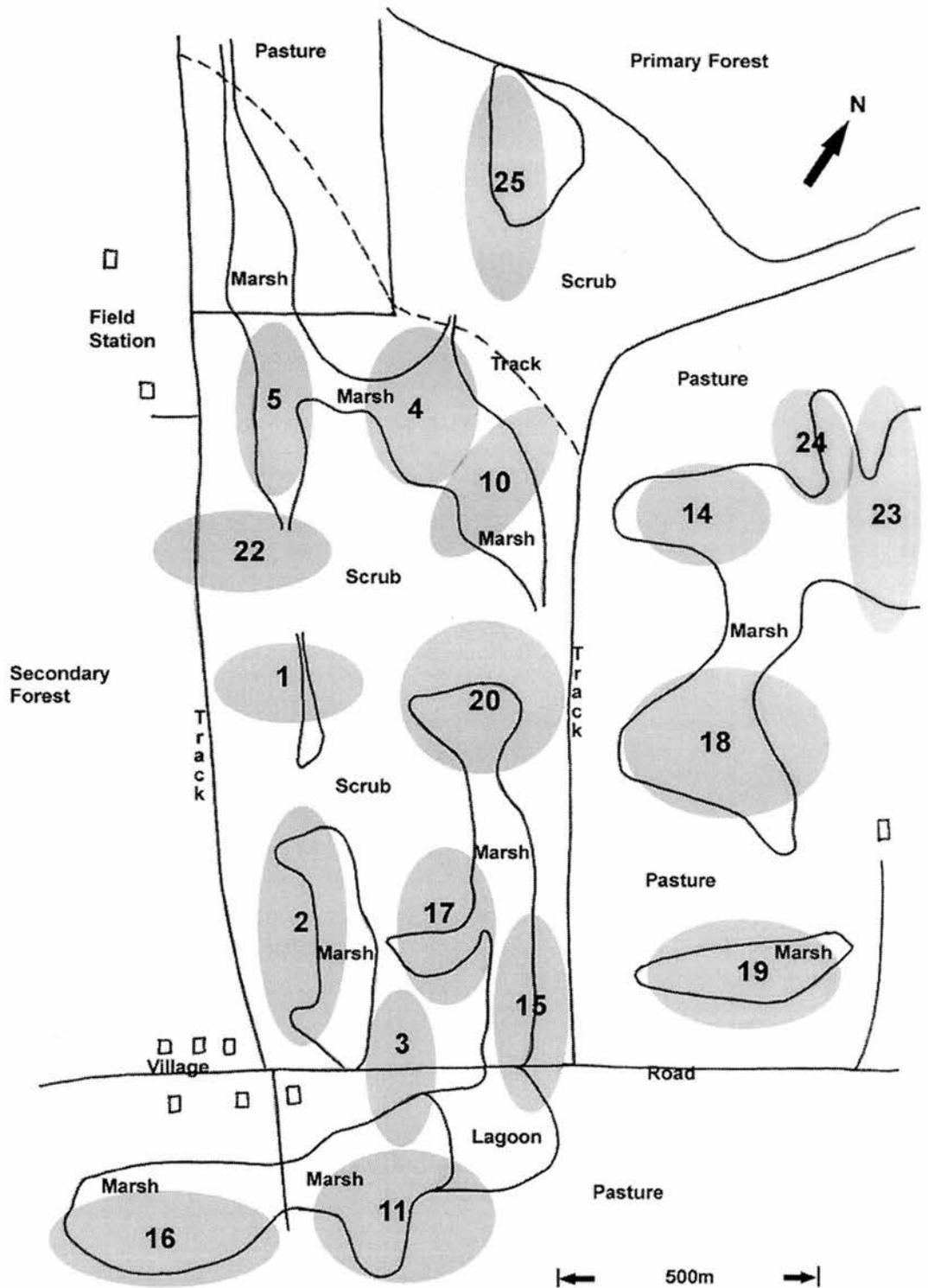


Fig. 2.3: Map of the southern La Suerte study area. Wren territories are shaded and are symbolic areas only. Lines represent tracks, fence-lines or marsh/forest edges. Measurements are approximate, taken from compass/rangefinder coordinates. Territories 11 & 18 were unoccupied in 2002, 25 became occupied in 2002, and 5, 15 & 24 were occupied by solo males in 2002. 23 & 24 were added to the study in 2002.

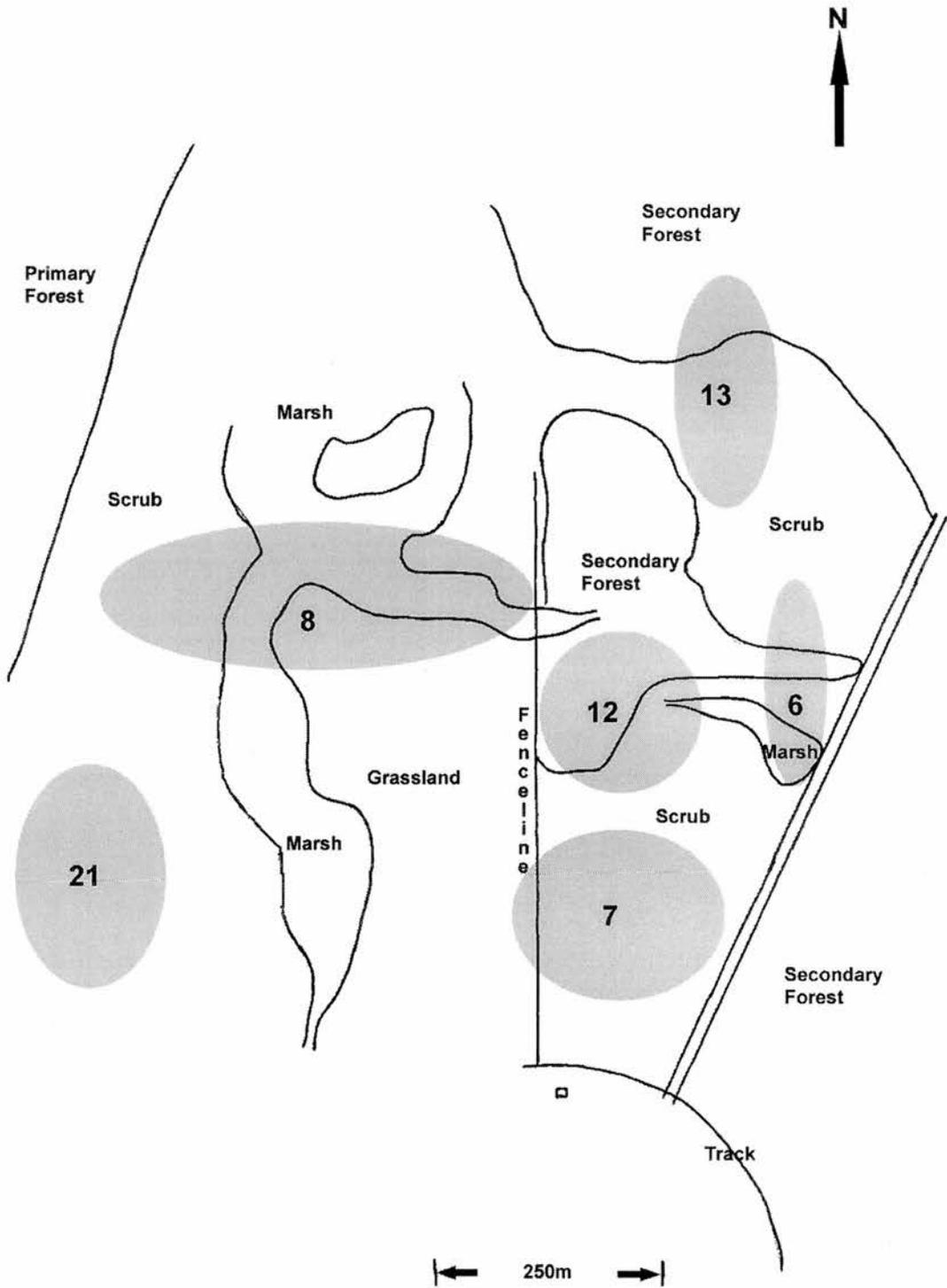


Fig. 2.4: Map of the northern La Suerte area. Wren territories are shaded and are symbolic areas only. Lines represent tracks, fence-lines or marsh/forest edges. This area lies northwest of the area in Figure 2.2, the track at bottom right leads back to the station. Measurements are approximate, taken from compass/rangefinder coordinates. Territories 6, 8, 13 & 21 were unoccupied in 2002.

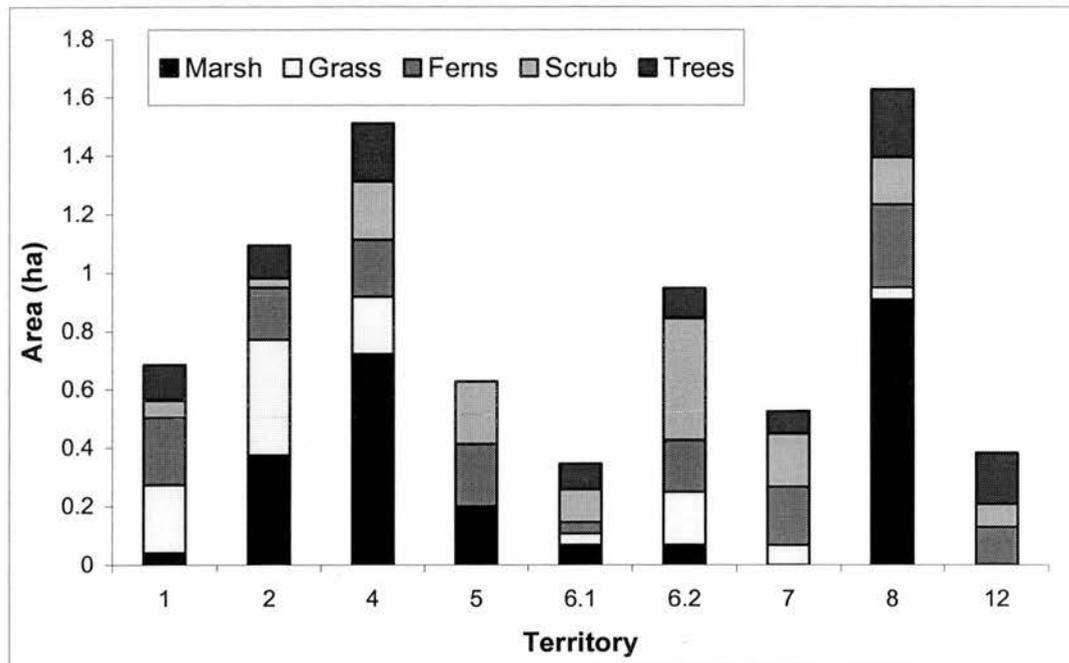


Fig. 2.5: Area of 8 territories at La Suerte, and composition by habitat type. Territory 6 is divided into 6.1: original pair, and 6.2: after female predation.

El Zota Biological Field Station is at 10°33'N, 83°44'W, and has land covering 1000 hectares. Only a small part of this site was cleared for cattle, although large areas of forest had been replaced with plantation trees for cultivation logging. Since the establishment of the field station (in 2001), all cattle had been removed, and the plantation trees are being gradually replaced by secondary re-growth and planted native forest trees. Most of the land therefore is forest covered, and the canebrake wrens are located in open marshes within the forest, but not forested marshes (swamps). This meant that the population was much less dense, and, including some neighbouring cattle pasture, I only had 10 occupied territories (Figs 2.6 and 2.7). This site was visited once, from July to August 2002.

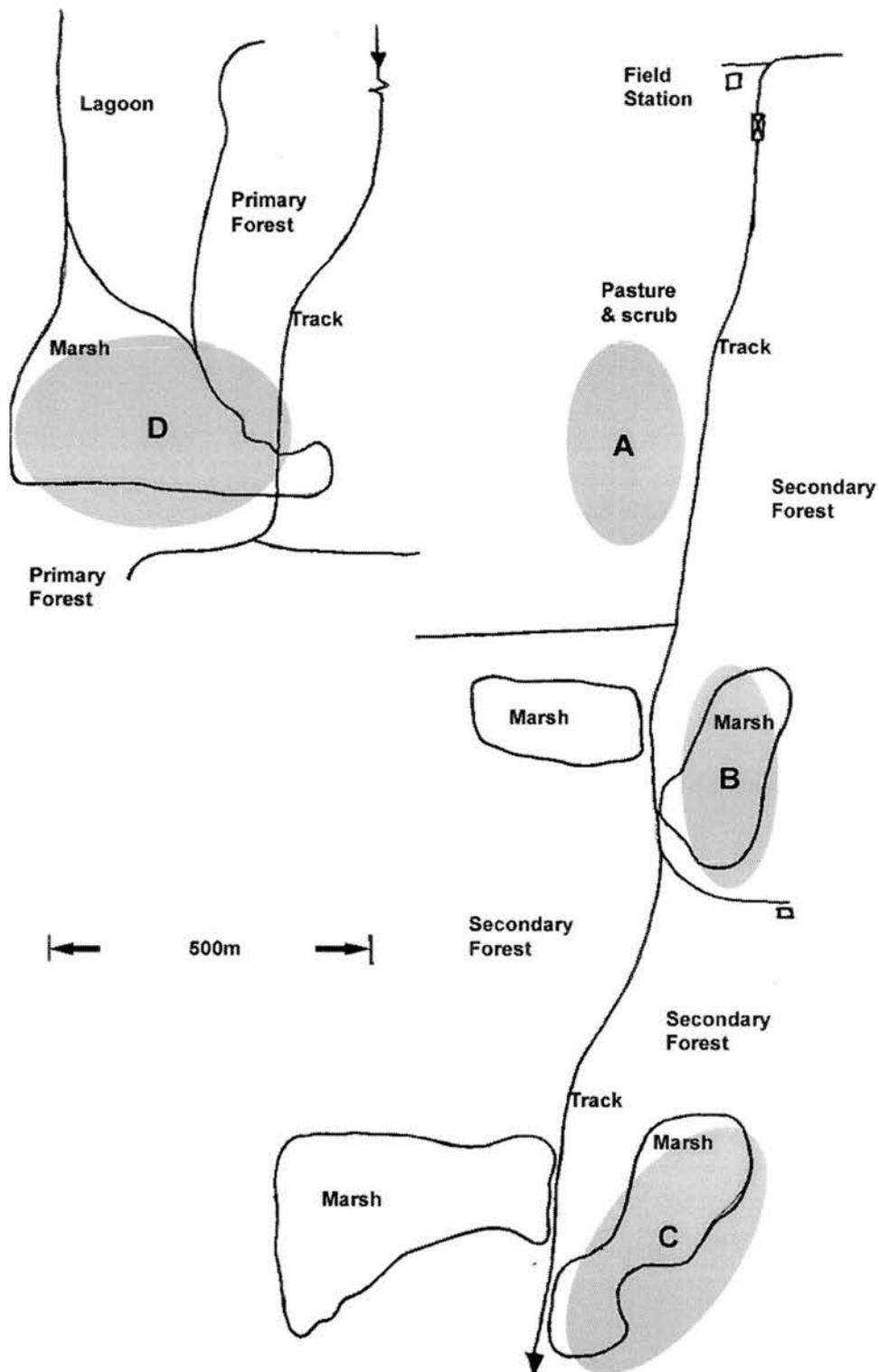


Fig. 2.6: Maps of the western half of the El Zota site. Wren territories are shaded and are symbolic areas only, solid lines indicate tracks or marsh/forest edges. Territory D lies 2km further south along the same track as the first three territories. A was occupied by a solo male. Measurements are approximate, taken from compass/rangefinder coordinates.

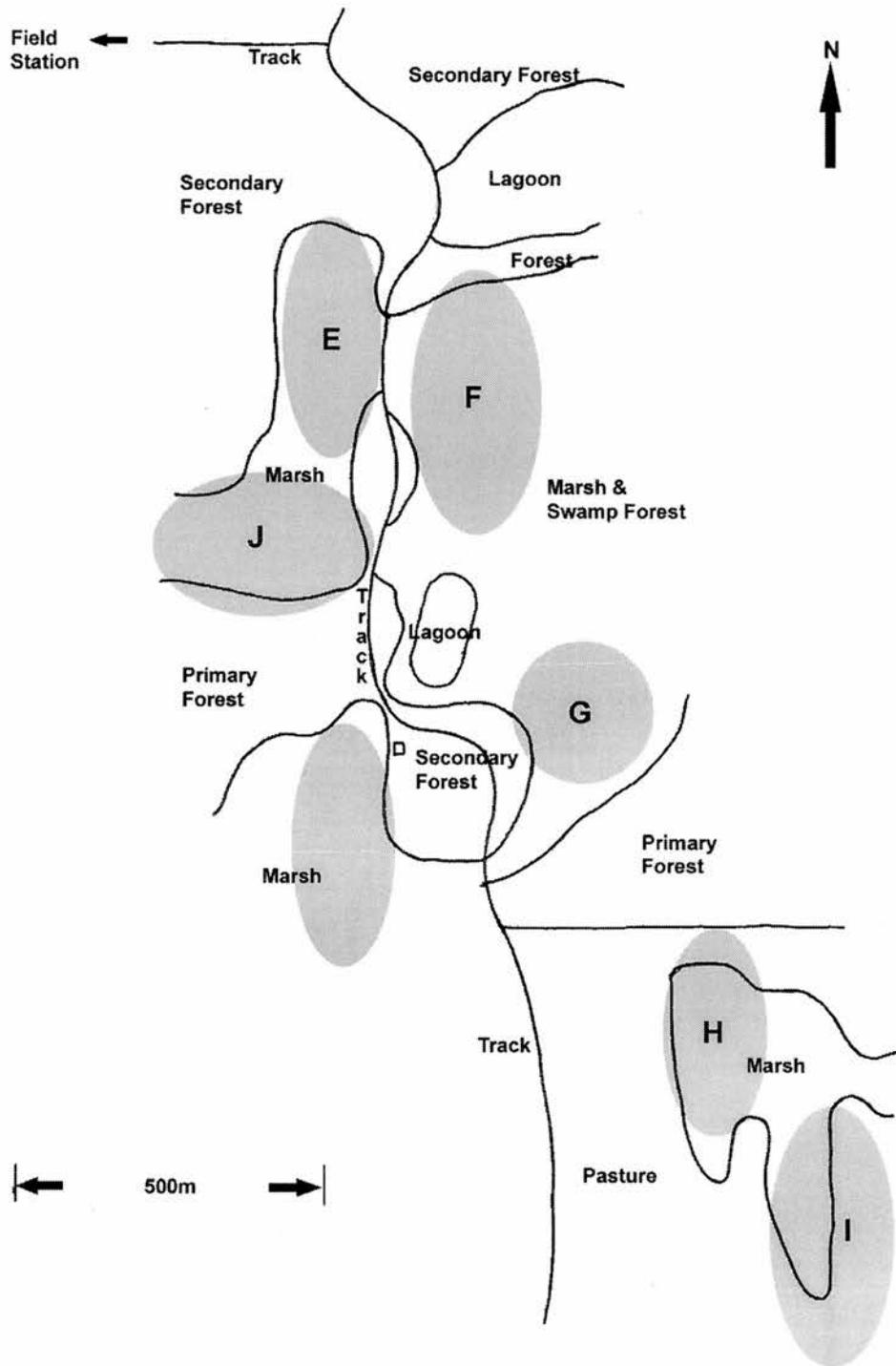


Fig. 2.7: The eastern area at the El Zota site. Wren territories are shaded and are symbolic areas only, solid lines indicate tracks or marsh/forest edges. The unnamed territory was not accessible. Measurements are approximate, taken from compass/rangefinder coordinates.

2.3 *Methods for mistnetting, ringing and blood sampling*

Mistnetting was carried out for the purposes of ringing the wrens and, in 2001, for collecting blood samples for DNA analysis. 18 and 30 foot Japanese mistnets from the British Trust for Ornithology were erected on bamboo poles locally collected. Nets were positioned well within territories and birds were lured to nets using the plain wren recording from a published CD (Ross and Whitney, 1996). Split ring plastic colour bands of 2.8mm diameter (A. C. Hughes, Middlesex, UK) were used in combination with metal alloy split rings of the same size, which were numbered between 0 – 100. A range of colours and patterns allowed each bird to have a unique combination of colour rings, with one colour ring on each leg and a metal ring on the right leg (2001) or left (2002).

Morphometric measurements taken were extended wing length (Svensson, 1992), using a wing rule, and tarsus length and bill length, using dial callipers. Bill length was taken from the corner of the nostril closest to the head, as the birds have no marked forehead, or cere against which to measure bill base. Eye colour was measured on a simple 3 level scale of dull, intermediate or bright. Birds were checked for brood patches, which were also assessed on a 3 level scale of absent, intermediate (some crêping and increased colour) or fully developed (highly veinated and crêped skin). Presence or absence of moult signs was noted along with any other unusual features, such as very worn feathers, or ecto-parasites. Body weight was taken by weighing the birds in cotton bird bags with 50g Pesola scales.

Blood samples were taken from the radial vein where it crosses the elbow on the underside of the wing. The vein was pierced with a medical needle (sterile, individually sealed), and then the blood extracted by holding a 0.75 μ l capillary tube against the blood droplets. Approximately 30 to 40 μ l of blood were taken and the wound then compressed with surgical alcohol (70%) for several seconds. The bleeding usually stopped within seconds of the vein being pierced, but I always ensured a bird was not released until there was definitely no more blood loss. The blood sample was then blown into an eppendorf containing 50 μ l of EDTA (100mM

EDTA, 100mM Tris, 2% SDS) buffer solution and stored until later extraction in St. Andrews.

2.4 Genetic sexing

DNA samples were extracted using standard protocols for blood and tissue samples, and an extraction kit for the feathers taken from nestlings (Griffiths *et al.*, 1998). The PCR protocol described by Griffiths *et al.* (1998) was found to work for the canebrake wren, and used for all sex determination.

2.5 Study methods

The analyses in this thesis primarily involve the use of General Linear Models for observational data, and Repeated Measures Mixed Models for experimental data (The SAS system, V8). The models were designed to allow the inclusion of repeated samples from the limited numbers of birds, thus although much of the observational data involve eight or fewer pairs, *season day* and *time of day*, as well as *pair*, were included as factors in the model. This meant that the non-independence of samples taken from the same pair was controlled for as much as possible. This method avoids the need to reduce data to means per observation period or even means per individual, which inevitably entail a massive loss of detail. The behaviour being examined through much of this thesis is only detectable when looking at changes in the behaviour of individuals under different circumstances. Simplifying the data to eliminate non-independence would have removed the detailed information on individual changes that was specifically of interest. Table 2.1 below shows an example of an ANOVA table from a GLM on observational data on song rates, demonstrating the inclusion of the factors mentioned above, as well as other potential influences that were under investigation.

Table 2.1: An example ANOVA table from the GLM designed for observational data. The factors 'season day' and 'time of day' are included to account for non-independence of samples from the same pair. This model shows a very significant effect on the dependent variable (1st line of results), and within the model, four of the five factors show a significant effect on the dependent variable (all except 'time of day'). Overall, 13.3% of the variance in the data is explained by the model (R-squared value).

Source	Degrees of Freedom	Sum of Squares	Mean Square	F value	P value
Model	118	10862.75	92.06	7.24	<0.0001
Error	5571	70790.12	12.71		
Corrected total	5689	81652.88			
Season day	77	2905.14	37.73	2.97	<0.0001
Time of day	1	21.29	21.29	1.68	0.1956
Breeding stage	11	1231.38	111.94	8.81	<0.0001
Pair	7	418.44	59.78	4.70	<0.0001
Pair*Breeding stage	21	517.18	24.63	1.94	0.0062
R-squared	0.133				

The experiments in this study were all designed around a randomised block design (see Chapters 5, 6 & 7 for more detail), with each subject being exposed to a set of stimuli in at least two trials (each trial containing all stimuli). Thus there were two levels of repeated sampling in this design: the trials, and the different experimental sections within each trial. A repeated measures GLM enabled the recognition of pairs as a subject in the model, and for trials and experimental sections carried out on each pair to be treated as repeated samples. This model provided a neat test of changes in response to stimuli within trials without the complication of variation in subject behaviour on different days and between different subjects. The basic structure of the repeated measures GLMs used throughout the thesis is therefore: Subject: *pair*, repeated measures: *experimental section* and *trial*, factors in the model: *experimental section*, *trial* and additional independent factors where appropriate.

Further specifics of field and analysis methods for both observations and experiments will be dealt with as necessary in chapters 3 to 7. Although this may mean some repetition, the chapters are designed to stand alone, and thus this was thought to be the most lucid approach to covering the very involved methodologies.

Chapter 3: Individual variation in duetting activity and accuracy.

3.1 Introduction

Singing, where it occurs, is possibly the most obvious of communicative signals, and is thought to transmit information ranging from a simple 'Here I am', to details of fitness (Galeotti *et al.*, 1997; Buchanan *et al.*, 1999; Buchanan *et al.*, 2003). Wherever song function requires the reliable recognition of individuals, the need arises for individual variation in song performance. In some situations, such as in group living species, the ability has been demonstrated for recognition of own group as opposed to other groups (Nowicki, 1983; Price, 1999). However, most studies in the subject have investigated the abilities of individual territory holding males to distinguish song of neighbour males from that of stranger males (Searcy *et al.*, 1981; Falls, 1982; Molles and Vehrencamp, 2001). Differentiation of neighbour from stranger song is thought to be advantageous in allowing a scaling of aggressive response, thereby not expending excess energy reacting to the lesser threat posed by a neighbours' broadcast song.

One aspect of individuality that has received little attention is that of female recognition of males, although mate recognition by song has been reported for dunnocks, *Prunella modularis* (Wiley *et al.*, 1991), great tits, *Parus major* (Lind *et al.*, 1997), and European starlings, *Sturnus vulgaris* (Eens and Pinxten, 1996). One study has further shown that female song sparrows (*Melospiza melodia*) were able to differentiate between songs of their own mate, neighbouring males, and stranger males (O'Loughlen and Beecher, 1999). Rather than the ability to identify individuals, studies of female response to male song have focused largely on female preferences for song type or quality. Such studies have demonstrated that females are able to reliably select better quality males on the basis of song cues alone (O'Loughlen and Beecher, 1997; Gil *et al.*, 2001; Forstmeier *et al.*, 2002; Hofstad *et al.*, 2002). These cited studies along with many others have investigated the aspects of male song that reflect quality, and have led to the theory that song can act as an honest signal of current fitness (Iwasa and Pomiankowski, 1994; Gil and Gahr, 2002). There are several proposed mechanisms by which song could be influenced by individual

quality, including developmental stress affecting learning (Nowicki *et al.*, 2002b; Buchanan *et al.*, 2003), parasite loads changing song performance (Buchanan *et al.*, 1999), and foraging needs reducing time spent singing (Greigsmith, 1982).

Few studies have investigated variation in song performance of duetting birds, even though these species provide the potential to look at song performance and responses to song of both sexes (Mebes, 1978; Wright and Dorin, 2001; Muller and Anzenberger, 2002). Most duetting research to date has involved only descriptions of song structure, with some work beginning to investigate the function of this behaviour (Vencl and Soucek, 1976; Todt and Fiebelkorn, 1979; Levin, 1996b; Hall, 2000; Seddon *et al.*, 2002). In this chapter, I describe the degree of individual variation in song rates, repertoire sizes and pair coordination in the canebrake wren. This will thus describe, for the first time in a species with complex duets, the extent of variation between pairs, and provide the setting for further study on information content of duet songs.

3.2 *Methods*

3.2.a Observations: Observations were carried out at La Suerte from March to August 2001. Eight pairs were used as subjects and were each visited every two days on a rotating observation timetable involving three morning periods between 0500 – 1000 hrs and occasional afternoon periods between 1500 – 1800 hrs, these times covering the most active parts of the day. Each observation period lasted 60 – 90 mins and involved gathering information on the variables summarised in Table 3.1. Song recordings were also taken during a series of playback experiments from May – August 2002, both at La Suerte and at El Zota field station, ~30km away. These recordings are used in the phrase matching analyses.

Table 3.1: A summary of the variables measured during observation periods detailing categories used and sampling frequency. Song descriptions: **I** male introductory phrase, **F** female duet phrase, **M** male duet phrase.

Variable	Measurement Scales	Recorded
<i>Behaviour</i>		
Male & female height	Estimated metre ranges: 0-1, 1-3, 3-6, 6-9, 9-12, 12-15, 15-20, 20-30 & 30+.	Start of each minute
M-F distance		
Male & female behaviour	Behaviour categories: Foraging, Singing, or Other.	Start of each minute
<i>Song data</i>		
No. of I phrases		No's per minute
No. of duets		No's per minute
Duet structure	i.e.: I(FM) ₅ , I(FM) ₂ M, I(M) ₃ (FM) ₄ , (FM) ₃ F	Every duet

3.2.b Recordings: During observations, sound recordings were made whenever new phrase types were sung, using a Sennheiser ME-66 gun microphone and a Marantz CP430 tape recorder (chrome tapes). During experiments in 2002, recordings were taken continuously. Songs were identified by eye from spectrograms and confirmed with Avisoft Correlator in Avisoft SASLab-Pro v5.1 (R. Specht, 2002).

3.2.c Data analysis: 13719 minutes of data were collected from the 8 pairs, representing more than 228hrs of observations. Residuals of the numerical data were tested for normality, and parametric GLM tests were performed (with *post-hoc* Tukey tests). The various song measures had high numbers of zero values from times when the birds were not singing. To avoid these zeros overwhelming changes in song rate when birds were singing, and preventing the detection of these changes, these variables were divided into two data sets: 'song occurrence', with singing and non-singing minutes being scored as 1 or 0, and original 'song rate' values for the minutes with song (with non-singing minutes removed). This meant that two types of song production would be looked at: 1) the proportion of minutes that contained song

– i.e. the time that the birds spent singing, and 2) the rate of song production when birds were singing. Using correlation statistics, song rate variables (duet rate, duet length, I phrases, incomplete I phrases, female initiated and irregular duets, song type switching) were condensed for further analysis into four that represented the initial set. These four were: *Song switching rate* (which correlated with none of the other variables), *female initiation* and *irregular duet rates* (which both correlated only weakly with duet rate; female initiated duets were (FM)_n, irregular duets were any that differ from the I(FM)_n or (FM)_n structure), and '*total song*'. This last variable allowed a direct assessment of the pair's singing rate by combining both male solo song and duetting rates. This was achieved by counting the numbers of I phrases, incomplete I phrases, and the total number of FM duet cycles within each minute sample, and then multiplying each by a mean duration for that category, i.e. the mean duration of one I phrase, one incomplete I phrase and one FM cycle. These mean durations were obtained from a random sample of 50 of each type from 6 pairs, and were as follows: I phrase, mean 1.209s ± 0.195s (SE), incomplete I phrase, 0.356s ± 0.031s, and one FM cycle, 0.712s ± 0.063s. Thus the following equation calculates an approximate time spent singing within each minute, based on the mean durations, and frequencies of the three different categories:

$$\text{Total song} = (\text{I calls} * 1.209) + (\text{inc. I calls} * 0.356) + (\text{FM cycles} * 0.712)$$

Although not a precise measure of time spent singing, this variable serves as an index of the total song production per minute, which combines the highly correlated variables of I singing rate, incomplete I singing rate and numbers of duets, along with the mean lengths of duets (which was highly correlated with total song, but not with I phrase or duetting rate). When male behaviour independent of duetting was of interest a fifth variable of *I phrase singing rate* was included in the analyses.

The GLM was designed to take account of the non-independence of repeated samples taken from the same pairs, thus the factors in the model were: *Season day*, *Time of day*, *Pair*, *Breeding state* and ***Pair*****Breeding state* (the importance of this last crossed factor is discussed in Chapter 4). See Table 3.2 for an example ANOVA table from the GLM model testing effects on the 'Total song' response variable. As

multiple variables (either 4 or 5) were being tested, the critical probability value was taken to be $p_{crit}=0.01$. When data did not involve multiple comparisons, $p_{crit}=0.05$ and correction factors were always applied (i.e. Williams correction factor for G tests).

Table 3.2: The results table produced from a GLM on Total song rate data, this shows the five factors that were entered into the model and the amount of variation in the data that is explained (the R^2 value). In this example, all factors except Time of day show a significant effect on total song rates at the $P=0.01$ critical value.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F value	P value
Model	118	10862.75	92.06	7.24	<0.0001
Error	5571	70790.12	12.71		
Corrected total	5689	81652.88			
Season day	77	2905.14	37.73	2.97	<0.0001
Time of day	1	21.29	21.29	1.68	0.1956
Breeding stage	11	1231.38	111.94	8.81	<0.0001
Pair	7	418.44	59.78	4.70	<0.0001
Pair*Breeding stage	21	517.18	24.63	1.94	0.0062
R-squared	0.133				

Consistent duet type construction from matching particular F and M phrase types was tested by comparing the distribution of observed and potential FM combinations against a predicted Poisson distribution.

3.3 Results

3.3.a Breeding behaviour: Breeding success in the whole population at La Suerte was low in 2001, with 22 pairs rearing only 3 juveniles to independence (Marshall-Ball and Slater, 2003). Among the 8 focal pairs, pairs 4 and 7 reared one each of these 3 juveniles. All pairs except pair 5 attempted to breed several times, pair 5 were a newly established pair and did not attempt to breed at all. Surprisingly, there was high adult mortality as well as nest failure, with females 6, 8 and 12 all being predated whilst incubating eggs. After mate loss, male 8 disappeared from the territory, whilst male 6 remained solo; and male 12 re-mated shortly after mate loss,

but did not attempt to breed again. Where both pairings in 12 are considered, they are referred to as 'a' and 'b': the initial female and new female respectively, otherwise, 12 refers to the second pairing, as more data were obtained from the second female.

3.3.b Positional behaviour: Males on average perched and foraged higher above the ground than females (means from 8 males: 1.8 – 4.4m, $n=8045$; means from 9 females: 0.7 – 2.1m, $n=2714$), and always averaged a higher position than their mate (paired t-tests from means per individual, pairs 12a and 12b both included: $T=7.51$, $p<0.001$, $n=9$). There was significant variation in the mean distance between members of each pair (see Fig 3.1) although overall, the birds generally remained close together (mean distance apart: $4.57\text{m} \pm 0.087\text{m(SE)}$, $n=2606$). Mates of pairs 5 and 12 tended to stay closer together than the other pairs, and mates of pairs 1, 6, 7 and 8 stayed further apart than others.

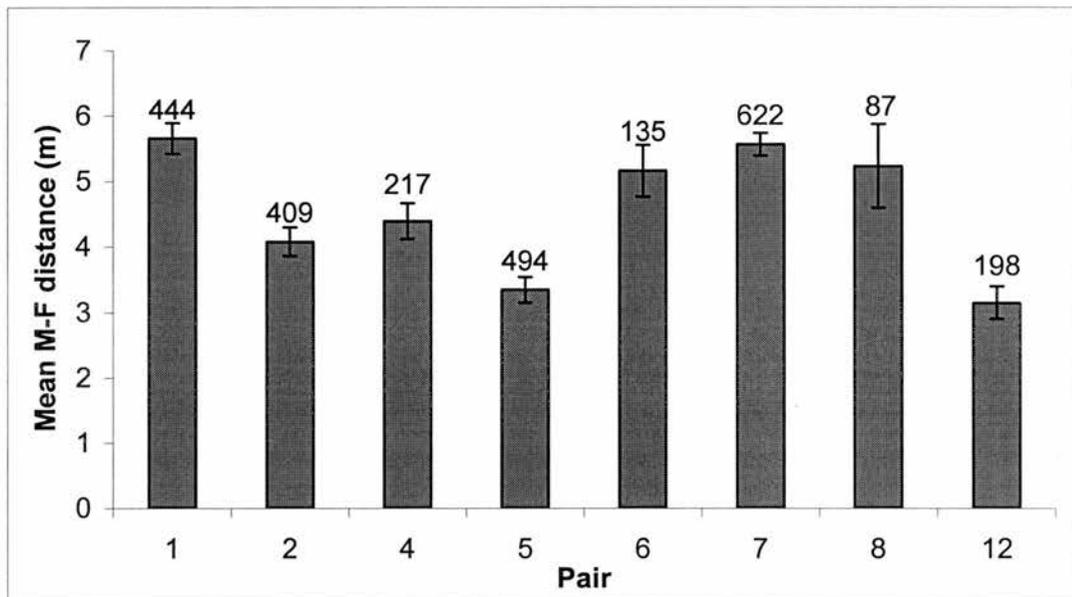


Fig 3.1: Mean distances between members of each pair (m), error bars show SE, numbers above each column show sample size per pair. GLM across pairs: $F_{7,2021}=7.84$, $p<0.0001$.

3.3.c Singing activity: These results are taken from both the scores for occurrence of different song behaviours, and the activity categories. The Other behavioural category included very diverse behaviours including roosting, brooding, preening, nest building and provisioning young. Many minutes contained both foraging and singing, and to allow a straightforward comparison of time allocated to the two

behaviours, the number of minutes involving both was divided equally between the two categories and added to those minutes containing only one or the other.

Over all breeding stages and pairs, both sexes spent more time foraging than singing, but females spent more time foraging (approaching significance) and less time singing than males (see Fig 3.2). Neither the time spent I phrase singing nor duet singing varied between pairs when proportions in different breeding stages were analysed.

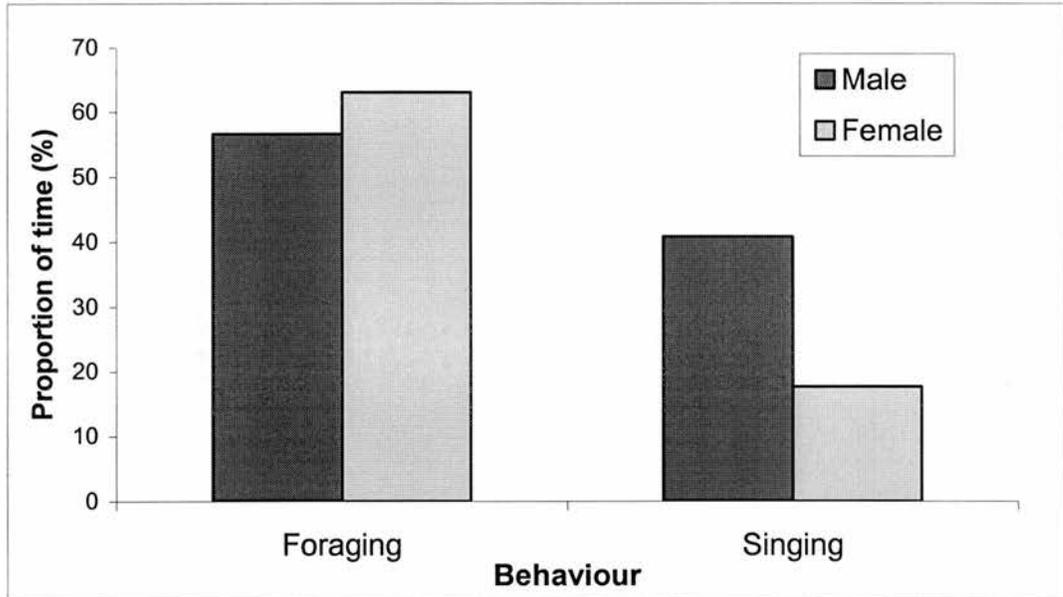


Fig 3.2: Proportion of minutes spent either foraging or singing for all birds, male $n=8$, samples=7191, female $n=8$, samples=2912. Mann-Whitney U tests were across proportions for each individual ($df=7$). Between the sexes, Foraging: $W=86$, $p=0.065$; Singing: $W=36$, $p<0.001$.

3.2.d Song rates: The difference in frequency of male solo song (I phrases) and of duets is demonstrated in Figure 3.3, solo song being much more frequent in all pairs. As described in the methods (section 3.2.c) the GLM for the song rate analyses was run on four variables: song switching rate, total song rate, female initiation rates and irregular duetting rates, the results discussed here are from the factor *Pair* (see Table 3.2). Total song rates, female initiation rates and irregular duetting rates all varied across pairs (see Figs 3.4 & 3.5), but there were no differences in phrase switching rates in different pairs. Total song was high in pairs 6, 8 and 12; and low in pair 7 (see Fig 3.4). Females 2 and 4 showed much lower duet initiation rates than other

females (see Fig 3.5). Figure 3.5 also shows mean irregular duetting rates, and there appears to be two groups to this data, with pairs 1, 4, 5 and 6 all showing low rates of singing irregular duets; and pairs 2, 7, 8 and 12 all having significantly higher rates.

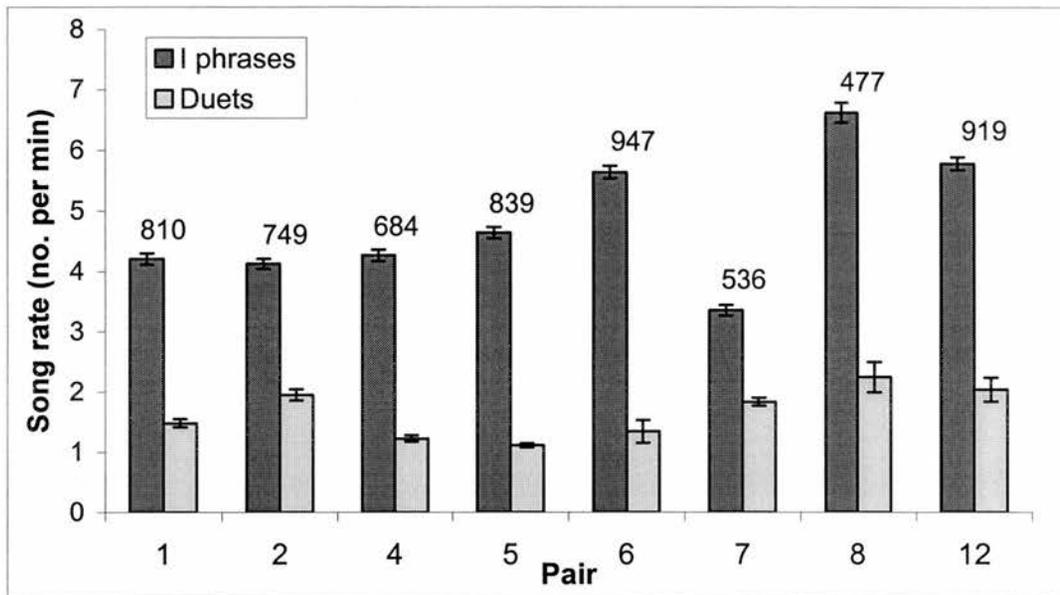


Fig 3.3: Rate (no. per minute) of I calls and duets for all pairs \pm SE, numbers above each pair show sample size. Paired t-test: $T=8.92$, $p<0.0001$, $df=7$.

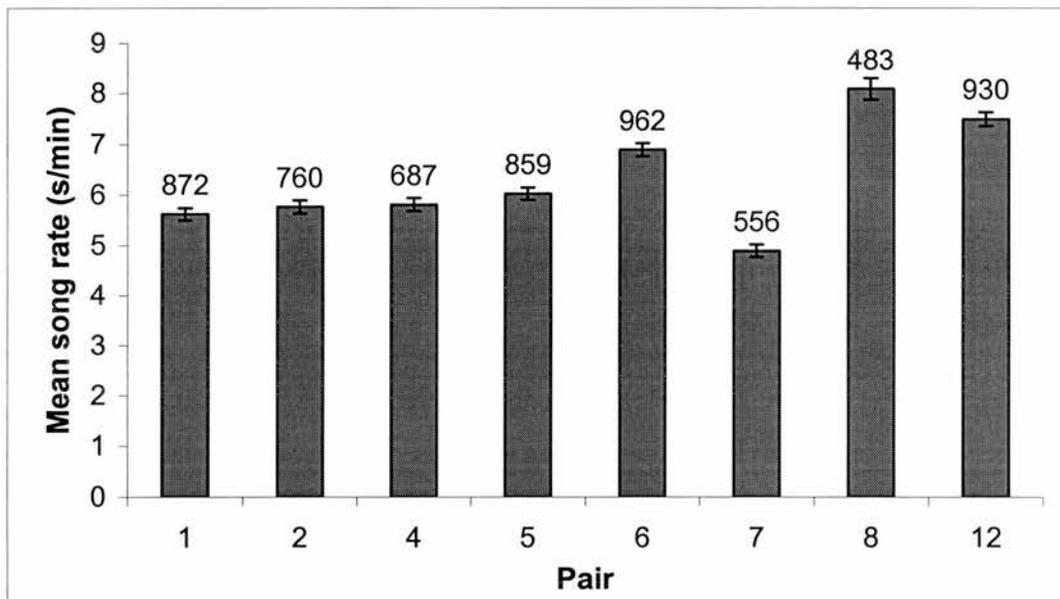


Fig 3.4: Total song rates in approximate sec/min for each pair, error bars show SE, numbers above columns show sample size per pair. $F_{7,5571}=4.70$, $p<0.0001$; Tukey *post-hoc* tests showed pair 7 < others and pairs 6, 8 and 12 > others.

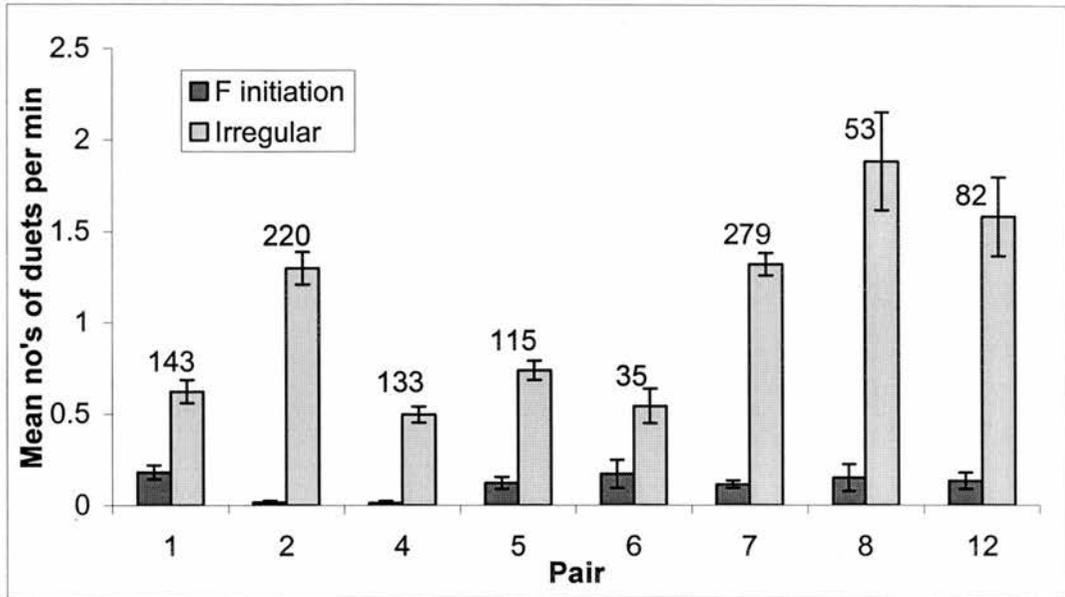


Fig 3.5: Rates of singing female initiated and irregular duets for all pairs, error bars show SE and numbers above columns show the sample size per pair for both variables. F initiation: $F_{7,928}=3.35$, $p=0.001$; Irregular duets: $F_{7,928}=5.99$, $p<0.0001$

3.3.e Song repertoires & duet phrase matching: Repertoires of I, F, and M phrases as measured from the 2001 observations are given in Table 3.3. Discovery rates of new phrases appeared not to be levelling off after 2001, rather, the addition of recordings from 2002 showed a continuing shift in repertoire content instead of an eventual asymptote (see Appendix 2). Thus repertoires from 2001 are presented, as this sample is constant in both observer effort and time period.

Table 3.3: Pair repertoires of song phrases and of duets (different FM combinations) identified from observations in 2001. 12a and 12b represent the original pair and later re-mated pair. Numbers in brackets show the number of song bouts (series of songs of the same phrase type) recorded for both I phrases and duets.

Pair	I phrase	F phrase	M phrase	Duets
1	24 (132)	16	12	20 (40)
2	24 (197)	19	16	19 (84)
4	24 (79)	14	13	18 (28)
5	31 (149)	10	13	22 (43)
6	24 (155)	16	15	11 (28)
7	24 (108)	15	14	20 (124)
8	23 (53)	7	8	9 (24)
12a	28 (136)	8	16	9 (22)
12b		14		18 (39)

Patterns in matching F with M phrase types to form duets were analysed through comparison with Poisson distribution frequencies, to indicate whether the occurrence of FM combinations differed from random i.e. whether birds consistently matched the same F and M phrases to give a limited number of set duets from the large number of potential FM combinations. This analysis was based on recordings from 2002, as these data included more pairs of birds, and two study populations. Table 3.4 and Figure 3.6 show two forms of results. The table shows G test results that compared the observed distribution of FM combinations against a random predicted distribution (i.e. the observed and expected numbers of duets that occurred zero times, once, twice, etc). In Figure 3.6, variance and means of each pair's FM distribution are presented, In a true random Poisson distribution variance = mean, thus where the variance exceeds the mean, the data are clustered, suggesting that consistent phrase matching is occurring. These two different methods are presented to show both the variation between pairs, and the greater robustness under small sample sizes of the mean:variance comparison method (see results from Pair 22 as an example).

Table 3.4: G test results comparing frequency distributions against Poisson predicted distributions. Numbered pairs were at La Suerte, and lettered pairs at El Zota, n shows the number of song bouts recorded for each pair (df=1).

Pair	n	G adj	p
2	71	8.2	0.003
7	33	18.1	<0.001
10	62	6.6	0.01
12	30	20.6	<0.001
14	37	12.5	<0.001
16	41	12.7	<0.001
17	94	4.0	0.03
18	54	10.3	0.001
20	50	6.3	0.01
22	22	27.9	<0.001
23	85	3.0	0.06
25	53	16.5	<0.001
C	57	11.0	0.001
D	34	24.8	<0.001
E	33	14.9	<0.001
F	59	7.3	0.005
G	53	12.5	<0.001

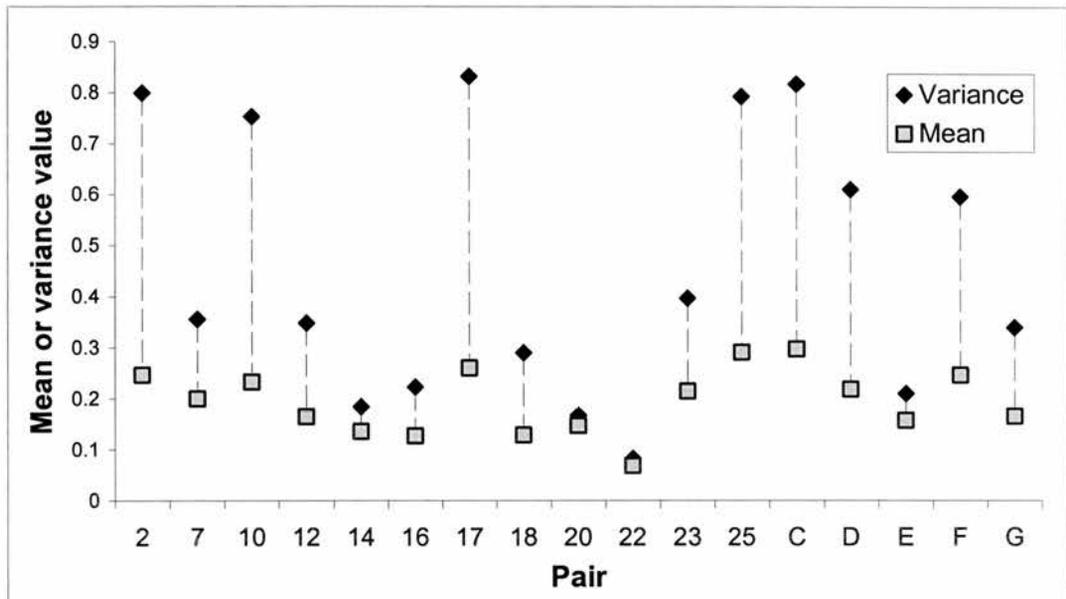


Fig 3.6: Mean and variance values per pair for the distribution of FM combinations across all potential combinations. Where variance > mean, the data are clustered, where variance = mean, the data are random. Numbered pairs were at La Suerte, lettered pairs at El Zota.

3.4 Discussion

This study followed 8 pairs of wrens very intensively through one breeding season, thus obtaining detailed information on the movements and singing activity of these pairs. The pairs demonstrated a considerable amount of variation in patterns of duetting behaviour, but also showed some consistencies that may be representative of more general behaviour.

3.4.a Sex-specific foraging patterns: One of the most marked trends observed in this study was the division of the foraging area vertically so that, although both sexes occupied a wide and overlapping range of heights, males consistently foraged above their mate as they moved together through the territory. This spacing behaviour whilst foraging may represent avoidance of disturbing prey, such as has been suggested for foraging flocks of waders (Goss-Custard, 1976; Goss-Custard, 1980), or may reduce competition between individuals (Bell, 1986). Foraging segregation according to sex has been reported in some species of *Phylloscopus* warbler, with

morphological differences being related to different foraging strategies (Morse, 1968; Busby and Sealy, 1979; Lynch *et al.*, 1985; Nystrom, 1990).

The tendency in sex-related differences for males to prefer higher foraging zones has been attributed to their greater role in territory defence (Morse, 1968; Busby and Sealy, 1979). This greater height allows for further transmission of song and better visibility. The preference could also be due to the male's greater need to mate-guard, with the higher position allowing better tracking of the female's movements. Although the canebrake wren, unlike the other passerines studied, demonstrates duetting and a high female involvement in territorial defence (Mann *et al.*, 2003), males do still invest more time in singing than females, and so the same function to the sex-specific division may apply here. There are slight morphological differences between members of a pair, with males generally being larger and heavier than their mate (Chapter 2, section 2.1). However, there were no obvious differences in foraging strategies, and overall heights used by each sex did overlap, so it seems unlikely that the separation was due to the use of different prey resources as was reported in the willow warbler, *Phylloscopus trochilus* (Nystrom, 1990).

3.4.b Song rates: Individual males and females did not show consistent differences in their time allocation to solo or duet singing, apart from the greater time spent singing by males, which was reflected in the very large difference between Introductory calling and duet singing time. However, within song bouts, song rates of introductory phrases and duets differed between individuals. Females were never observed singing solos, when they produced a song phrase it always elicited a male response, thus forming female initiated duets. The greater male song rates, and male responsiveness to female song are both suggestive of a role in mate-guarding, a possibility which will be tested in Chapters 4 and 7. Variations in female responsiveness to her mate's introductory calls, and in her initiation rates led to significant between-pair variation in duetting rates. The performance of irregular duets, where one or both members of the pair make a 'mistake' in the duet performance, also varied widely between pairs. This considerable degree of individual and pairwise variation in investment in singing raises the possibility that the motivation of both members of the pair to sing and to duet, and also the precision with which the pair are able to duet, could provide a mechanism for information

transfer on the state of the individuals and the pair-bond (Lynch *et al.*, 1985; Geissmann and Orgeldinger, 2000; Hofstad *et al.*, 2002; Nowicki *et al.*, 2002a). Chapter 4 takes a different angle on the variation in song rates, by looking at the patterns of duet use through different breeding stages, and provides an insight into individual fitness information that may be contained in singing activity patterns.

3.4.c Song repertoires: Canebrake wrens appear to demonstrate open-ended learning (Catchpole and Slater, 1995), in that individual repertoires of phrase types appeared to shift with new phrases replacing abandoned ones (see Appendix 2). This behaviour makes it difficult to calculate entire repertoire size at any one point in time, and thus the effort- and time-controlled 2001 subset was used. The small repertoires presented for pair 8, and for the female 12a are probably due to a smaller sample of recordings from these birds as both females were predated part way through the season, and as male 8 disappeared shortly after losing his mate. The repertoires and duet type consistency trends all extend preliminary data already published (Mann *et al.*, 2003), and demonstrate the large individual repertoires of both male and female wrens. These phrase repertoires are consistently used in specific combinations to form a limited, but still sizable, repertoire of duets. The large individual and pair repertoires provide several mechanisms by which individual recognition could take place: 1) That each individual or pair could have a unique set of song types (Beecher *et al.*, 1994); 2) That shared song types could carry characteristic variation between individuals (Brooks and Falls, 1975; Gentner and Hulse, 1998); and 3) that the sequence of performance of multiple song types could be unique (Gentner and Hulse, 1998). In Chapter 6 I will investigate further aspects of individual variation and study repertoire sharing in relation to micro and macro geographic scales.

3.4.d Conclusions: This chapter was intended to provide an initial insight into the duet use of canebrake wren pairs. The observational study allowed me to build an understanding of the variation in behaviours occurring naturally, and to develop hypotheses for duet function to be tested in later seasons. Looking simply at the differences in duet use between pairs has shown marked sexual differentiation in foraging patterns and, probably linked to this, a very strong divergence in the investment in singing. This species performs outstandingly intricate duets, with large

repertoires, tight coordination and complex phrase structure; and with an equally highly developed contribution from both sexes. However it still displays the typical sexually biased role in singing (Yasukawa *et al.*, 1987; Levin, 1996b; Perez-Villafana *et al.*, 1999; Nealen and Perkel, 2000), in that the male spends much more of his time singing than the female, and thereby that the male always responds to his mate's song, but the female's responsiveness is generally low and very variable.

Chapter 4: Influences of breeding state on duetting behaviour

4.1 Introduction

Changes in male song rates or song types with breeding state have been reported in a variety of birds and mammals (Eens *et al.*, 1994; Steenbeek *et al.*, 1999; Leitner *et al.*, 2001; Amrhein *et al.*, 2002; Foerster *et al.*, 2002), with several reasons being suggested for the observed trends. The importance of male song in attracting mates is well documented (Kroodsma, 1991; Catchpole and Slater, 1995), and it is therefore not surprising that observed differences in song rates correlate well with mated status (Mitani, 1988; Staicer, 1996; Hunt *et al.*, 1997; Gil *et al.*, 1999; Amrhein *et al.*, 2002). Møller (1991a) proposed that a male's song could be an honest indicator of his mate's fertility through its use in mate-guarding. He predicted that a peak in male song coinciding with the mate's fertile period would serve to keep out rival males when the risk of losing paternity was greatest. Evidence was presented from general song trends across populations, but this 'fertility announcement hypothesis' has not been supported by studies which have followed individual pairs (Krokene *et al.*, 1996; Rodrigues, 1996; Gil *et al.*, 1999; Hall and Magrath, 2000). Some authors have presented evidence that mated males use song to attract extra-pair copulations, particularly once their mate is incubating (Krokene *et al.*, 1996; Forstmeier and Balsby, 2002; Forstmeier *et al.*, 2002). However, this behaviour appears to be associated not so much with an increase in song, but with more movement around or even outside the territory (Currie *et al.*, 1998; Stutchbury, 1998; Forstmeier and Balsby, 2002).

In species with joint parental care, males may assist in provisioning the young until post-fledging independence. Several studies have looked for a drop off in song rate due to time spent provisioning young, or experimental evidence of a trade-off in time allocated to singing and parental care (Hegner and Wingfield, 1987; Dittami *et al.*, 1991; Hunt *et al.*, 1999). Results have been mixed, and even when male song rate has been increased by testosterone implants, some species have shown no negative impact on nestling provisioning or growth rates (Van Duyse *et al.*, 2000; Lynn *et al.*, 2002; Van Duyse *et al.*, 2002). Some of the theories outlined briefly here have very

limited supporting evidence or have as yet been the subject of few investigations. Regrettably, much of the work on breeding and song has focussed on experimental hormone treatments of breeding males, which, although providing important insight into the hormonal control of song, have done little to reveal the natural changes in song use and function with breeding state.

This chapter follows changes in the duetting behaviour of the canebrake wren through different breeding stages. To date, very little work has been done on changes in female song or duets with respect to breeding, even though the integration of song between members of a pair provides the potential for a greater array of song functions. Two areas that have received some attention are female mate guarding, and mate solicitation. Female song and female involvement in duet song has been demonstrated in some species, as functioning to mate-guard, or keep out same-sex territory intruders (Beletsky, 1983; Arcese *et al.*, 1988; Baptista *et al.*, 1993; Appleby *et al.*, 1999). Females in promiscuous or polyandrous mating systems appear to use solo calls or song to attract males, thereby increasing their number of copulations, and indirectly improving their mate quality through male-male competition (Wiley *et al.*, 1991; Langmore *et al.*, 1996; Semple and McComb, 2000).

One of few detailed studies into duetting and breeding behaviour comes from field and captive work on the slate-coloured boubou, *Laniarius funebris* (Sonnenschein and Reyer, 1983; Schwabl and Sonnenschein, 1992) which performs relatively simple antiphonal duets. These studies showed that, although male song rates were independent of breeding state, female song rates varied through the season, with highest duet rates during courtship and pre-breeding stages, and lowest rates during incubation and nestling care. Despite an absence of seasonal trends in male song or female hormone levels, Schwabl and Sonnenschein (1992) further proposed that male song rates of a certain phrase type (M1) were linked to their mates' oestradiol levels, and were independent of male testosterone levels. Although a tenuous possibility, the authors suggested that this was due to the long pair bond and high degree of cooperation seen in duetting species, where high male involvement in parental care may have lead to a system of regulating male song according to female reproductive state rather than testosterone levels. The canebrake wren has a similar breeding system to the boubou, being territorial and monogamous with joint parental

care and sexual monomorphy. However they possess very different and more complex antiphonal duets, and I will present here data on the patterns of duet use in a wild population through one breeding season.

4.2 Methods

4.2.a Observations: Observations were carried out at La Suerte from March to August 2001. Eight pairs were randomly selected from the population as subjects and were each visited every two days on a rotating observation timetable of three morning periods between 0500 – 1000hrs and occasional afternoon periods between 1500 – 1800hrs, times that covered the most active parts of the day. Each observation period lasted 60 – 90mins and involved gathering the variables summarised in Table 3.1 (Chapter 3, pg 3.3). Song descriptions will follow previous chapters, with a duet I(FM)n involving the male introductory phrase **I**, and the female **F** and male **M** cyclic duet phrases. Data other than song were gathered through instantaneous sampling whereby all measurements were noted once a minute. Song data were gathered in minute segments, coinciding with the instantaneous sample time, but with all song behaviour being noted. To minimise disturbance, nests were checked every three days when the female was away foraging.

Breeding states were defined according to nest observations and other indicative behaviour and categorised as follows: *Pre-fertile* non breeding or pre-breeding, *Fertile* nest-building and laying (Birkhead and Møller, 1992), *Post-fertile* incubation, and with nestlings and fledged young still being fed by parents, *Juvenile* independent juveniles on natal territory. Additional categories that will be referred to are: *Termination* cessation of breeding attempt either through desertion or nest predation, *Mate-loss* after predation of females during incubation, *Re-mating* up to six weeks after the pair formation, and *Intrusion* during an intrusion into the territory. Repeats of the same breeding stage in subsequent attempts were pooled for each pair.

4.2.b Data analysis: More than 228hrs of observation data were collected from the 8 pairs. Residuals of the numerical data were tested for normality, and parametric GLM tests were performed (with *post-hoc* Tukey tests). The various song measures had high numbers of zero values from times when the birds were not singing. As in

the previous chapter, to avoid these zeros overwhelming changes in song rate when birds were singing, and preventing the detection of these changes, these variables were divided into two data sets: 'song occurrence', with singing and non-singing minutes being scored as 1 or 0, and original 'song rate' values for the minutes with song (with non-singing minutes removed). Song rate variables (duet rate, duet length, I phrases, incomplete I phrases, female initiated and irregular duets, song type switching) were condensed after correlation into five that represented both male and female behaviour: *Mean duet length* (which was not correlated with duet or I singing rates), *total song rate* (calculated from I phrase and duet rates, see Chapter 3), *I phrase singing rate* (which although expressed in total song, was an independent measure of male behaviour), *song type switching* (which was not correlated with any song rate) and *female initiated duet rate* (which was only weakly correlated with duet rate, and strongly indicative of female motivation).

Wherever multiple variables are considered (including *post-hoc* tests), the critical probability value was taken to be $p_{\text{crit}}=0.01$. For data tested through Mann-Whitney U tests, $p_{\text{crit}}=0.05$ and was adjusted for ties. As described in the previous chapter (section 3.2.c), the GLM used for both chapters was designed to account for the non-independence of repeated samples from the same pair, by including the factors *Season day* and *Time of day*. The GLM factor discussed in this chapter is *Pair*Breeding stage*, which, being crossed, looked at changes according to breeding stage *within* each pair (other factors in the model: *Breeding stage*, *Pair*, *Season day*, and *Time of day*). Where non-significant results are mentioned without statistics, the p-value exceeded $p=0.1$, and thus did not approach significance.

4.3 Results

For a summary of breeding success in 2001, see section 3.3.a in the previous chapter. Two intruder events were observed both involving un-ringed solo males, and both lasting 1-2hrs (observation periods were continued until the intrusion ended). Remating data are presented from both pair 12 and pair 6, the male at 6 was accompanied by a new female for two days, but otherwise remained solo.

4.3.a Positional behaviour: There were some marked changes in the heights that males and females tended to frequent during different parts of the breeding season (Fig. 4.1). Males perched much higher after mate loss than at any other time, and females stayed lower when they had nestlings or fledged young (post-fertile). Note that here and later on in the results, only two pairs underwent some stages, and this renders the observed patterns less valid as indicators of general behaviour. However the GLM models looked at behaviour within pairs, and *post-hoc* analyses (both within the GLM, and in addition when $n=2$, two sample t-tests compared breeding stages) were carried out on each pair, so the analyses take account of this problem as much as is possible. The small error bars, for example during intrusions (see Fig. 4.1) reflect the considerable regularity of the behaviour of birds during this time.

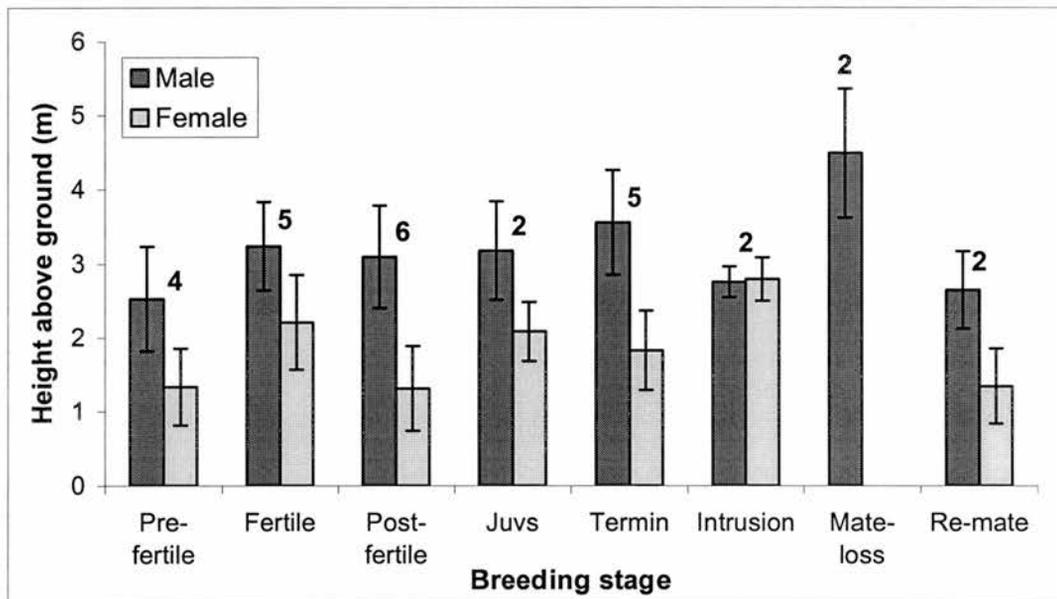


Fig. 4.1: Mean heights above ground (m) for males and females in all states. Error bars show SE, numbers above columns show number of pairs for each state. Male sample $n=7504$, female sample $n=2605$. GLM models for '*pair*breeding state*': Male height: $F_{19,7518}=63.1$, $p<0.0001$; Female height: $F_{17,2602}=30.63$, $p<0.0001$.

4.3.c Song behaviour: Time allocation to singing showed marked differences both between sexes, and across breeding stages. Figure 4.2 shows that the proportion of time spent singing tended to be greater for males than for females ($W=36.0$, $p<0.001$, $df=7$), matching results from previous analyses (Chapter 3). No robust statistics could be carried out on these proportions, as the number of pairs going through

matching sequences of breeding stages was to small. However a few patterns can be seen, particularly the peak in male time spent singing after mate loss, compared to all other stages, and the female peak during her fertile period and subsequent drop during parental care.

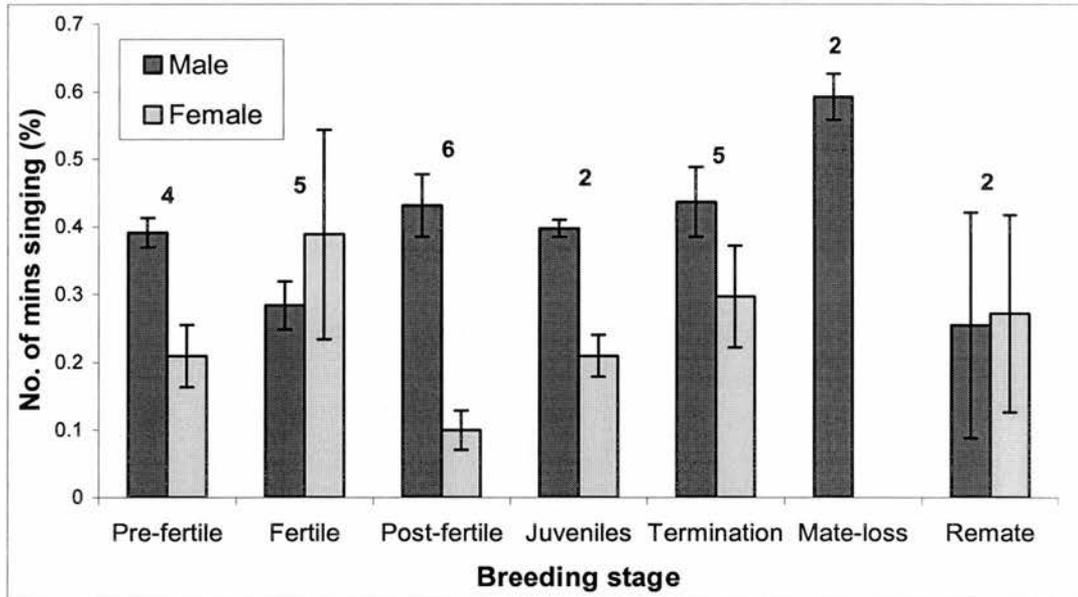


Fig. 4.2: The proportion of time spent singing for both males ($n=8$ birds, 7191mins) and females ($n=10$ birds, 2912 mins) in each breeding stage. Numbers above columns show the number of pairs in each breeding stage.

The rate at which males sang Introductory phrases varied significantly across breeding stages (Table 4.1 shows the results table from the GLM as an example, see also Fig. 4.3), post-hoc testing showing a peak after mate-loss, and the lowest point during the female's fertile period. Termination of a breeding attempt and re-mating both had higher I singing rates than pre-fertile, fertile and post-fertile stages. Male I phrase singing rates did not correlate with male height in any breeding stage (Pearson's correlations within individuals, all $p>0.4$), except in mate-loss and re-mating, where the two were positively correlated (Mate-loss: *male 6* $R=0.096$, $p=0.054$, $n=549$; *male 12* $R=0.226$, $p<0.001$, $n=322$. Re-mate: (male 6 non-significant); *male 12* $R=0.26$, $p<0.001$, $n=267$).

Table 4.1: The results from the GLM for male Introductory phrase singing rates, showing the overall model, as well as the effect of each of the five factors. All five factors show a significant influence on I phrase singing rates, and the model explains 19% of the variance in the data.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F value	P value
Model	118	8554.27	72.49	10.90	<0.0001
Error	5426	36101.36	6.65		
Corrected total	5689	44655.63			
Season day	77	1848.33	24.0	3.61	<0.0001
Time of day	1	84.94	84.94	12.77	0.0004
Breeding stage	11	697.53	63.41	9.53	<0.0001
Pair	7	321.61	45.94	6.91	<0.0001
Pair*Breeding stage	21	301.89	14.37	2.16	0.0016
R-squared	0.192				

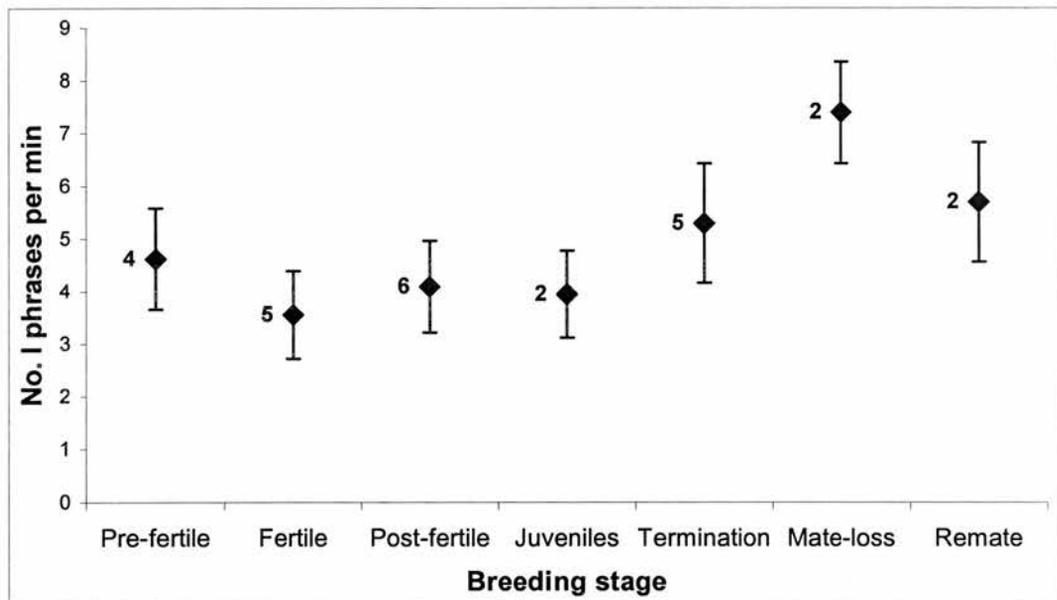


Fig. 4.3: The rate of male introductory (I) phrase singing by males ($n=8$) during different breeding stages. Numbers next to points show the number of pairs at each stage. GLM model with *pair*breeding stage*: $F_{21,5426}=2.16$, $p=0.002$.

Total song rate showed an identical pattern of change with breeding state as I phrases ($F_{21,5426}=1.94$, $p=0.006$, *post-hocs* as with I phrase results), reflecting the greater

level of solo male singing than of duets (see section 3.3.c, previous chapter). Rates of switching song types did not change significantly between breeding states.

Three aspects of duet performance all showed significant change with breeding stage. Duet rates (as numbers of duets per minute, see Fig. 4.4) were highest when the pair had independent juveniles, and rates after a termination of breeding were higher than during the breeding stages (fertile and post-fertile). Rate of female initiation of duets is also plotted on Figure 4.4. As these duets were much less common than male initiated, the rate shown is from all minutes that contained duets (whereas overall duet rates are taken from all minutes that contained song of any type). Female initiation was highest during the fertile period than during any other stage.

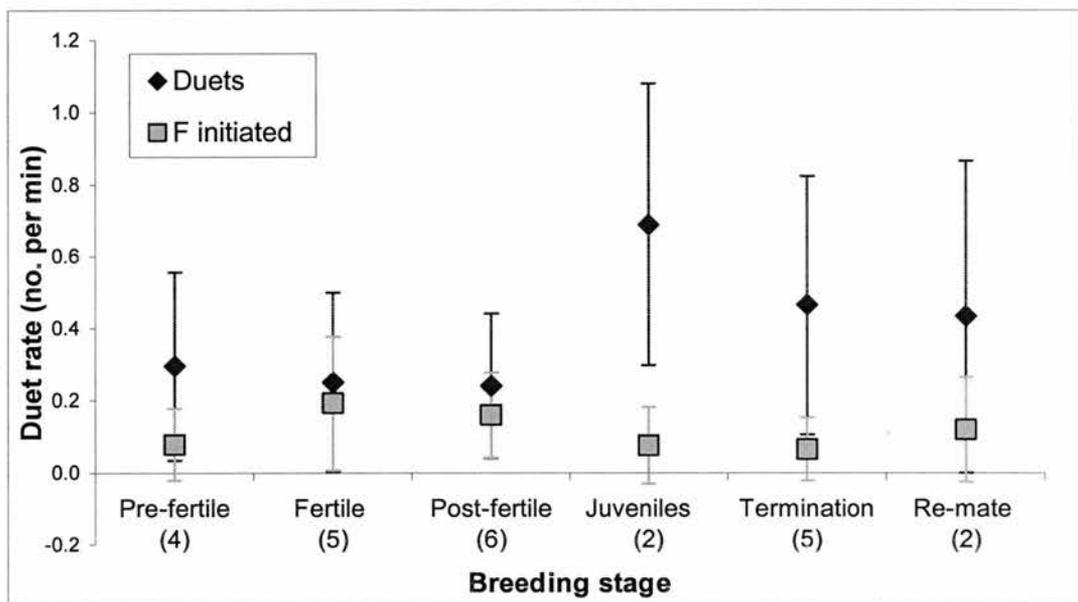


Fig. 4.4: Rate of singing duets (from all minutes with song) and female initiated duets (from all minutes with duets) as duets per min for different breeding stages. Error bars show SE, numbers in brackets show the number of pairs at each breeding stage (overall $n=10$). GLM model with $pair*breeding\ stage$: Duets: $F_{19,5476}=27.4$, $p<0.0001$; F initiated duets: $F_{17,928}=6.9$, $p<0.0001$.

The mean length of duets (no. of FM cycles per duet) was greatest during the female fertile period, with a decrease through post-fertile to juveniles. Duets were shorter in the pre-fertile periods than all other stages, including re-mating (see Fig. 4.5).

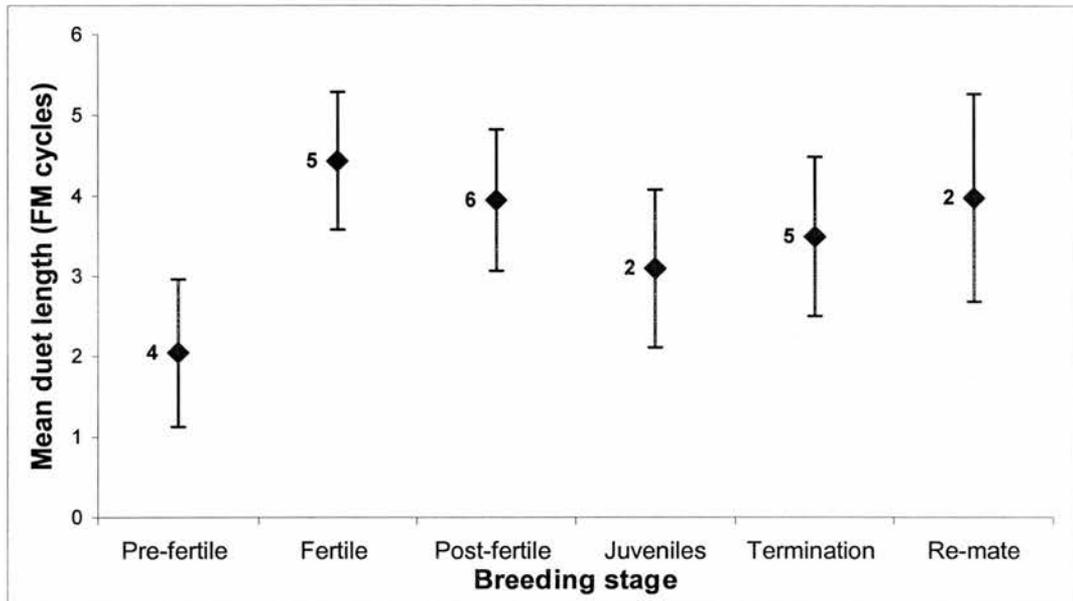


Fig. 4.5: Mean length of duets (no. of FM cycles per duet) in different breeding stages. Error bars show SE, numbers adjacent to points show number of pairs in each stage. GLM with *pair*breeding stage*: $F_{17,1019}=14.70$, $p<0.0001$.

4.4 Discussion

The pairs followed through the 2001 breeding season showed marked variation in duetting behaviour across different stages of the breeding cycle, and in other circumstances such as mate loss, re-mating, and during territorial intrusions by solo males. There was an apparent discrepancy between the lack of variation in the amount of minutes in which male song occurred (relative to female song, Fig. 4.2), and the significant variation in song rates within singing bouts (as no's per minute, see Figs 4.3 & 4.4). This may be due to an energetic constraint on the male which limits the amount of time he can spend singing, but that doesn't affect the rate at which he can produce song within the allotted time. This is a possibility that would require further study, so here, the considerable changes in song rates within the singing time will be discussed with respect to some of the theories for song change outlined in the Introduction (Section 4.1).

4.4.a Mate attraction: The canebrake wren forms long-term pair bonds, with new pairs not commencing breeding until their second season together (pers. obs.), thus the pre-laying courtship period observed in annually re-mating species does not occur. The female predations, however, allowed the study of the re-mating process,

and evidence is presented that supports the predictions for male song being used in mate-attraction. Both measures of male song production (song occurrence: Fig. 4.2, song rate: Fig. 4.3) were higher following mate loss in two males who underwent this, than during any other breeding stage, and most importantly, declined significantly after re-mating (i.e. Gil *et al.*, 1999; Amrhein *et al.*, 2002). After mate loss, males also tended to perch higher than during any other stage (Fig. 4.1). Perching higher when singing has been suggested as a mechanism for furthering the broadcast range of song, and thus improve the chance of mate-attraction (Morse, 1968; Holland *et al.*, 1998). If males always sang from a higher perch, more time spent singing, as seen following mate-loss, would increase the mean perching height without changing the actual height used for singing. After mate-loss and re-mating, the two males did tend to sing more when they were perched higher; however this correlation was not present during any other time. In other words, male perching height was not usually influenced by song rate; but it was following mate-loss. This again suggests a change in behaviour to increase the chance of re-mating, although it does not explain the continued correlation after re-mating in male 12. Despite the small sample size for mate-loss and re-mating, the trends are sufficiently strong within both males for the predictions of song use in mate-attraction to be upheld.

4.4.b Mate-guarding and EPCs: The fertility announcement hypothesis by Møller (1991) predicted a peak in male singing coinciding with the mates' fertility; which was not supported by this study. There was, in fact, a decline in I phrase singing (Fig. 4.3) and total song rates (as no's per minute) during the female fertile period. Higher rates of I phrase singing and duetting were seen following nest predation (Figs 4.3 & 4.4), which could be interpreted as a response to the female becoming fertile again for another breeding attempt, despite the decline during definite fertile periods. However an observed change in movement patterns unique to post-nest predation (both members of the pair moving together very rapidly around the territory with frequent alarm calling), suggests that the peak in singing for both males and females is likely to be a response to the predator attack (Scheuerlein *et al.*, 2001). This is supported by the apparent high risk to females during incubation that is demonstrated by the loss of three from seven breeding females in this study. In a genetic study on a congener with similar social structures, Haggerty *et al.* (2001) found no evidence of extra-pair parentage in 23 nests and 84 offspring of the Carolina wren (*Thryothorus*

ludovicianus). In the absence of any other information, it seems possible that EPCs might be equally rare or absent in the canebrake wren, although the small clutches and low success rates would make any EPC a highly costly event to the cuckolded parent.

Males increased their movement around the territory (pers. obs.) once their mate was incubating, and solo song during this period could have been used to attract extra-pair matings. Associations with females other than the mate were never observed (although non-singing encounters may have gone undetected), males were never seen to leave their territory, and they remained 100% responsive to their mate at all times (female solo song never occurred, so every female initiated song was replied to by her mate). The low female responsiveness to male song suggests that females also do not mate guard through song, possibly because the usual close association of the mates (see Chapter 3) allows constant contact and removes the need for vocal mate guarding. The only period when males were unaccompanied for long periods was during incubation, but it seems unlikely that females would attempt to mate-guard during this time. First, there is no evidence to suggest that males of the species divide paternal care between females, thus the female does not stand to lose paternal investment. And second, forcing male attentiveness in the brief intervals when she is off the nest still leaves much time when the male is unaccompanied, thus not considerably reducing any EPC opportunities. During incubation the pair's behaviour pattern was for the female to sing an F phrase whenever she came off the nest to forage. In response to her, the male would reply to form a duet, and instantly fly towards her; accompanying her and duetting frequently until she returned to the nest (pers. obs.). Female song during laying and incubation may inform the male of the status of herself and brood, which would facilitate the onset of paternal care after hatching. The equal division of parental care after hatching and the closely cooperative nature of pair behaviour, suggests that the female would benefit from re-establishing contact as often as possible both for pair-bond maintenance and to provide information on the status of the nest.

4.4.c Signalling fertility: Use of song by females to solicit matings has been shown only in species with polyandrous or promiscuous breeding systems (Wiley *et al.*, 1991; Langmore *et al.*, 1996; Semple and McComb, 2000). However, the female

wrens in this study showed distinct changes in song behaviour when they were fertile. During nest building and laying, females spent more time singing than during any other stage (Fig. 4.2), rate of female initiation of duets was highest (Fig. 4.4), and duets were longer (Fig. 4.5). Overall duet rate, however, did not change. Duet length is normally a female driven behaviour – the male is usually the last bird to sing, with the female choosing to end the duet by failing to reply. Thus the two indicators of female motivation to duet, female initiation and duet length, both peaked during fertility, and females also appeared to spend more time singing at this stage.

Female canebrake wrens may be signalling their fertility to their mate through this increased motivation to sing. This signalling could allow the male to invest more efficiently in copulation, and could also provide paternity assurance to her mate to ensure his later contribution to parental care (Iwasa and Harada, 1988; Møller, 1991b; Markman *et al.*, 1995). Mate-guarding is thought to be a costly behaviour (Alberts *et al.*, 1996; Komdeur, 2001), and if the male's contribution to parental care is important (Lack, 1968; Winkler, 1987; Markman *et al.*, 1996), it may be beneficial for the female to ensure that her mate is fully committed to his brood by being sure of his paternity. Female preference for her own mate has been demonstrated experimentally using song recognition (Lind *et al.*, 1997; O'Loughlen and Beecher, 1997; O'Loughlen and Beecher, 1999), and observations of wild birds (Currie *et al.*, 1998), which would support the idea that mating only with your own mate may sometimes be the best option. The females in this study appear to signal their fertility without any behaviour suggestive of seeking EPCs, and therefore it seems probable that females are seeking to ensure adequate fertilisation of their eggs, and may be providing paternity assurance to their mate to guarantee later parental care.

The fact that overall duet rates did not change during the fertile period may reflect an energetic constraint during this time, as has been suggested for the time males spent singing. Such that although the female could shift her duetting behaviour, the demands of nest building, egg formation and laying prevented an overall change in duet rates.

4.4.d Parental care: There is mixed evidence for a trade off between song and provisioning young (Greig-Smith, 1982; Hegner and Wingfield, 1987; Dittami *et al.*, 1991; Hunt *et al.*, 1999; Van Duyse *et al.*, 2000; Lynn *et al.*, 2002). This study shows that males did not appear to be subject to such a trade-off, as I phrase singing rates actually increased from the fertile period to the male parental care periods (contributing roughly equally with the female in nestling and fledgling stages, pers. obs.), and did not differ between pre- and post-fertile periods (Fig. 4.3). Females, however, appeared to show a shift in time spent singing, with very low levels during the post-fertile stage relative to both fertile and pre-fertile stages (Fig. 4.2). Duet length also declined from fertile through post-fertile to juvenile stages, although it was lowest during pre-fertile periods (Fig. 4.5). This trend for females to spend less time singing (song occurrence), and sing shorter duets during parental care suggests that female singing behaviour was affected by a trade-off with parental care. This fits with non-song evidence from sunbirds (*Nectarinia osea*, Markman *et al.*, 1996), but contrasts totally with data from reed warblers (*Acrocephalus scirpaceus*) where the male suffers a trade-off between sexual and parental behaviours, but the female does not (Dittami *et al.*, 1991).

It is possible that instead of an energetic trade-off, low female vocalisation activity and low foraging heights during parental care could be an adaptive response to the vulnerability of the young to predation. Broods were mostly predated during nestling care, but also during incubation or just following fledging. Provisioning nestlings involves both members of the pair visiting the nest much more frequently than during incubation, and nestlings also produce both calls and odours that eggs do not. The wren chicks in the study fledge before they can fly (Marshall-Ball and Slater, 2003), and are adept at scrambling through scrub, which points to the nestling stage being one of high predation risk. Reducing the amount of time spent singing, and approaching the nest more furtively may reduce the conspicuousness of the female's provisioning. Males, although provisioning silently, would sing from high perches following each delivery to the nest and thus separated their singing and parental behaviour in a way that females did not.

Pairs duetted at a greater rate when they had independent juveniles (loosely associated with the parents) on the territory than during any stage other than

territorial intrusion (Fig. 4.4). If this were purely a response to the decreased predation pressure or the cessation of a trade-off between care and singing, duet rates should not exceed non-breeding levels. However as they do, I suggest that the parents may be providing a stronger song stimulus to their offspring during the initial learning period of the young. Young at this time produce a lot of sub song, and as their song develops, they start to join in with the parents' duets (Appendix 2). The immense complexity of the duets of this species, both in repertoire and timing, appears to incur a long learning period (year old male song phrases were still distinguishable from older males, unpubl. data), and maybe tutoring provides the juveniles with greater learning potential. The role of parental song in tutoring young could only be precisely determined through lab studies, but the ontogeny of duetting in wild birds, looking at tutor choice and innovation, is a subject that warrants further investigation.

4.4.e Predation and the pair-bond: Termination of a breeding attempt through nest predation or desertion occurred 9 times in 5 pairs. An actual desertion event was observed once, and was apparently due to the presence of a snake on the ground beneath the nest. Terminations lead to higher male introductory singing rates (Fig.4.3), and higher duetting rates relative to the preceding breeding stage (any of the post-fertile stages, Fig. 4.4). I phrase rates and duet lengths (Fig. 4.5) were elevated above levels seen during pre-fertile and fertile stages, and thus the change did not represent a return to non-breeding singing behaviour. The increased song rates may be a way of re-affirming, or assessing the pair-bond following the failure to breed. Divorce, although rare, was seen in the population, and having failed in one attempt to breed, perhaps a re-affirmation of partner commitment is needed before another attempt is made. The observed desertion, and behaviour following nest predations, was characterised by very high occurrence of alarm calling, particularly by the female. This could have been a mechanism of informing the male of the event, or could be a response to the predation risk to the female. Experiments investigating the role of duets in pair-bond formation and advertisement are discussed in a later chapter (Ch. 7).

4.4.f Conclusions: This chapter has presented a detailed study of changes in song behaviour in a duetting species. Some of the results have matched an early study on

African shrikes (Sonnenschein and Reyer, 1983), but the comprehensive nature of the data in this study have allowed a greater insight into duet use with breeding. Evidence from the canebrake wren appears to support theories of song being used as a mate attractant, and to coordinate reproductive behaviour within the pair. The study adds more evidence against the fertility announcement hypothesis (Møller, 1991a; Gil *et al.*, 1999), and also against a trade-off between singing and parental care in males. Shifts in female singing behaviour appear to be driven by fertility signalling to her mate, and later by an apparent trade-off between parental care and time spent singing, and possibly by the considerable predation risk to herself and her brood.

Chapter 5: Species recognition and inter-specific aggression.

5.1 *Introduction*

The species-specificity of song and calls in birds is a well-described phenomenon (Catchpole, 1982; Kroodsma, 1991), maintained by the need to identify conspecifics from heterospecifics (Becker, 1982). Distinguishing between own-species calls and those of other species is important in identifying threats to a defended resource such as a mate or territory (Catchpole, 1982; Falls, 1982). This is also an underlying requirement for mate-choice, although the two functions can lead to conflicting selection, where species recognition requires consistency in a signal within a species, but sexual selection drives towards individual variation (Endler, 1992; Kroodsma, 1991). Distinguishing between own and related species during mate selection will also be reinforced where hybridisation incurs a loss of fitness (de Kort *et al.*, 2002; de Kort and Ten Cate, 2001; Grant and Grant, 1992).

Mate-choice, hybridisation avoidance and mate or resource defence all require the ability to identify conspecific calls from a background of other sounds. However, specific recognition of the vocalisations of other species may also be advantageous for several reasons. Although other species do not usually represent a risk to mate-defence, they may compete for food or nest sites (Cody, 1978; Garcia and Arroyo, 2002; Orians and Willson, 1964). In this situation, aggression between species, and displacement of less competitive species can occur (Garcia and Arroyo, 2002; Pearson and Rohwer, 2000; Piper and Catterall, 2003). In contrast to interspecific aggression, however, multi-species assemblages in birds require heterospecific attraction (Greig-Smith, 1978; Krams and Krama, 2002) with birds being attracted by the calls of their own and other species. For example, the ability to recognise alarm calls of other species appears to be involved in anti-predator behaviour such as recruiting to mobbing a predator or fleeing to safety (Forsman and Monkonnen, 2001; Krams and Krama, 2002; Shriner, 1998).

Several factors may lead to territorial segregation between pairs of sympatric, closely related species (Murray, 1988; Orians and Willson, 1964). In some studies, this

separation appears to arise through divergent habitat requirements or foraging behaviours (Hoi *et al.*, 1991; Martin and Thibault, 1996). However, many species demonstrate inter-specific aggression in response to playback, which suggests the presence of direct competition (Robinson and Terborgh, 1995; Rolando and Palestini, 1991). When unequal, this competition can lead to the competitive exclusion of one of the species in areas of sympatry (Halley and Gjershaug, 1998; Pearson and Rohwer, 2000).

The canebrake wren (*Thryothorus modestus zeledoni*) and black-throated wren (*T. atrogularis*) are sympatric in much of their range (Brewer, 2001), and occur together at the two study sites in north-eastern Costa Rica. *T. m. zeledoni* territories tend to be centred around marshes and adjacent secondary growth, where-as *T. atrogularis* occupies secondary growth at forest edges. The two species often have abutting or slightly overlapping territories. Both species sing duets, but *T. atrogularis* sings overlapping songs with males and females having similar song types, as opposed to the strictly sex-differentiated and antiphonal duets of *T. m. zeledoni* (Fig. 5.1). In contrast to the similarities in habitat use and foraging behaviours, the species are very distinct, with the duets covering almost non-overlapping frequency ranges, and with markedly different plumage colouration and patterning. This chapter presents an experimental study of recognition of songs from sympatric and potentially competing congeners. The prediction was that canebrake wrens would be able to recognise songs of black-throated wrens as well as those of their own, and would react aggressively based on the assumption that the other species represented a resource threat.

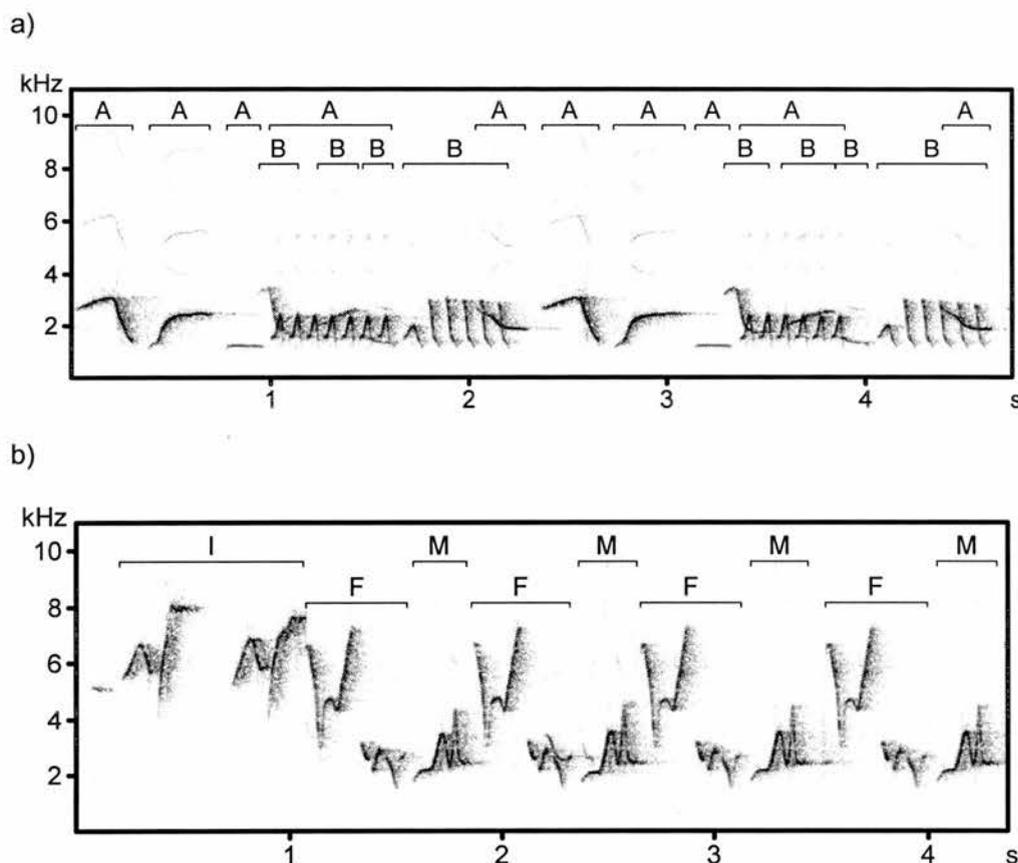


Fig. 5.1: Sonograms showing examples of duets of a) *T. atrogularis*, and b) *T. m. zeledoni*. In a) letters A and B indicate syllables (with trills marked as one section) sung by the two birds (sexes not known), and in b) letters show the male introductory phrase (I), followed by cycles of female and male duet phrases (F and M). Both recorded at La Suerte in 2001.

5.2 Methods

Birds at both field stations were used in this study, please refer to Chapter 2 for habitat structure at each site. Playbacks were carried out to pairs of the canebrake wren, with 4 pairs studied in July-August 2002 and 6 pairs in November-December 2002 at La Suerte; and 4 pairs at El Zota during July-August 2002.

5.2.a Playback design: Playback experiments consisted of two 5 minute stimuli: one of a series of duets of *T. m. zeledoni*, and one of a series of duets of *T. atrogularis* (Fig. 5.1), the two stimuli being separated and followed by 10 minutes of silence (total trial duration, 30 mins). Stimuli were constructed using Avisoft

SASLab v5.1 (R. Specht, 2002) and were constant in amplitude, length and timing of the individual duets and overall amount of singing time. Each 5 minute stimulus was taken from one original duet recording, with 6 duet samples for each species, each from different pairs. Stimuli were allocated to subject pairs in a randomised block design. This means that the sequence of the two stimuli was randomly assigned in the 1st of two trials on each subject pair, and reversed for the 2nd trial. To further avoid pseudo-replication (Kroodsma, 1989; Kroodsma *et al.*, 2001), the original recordings that the stimuli were constructed from were also randomised, and no subjects ever heard the same recording twice. Subjects only heard recordings taken from non-neighbours to avoid confounding effects of familiarity.

Playbacks were carried out using a 40W amplifier (T1140), portable Panasonic CD player (SL-SX228), and 5" Visaton passive full range speaker. The speaker was positioned approximately 10m within the subjects' territory boundary and facing into the territory centre. Playback site was kept the same across replicates to control for perch distances affecting approach behaviour. Playback amplitude was controlled in all experiments by occasional testing with a sound pressure level meter (Beha 93411), with the volume being set to a level equal to that measured from the wrens themselves. Continuous sound recordings were made throughout all trials, using a Sennheiser ME-66 gun microphone and a Marantz CP430 tape recorder (with chrome tapes). These were positioned about 5m from the speaker, again facing into the territory. Observations were made from concealed points, using instantaneous sampling (once per minute) to record position throughout the 30 mins, additionally all songs and their structures were noted for each minute (see Table 5.1 for a list of variables measured).

Playbacks were all carried out between 0700-0830 hrs, and were preceded by a 30 min observation period to check the status of the pair (playbacks were not carried out if the subjects were showing alarm behaviours from intruders or predators). Replicate trials on the same pair took place at least a week apart.

Table 5.1: A summary of the variables measured during experiments detailing categories used and sampling frequency. Switches in song type included either I, F or M phrases, however a switch in one always led to switches in the others.

Variable	Measurement Scales	Recorded
<i>Behaviour</i>		
Male & female height	All distances were estimated in metre ranges: 0-1, 1-3, 3-6, 6-9, 9-12, 12-15, 15-20, 20-30 & 30+.	Start of each minute
Male & female distance to speaker		
M-F distance		
<i>Song</i>		
No. of I calls		No's per minute
No. of duets		No's per minute
Duet structure	i.e.: I(FM) ₅ , I(FM) ₂ M, I(M) ₃ (FM) ₄ , (FM) ₃ F	Every duet
Phrase type switches	In either I, F, or M phrases	No's per minute

5.2.b Data analyses: Responses were compiled as means for each of the four experimental sections (Stimulus 1 playback, subsequent interval, stimulus 2 playback & subsequent interval) for each subject pair and trial. These were then tested through repeated measures GLM models (residuals fitting normality), which tested for relative changes in behaviour whilst controlling for effects of day and subject pair (subject: *pair*, repeated measures: *trial* and *experimental section*, independent factors: *site* and *season*). Site and experimental section were crossed in the model to test for differences in response to *T. atrogularis* in the two sites.

An initial set of 12 variables (male and female distance to speaker, male-female distance, male and female height, I phrase rate, incomplete I phrase rate, duet rate, mean duet length, female initiated duet rate, phrase type switching rate and total song rate), was reduced to 5 that best represented the responses of the pair: Male distance to speaker (highly correlated with female distance and had larger sample size), male-

female distance (not correlated to distance to speaker or song rates), mean duet length (not correlated with duet rates, or total song), phrase switching rate (correlated with song rates, but of interest as a separate response), and total song (combines I phrase and duet singing rates for an index of the pair's song production per minute, see Chapter 3, pg 3.4). As the GLM model (with *post-hocs*) was run on these five variables, the critical probability value was taken as $p_{crit}=0.01$ in all analyses.

5.3 Results

No differences were found in responses to playback between the summer and winter seasons. The only behaviour that differed between the two sites was male I phrase singing rate, which was tested separately from Total song as field observations suggested that the difference in male song was present. Males at El Zota sang more in response to *T. atrogularis* playback than at La Suerte, but their response to *T. m. zeledoni* playback did not differ (Fig 5.2, playback sections only). Table 5.2 shows the full GLM results table as an example of the structure of the model and showing the relative importance of the three factors.

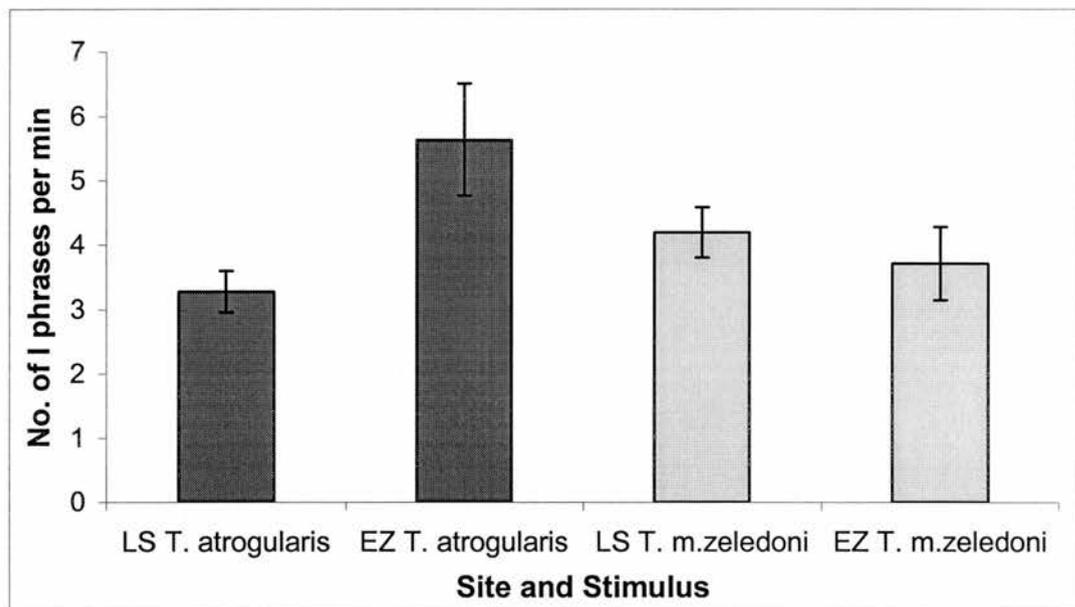


Fig 5.2: Rate of male Introductory phrase singing in response to *T. atrogularis* and *T. m. zeledoni* at La Suerte (LS) and El Zota (EZ). GLM *experiment section*site*: $F_{4,29}=3.81$, $p=0.011$, $n=14$, *post-hoc*: La Suerte *T. atrogularis* < El Zota *T. atrogularis* & La Suerte *T. m. zeledoni*.

Table 5.2: The full results table from the repeated measures GLM testing the effects of site, season, experimental section and trial on male Introductory phrase singing rates. 'Pair' is the subject factor in the model, and thus is not tested as an experimental factor, experimental section and trial were the repeated measures.

Null model	Degrees of Freedom	Chi-squared value	P value	
	1	34.32	<0.0001	
Effect	Numerator d.f.	Denominator d.f.	F value	P value
Season	1	11	0.32	0.583
Trial	3	91	2.75	0.047
Expt. Section	1	91	5.15	0.026
Expt. Section*Site	4	29	3.81	0.011

Duets were longer (more FM cycles) during playback of either stimulus than they were during intervals (see Fig. 5.3), but did not differ between the two stimuli. The rates of switching phrase types were greater during playback of both stimuli than during the intervals, and were higher in response to *T. m. zeledoni* stimuli than to *T. atrogularis* (Fig. 5.4). The pairs also sang more in response to *T. m. zeledoni* playback than to *T. atrogularis* (Fig. 5.5a). As the rate of song switching showed a different pattern of response to total song rate despite being correlated, it is presented as a separate behaviour. The distance between members of the pair were less, and male distance to speaker was less during *T. m. zeledoni* playback, than during that of *T. atrogularis* (Figs 5.5b & c). Figure 5.5 shows the response of the wrens during the playback periods only (excluding the intervals).

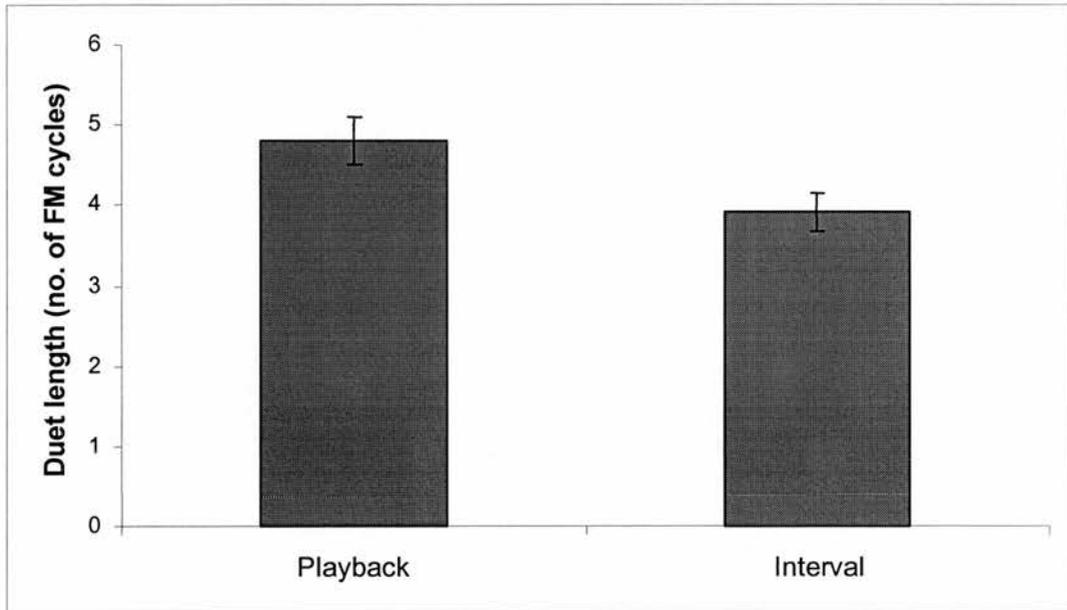


Fig. 5.3: Mean length of duets sung in response to playback and during the silent intervals. $F_{3,78}=4.46$, $p=0.006$, $n=14$ pairs (both stimuli combined as they did not differ significantly).

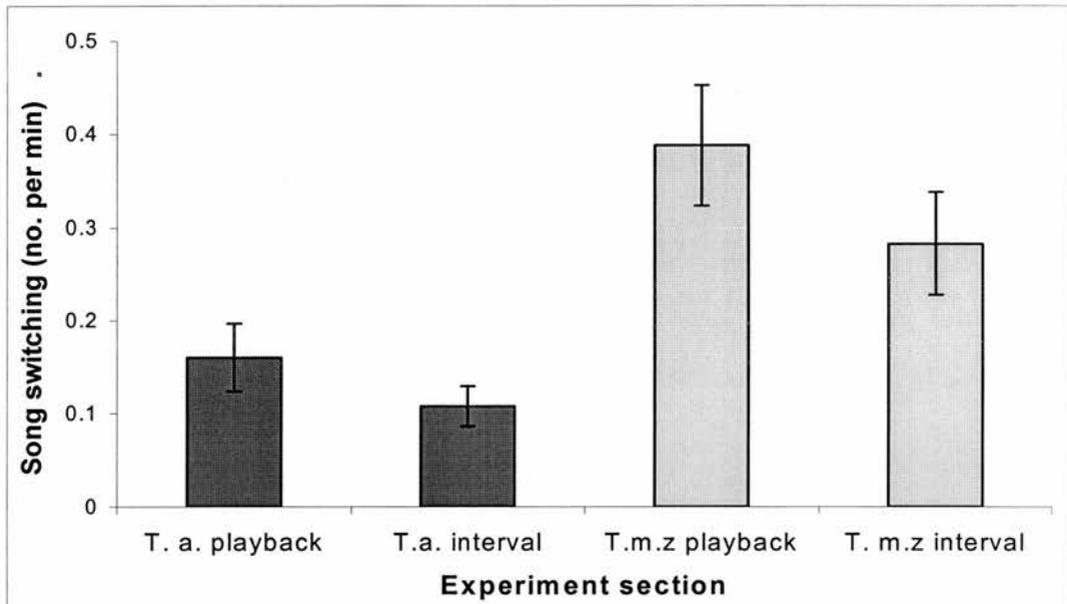


Fig. 5.4: Song type switching rate (no's per min) during the four experimental sections: *T. atrogularis* (T.a.) playback and interval, and *T. m. zeledoni* (T.m.z) playback and interval. $F_{3,63}=9.45$, $p=0.0055$, $n=14$, *post-hoc*: all sections differ.

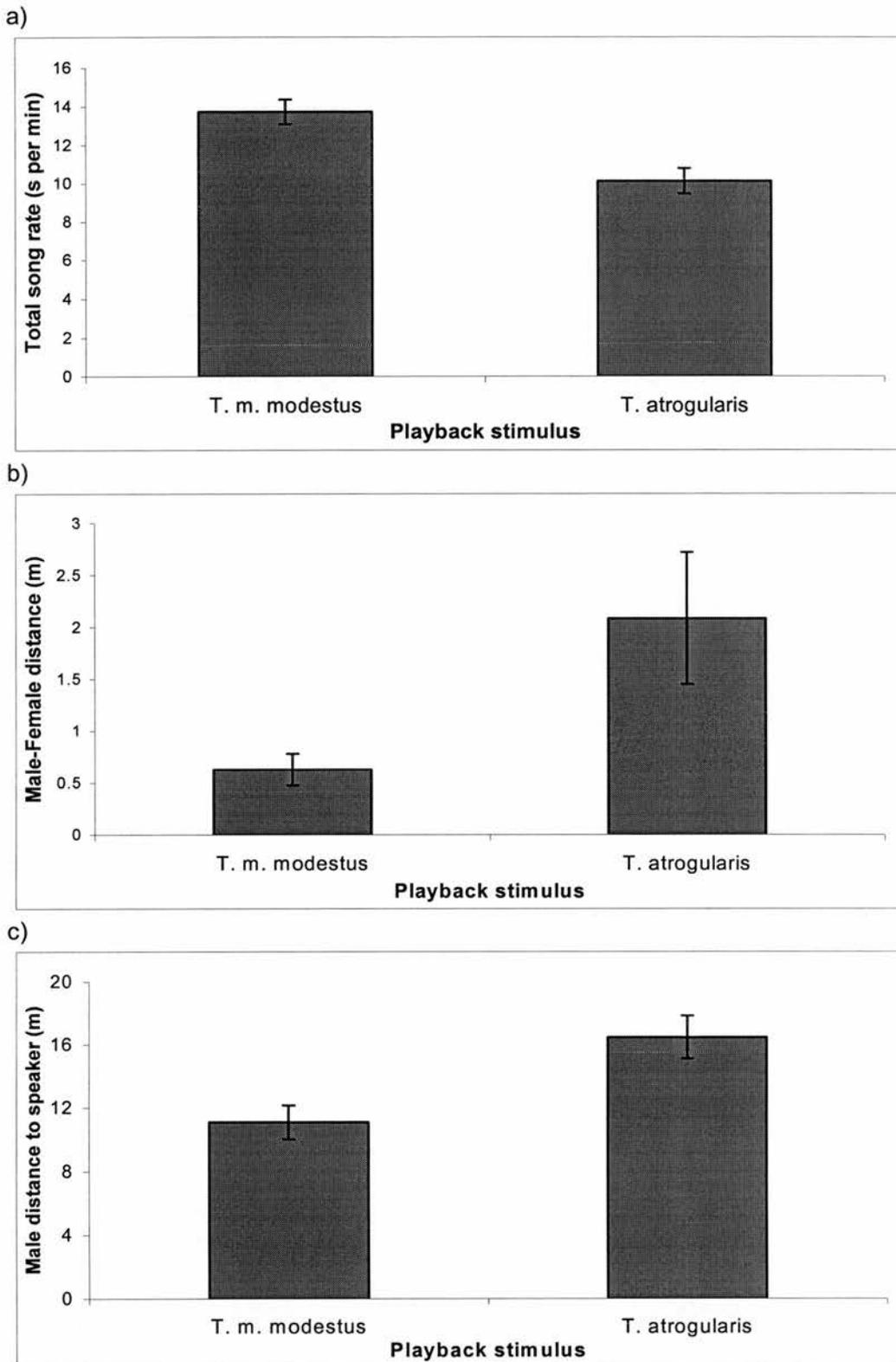


Fig. 5.5: Responses of subject pairs ($n=14$) to the two playback stimuli, a) total song rate (s per min), $F_{1,95}=31.58$, $p<0.0001$; b) mean distance (m) between members of the pair, $F_{1,52}=52.4$, $p=0.0036$; and c) male distance to speaker (m), $F_{1,94}=8.29$, $p=0.0049$ (playback sections only, intervals did not differ significantly).

5.4 Discussion

This chapter presents results from a playback experiment that suggests an ability in the canebrake wren to identify duets of the sympatric black-throated wren in addition to those of its own species. The wrens reacted highly aggressively to conspecific song, sometimes even attacking the speaker, and to a lesser degree reacted similarly to congeneric song.

One theory for interspecific aggression is that birds simply mistake the song of other species as that of their own (Møller, 1992; Murray, 1976; Murray, 1981). Convergence to, or mimicking songs of another species may however be used to interact with and exclude another species, by simulating another member of the recipient species (Baptista and Catchpole, 1989; Cody, 1973; Sorjonen, 1986). The two species in this study have very different songs, with both the duet structure and frequency ranges being highly distinctive. This makes it unlikely either that the canebrake wrens were mistaking heterospecific song as conspecific (Cody, 1973; Møller, 1992; Murray, 1976), or that the birds are currently competing for acoustic space (although evolutionary divergence may have stemmed from the need for species-specificity) (Doutrelant *et al.*, 2000a; Doutrelant *et al.*, 2000b; Wallin, 1986). This therefore suggests that some inter-specific territoriality is taking place, with the two species probably needing to exclude each other due to a high dietary overlap (Brewer, 2001; Skutch and Stiles, 1989). The difference in male *T. m. zeledoni* response to *T. atrogularis* song between the two study sites supports this suggestion. Subject males at El Zota even appeared to respond more strongly (but non-significantly so) to heterospecific song than to conspecific.

El Zota was a much more forested site than La Suerte, with marshes bordered immediately by forest, and hence potentially considerable contact between the two species. La Suerte, by contrast had previously been heavily cleared, and marshes were bordered by scrub that extended to the forest patches. The greater heterogeneity at La Suerte meant that the preferred habitats of the two species only occasionally abutted. The fact that the higher response to *T. atrogularis* was at the site where overlap was greatest goes against predictions that higher contact would lead to habituation towards a non-competing species and a decline in response to their song

(i.e. Kreutzer and Guttinger, 1991). As in this study, other passerine species such as *Acrocephalus* warblers, nightingales and treecreepers have shown a relationship between greater sympatry leading to greater interspecific aggression (Catchpole, 1978; Catchpole and Leisler, 1986; Gil, 1997; Sorjonen, 1986). That male canebrake wrens at El Zota were more aggressive to congeners suggests that interspecific competition at this site was a greater threat than at La Suerte. That female canebrake wrens did not differ in their responsiveness between the two sites may be due to their greater involvement in breeding behaviour in the summer (males do not nest build or incubate), and their lower involvement in territorial defence (outwith playback experiments, see Chapter 3).

Inter-specific spacing has been described for several groups of birds including wader flocks (Shubin, 1999), small passerines (Gil, 1997; Hudman and Chandler, 2002; Rolando and Palestini, 1991), and multi-species communities (Clarke and Schedvin, 1999; Piper and Catterall, 2003; Robinson and Terborgh, 1995). In some cases the lack of evidence for inter-specific aggression suggests that species are isolated as a result of differences in foraging or habitat requirements (Hoi *et al.*, 1991; Hudman and Chandler, 2002; Martin and Thibault, 1996). However, several playback studies have not only shown aggressive responses to song of sympatric species, but also a difference in levels of response from the competing species. These studies lend support to the theory that asymmetrical aggression can lead to displacement of one species (Clarke and Schedvin, 1999; Dearborn, 1998; Piper and Catterall, 2003; Robinson and Terborgh, 1995). The few available data (Brewer, 2001; Skutch and Stiles, 1989), and my own observations indicate that both species of wrens in this study forage in the same way and for the same prey range. They do not differ greatly in body mass (23g and 24g, Skutch and Stiles, 1989) and thus it seems unlikely that an ecological separation other than that of habitat use exists. Competition when overlapping would be the expectation between such similar species, and further work would be needed to ascertain whether either species is excluding the other from a preferred habitat.

5.4.a Conclusions: The experiment presented here is far from conclusive proof of inter-specific territoriality, as it was initially planned simply to serve as a control for conspecific recognition. Further experiments using other sympatric, but less

overlapping congeners, as well as sympatric but non-insectivorous passerines had to be stopped due to low responsiveness of some species in the winter and an extremely wet season. These experiments would have examined the possibility that the subject wrens would react to any loud song, and were not specifically recognising a competitor species. They would also have looked further at the hypothesis of inter-specific competition by comparing relative aggression to a range of congeners, which share varying degrees of niche overlap. Despite its limited scope, this study is the first to provide experimental evidence for inter-specific responsiveness in a duetting species. The potential implications of this study are discussed further in Chapter 8 (section 8.3).

Chapter 6: Repertoire sharing, geographic variation and aggressive signalling in duet song.

6.1 Introduction

The two fundamental functions of bird song: mate-attraction and territorial defence, have both been well documented (Catchpole and Slater, 1995). In many species, these functions are fulfilled by the possession of a single song type. Thus the widespread occurrence of song repertoires, sometimes very large, has received much theoretical and empirical scrutiny (Searcy and Andersson, 1986; Kroodsma, 1991; Catchpole and Slater, 1995). Most of the theories for the evolution of repertoires are based on the advantages of diversity, and thus the focus is simply on the repertoire size rather than the specific content. This simple selection for variety could arise through a female bias (Endler and Basolo, 1998; Collins, 1999; but see Gray and Hagelin, 1996), or could convey information about the individual. Repertoires may serve as an honest signal of singer quality, if the development of larger repertoires incurs a cost or is affected by developmental stress (Buchanan *et al.*, 1999; Doutrelant *et al.*, 2000; Gil and Gahr, 2002; Nowicki *et al.*, 2002; Buchanan *et al.*, 2003). Unique repertoires have the potential to provide individual identification (Molles and Vehrencamp, 2001a), but many studies exploring individual recognition by song have shown that birds are able to distinguish between individual conspecifics on the basis of characteristics of shared song types (Falls, 1982; Stoddard, 1996; Wanker and Fischer, 2001; Bard *et al.*, 2002; Phillmore *et al.*, 2002; but see Brooks and Falls, 1975).

Many species appear to have regional or even very localised dialects in the song structures or repertoires of different populations (Kroodsma, 1974; Slater *et al.*, 1984; Trainer, 1989; Briskie, 1999; Harbison *et al.*, 1999; Tracy and Baker, 1999; Rich, 2002). In some cases the dialect groups are very small, and the boundaries between groups are precise (i.e. Latruffe *et al.*, 2000; Leader *et al.*, 2000), rather than following a gradient (i.e. Vehrencamp *et al.*, 2003). One of several theories (for review see Slabbekoorn and Smith, 2002) for the occurrence of such geographic variation is that of delayed learning, whereby males establish their repertoires after

settling on a territory, and develop their repertoire based on the songs of the local population (for review see Kroodsma, 1982). The development of songs matched to neighbours can occur in two different ways: first, song is not learnt until the male is settled, and is then learnt through direct imitation of neighbours; and second, song is learnt before settling, and then selective attrition of songs occurs leaving those shared with near neighbours (Kroodsma, 1974; Marler and Peters, 1982; Cunningham *et al.*, 1987; Nelson, 2000). The imitation hypothesis predicts much more similarity between individuals than the selective attrition hypothesis, as in the former, neighbours are always the tutors whereas they are unlikely to be in the latter case. Two recent studies have shown that, within species, these two mechanisms may both occur depending on the local pattern of dispersal and migration (Kroodsma *et al.*, 1999; Nelson *et al.*, 2001). Both studies showed that sedentary, long-term territory holding populations appeared to fulfil predictions for imitation, whereas migratory or nomadic populations showed less repertoire sharing and lower similarity within shared songs, which was suggestive of selective attrition.

The possession of a repertoire of songs permits differences in performance of the song types, such as changing the rate of switching between types. Song type switching can occur with 'immediate variety', where each type is sung only once before switching and thus every song differs from the previous one. But it can also occur with 'eventual variety', where one song type is sung a number of times (called a 'song bout') before switching to a new type (Catchpole and Slater, 1995). The length of a bird's song bouts (and its inverse, the rate of song switching) varies within individuals, but can also be a characteristic of species (i.e. the song thrush *Turdus philomelos* sings with eventual variety, but the blackbird *T. merula* sings immediate variety song, Cramp, 1988). Switching rapidly between song types may be a way of signalling repertoire size (Horn and Falls, 1991), and in some cases appears to be directed only at female attraction and not male-male interactions (Kroodsma, 1977; Balsby and Dabelsteen, 2001). A slightly different angle was put forward in the idea that song switching is an indicator of motivation to attack or approach (Falls and D'Agincourt, 1982; Kramer and Lemon, 1983; Stoddard *et al.*, 1988). With this theory, increased song switching rates correlate with increases in motivation.

Contrary to the above hypotheses, switching song types frequently may not perform a direct function. Two theories propose that the behaviour arises through more basic physiological mechanisms. The anti-exhaustion hypothesis (Lambrechts and Dhondt, 1988) proposes that repetition of the same behaviour may lead to muscle fatigue and therefore limit the rate of performance, thus by switching to different types such fatigue is prevented. The theory further predicts that if higher song rates are required, song switching must increase in direct proportion, and thereby that song switching is related solely to song rate and is independent of context. The second theory is the monotony threshold hypothesis (Hartshorne, 1956), which argues that listeners will habituate if the same song type is repeated many times, and that this habituation is reduced by switching song types. The occurrence of habituation is the basis of standard techniques to test for discrimination between song types (Searcy and Marler, 1981; Yasukawa, 1981; Latruffe *et al.*, 2000). However, as with anti-exhaustion, if prevention of habituation is the only function involved, changes in switching rate should be dependent on song rate rather than context (although a monotony threshold function does assume a receiver is present).

Where neighbouring birds share song types, this sharing can lead to song matching during counter-singing (Krebs *et al.*, 1981; Beecher *et al.*, 1994). In a thorough study of the song and behaviour of song sparrows (*Melospiza melodia*, Beecher *et al.*, 1994; Beecher *et al.*, 1996; Beecher *et al.*, 2000a; Beecher *et al.*, 2000b; Burt *et al.*, 2001; Burt *et al.*, 2002), the tendency for neighbouring males to share part of their repertoires was shown to result in two types of song matching. 'Repertoire matching' was proposed to be a low level aggressive signal, where a song type is sung that is shared with the neighbour but differs from the type the neighbour is currently singing, whereas 'song type matching' where the exact match is sung in reply to a neighbour's song, was thought to be a higher level of aggression (Burt *et al.*, 2002). Studies on other species also support the theory that was first presented by Krebs *et al.* (1981) that song type matching is a signal of aggression (Lemon, 1968; Falls *et al.*, 1982; Falls, 1985; Vehrencamp *et al.*, 2003). Molles and Vehrencamp (2001b) further discuss the retaliation costs of falsely signalling aggressive intent through song type matching, and propose that these costs enforce an honesty to the use of matching behaviours in agonistic interactions. The evidence from song sparrows

suggests that repertoire matching and song type matching act as a set of graded signals of aggression, and further studies on this are needed on other species.

This study aimed to investigate patterns of song type sharing, and the use of repertoires in aggressive signalling in the canebrake wren (*Thryothorus modestus zeledoni*) (for duet description, see Chapter 3 or Mann *et al.*, 2003). Owing to the absence of previous data on repertoire sharing or aggressive signals in the canebrake wren or duetting birds in general, this study necessitated the inclusion of a wide range of data. First, a foundation of information on repertoire sharing and individual characteristic patterns were required; which were then built upon to investigate mechanisms for aggressive signalling. As pairs in this species are territorial year-round, initial predictions were that there would be both song type sharing, and very close similarity of song types between neighbours, matching predictions for imitative learning after settlement. If repertoire sharing with direct neighbours were involved in reduced aggression, neighbours would be expected to share more duets than non-neighbours. The canebrake wren sings antiphonal duets with eventual variety, thus increased song switching rates and song type matching may both occur in response to simulated intrusions, and would be correlated to the level of intrusion threat perceived by the subject.

6.2 *Methods*

Experiments were carried out at both field stations between May-August and October-December 2002. They involved 23 pairs and 5 territory holding solo males (14 & 4 respectively at La Suerte, and 9 & 1 at El Zota).

6.2.a Playback experiments: Stimuli were constructed using Avisoft SASLab v5.1 (R. Specht, 2002) from recordings made in 2001. They were constant in amplitude, length and timing of the duets and overall amount of singing time, and stimuli were allocated to subject pairs in a randomised block design. This means that the sequence of the stimuli were randomly assigned in the first of two or four trials on each subject pair, and reversed or differently sequenced for following trials. To further avoid pseudo-replication, the original recordings that the stimuli were constructed from were also randomised, never used more than twice overall, and no subjects ever

heard the same recording twice. Original recordings were also non-neighbour to the subject, to avoid confounding effects of familiarity. Replicate trials on the same pair were at least one week apart, all pairs were used in two experiments, solo males in just one. Different experiments on the same pair were separated by at least two weeks.

Playbacks were carried out using a 40W amplifier (T1140), portable Panasonic CD player (SL-SX228), and 5" Visaton passive full range speaker. The speaker was positioned approximately 10m within the subjects territory boundary and facing into the territory centre. Playback site was kept the same across replicates to control for perch distances affecting approach behaviour. Playback amplitude was controlled in all experiments by occasional testing with a sound pressure level meter (Beha 93411), with the volume being set to a level equal to that measured from the wrens themselves. Continuous sound recordings were taken throughout all trials, using a Sennheiser ME-66 gun microphone and a Marantz CP430 tape recorder (chrome tapes). These were positioned about 5m from the speaker, again facing into the territory. Observations were made from concealed points, using instantaneous sampling (once per minute) to record positions of birds, and also recording all songs and their structures in one minute segments (see Table 5.1, page 5.5 for a list of variables measured).

The principal playback experiment discussed in this chapter investigated song switching function, by testing responses to a stimulus with 2 song switches (3 duet types) in 6 minutes (a natural but very high rate), against a stimulus with no switches (one duet type) in six minutes (with 10 minute intervals, giving a 32 minute trial). Some data are also presented from four other experiments discussed in Chapters 5 and 7, all testing different hypotheses. The basic structure and set up of these five experiments are shown in Table 6.1, where the number and type of stimuli, number of trials on each subject, and number of subjects involved are all indicated. All trials were preceded by a 30 minute observation period which was used to determine the breeding state of the pair, and check that the birds were not disturbed by intrusions or predator presence prior to the playback.

Table 6.1: The stimuli and design for five playback experiments, chapter numbers in brackets refer to the chapters in which those experiments are discussed fully.

Experiment	Stimuli	No. Subjects	No. trials per subject	Time of day
Song Switching Rate	1.3 phrase types (2 switches) 2.1 phrase type (no switches)	8 pairs, 5 solo males	2	7.00-8.30am
Species Recognition (Ch 5)	1.Canebrake wren duet 2.Black-bellied wren duet	14 pairs	2	5.30-7.00am
Pair-Bond Strength (Ch 7)	1.Long duet 2.Short duet 3.Fast duet 4.Slow duet	15 pairs	2	5.30-7.00am
Mate-Guarding (Ch 7)	1.Male solo song 2.Female solo song	6 pairs	4	3.30-5.30pm
Joint Territory Defence (Ch 7)	1.Male solo song 2.Female solo song 3.Duet	5 pairs	1	5.30-7.00am

6.2.b Data analyses: Song recordings were processed in Avisoft SASLab Pro (v. 5.1, R. Specht 2001) and song types identified by eye using spectrograms with a sample confirmed through spectrogram correlations in Avisoft Correlator. Once all song phrases (>15000) were identified, population and individual repertoires could be counted. Mean individual song type sharing was calculated by scoring the proportion of each individual's repertoire that was shared with each other individual in the population. As the subjects always sang with eventual variety (Catchpole and Slater, 1995), for further analyses, only one song sample was taken from each bout (a bout being a series of songs of the same type). Parameters of phrases were measured using inbuilt cursors and automatic measurement settings, and were (for each syllable): duration, interval from the previous syllable, start, end and middle frequency, max and min frequency and frequency of peak amplitude. Thus depending on the number of syllables in the phrase, the number of initial variables ranged from 7 to 31 (the number of syllables was constant within a phrase type). These data were reduced to four components in a Principal Component Analysis, and analysed in ANOVA models.

Responses to playbacks used in the analyses were: *male distance to speaker* (highly correlated with female distance, and more complete data), *male-female distance*, *total song rate* (explains I phrase and duetting rates and mean duet length, see Chapter 3), *I phrase singing rate* (for solo males), and *song switching rate*. These were compiled as means for each experimental section (Stimulus 1 playback, subsequent interval, stimulus 2 playback & subsequent interval etc.), for each subject pair and trial. These responses were then tested through repeated measures GLM models (residuals fitting normality), which were designed to overcome the effects of variation between days and pairs, allowing the data to be tested for relative differences within each trial. Thus *pairs/solo males* were subjects in the model, with *experimental section* and *trial* acting both as repeated measures, and as effects. Wherever multiple comparisons were being analysed, the critical probability value was taken as $p_{\text{crit}}=0.01$ (including *post-hocs*); in tests involving a single comparison, $p_{\text{crit}}=0.05$ and appropriate correction factors were applied (i.e. Williams correction factor for G tests).

6.3 Results

Repertoires were taken from all recordings made during 2002, and only those individuals with >70 song bouts recorded (series of songs of the same type) were included in the analyses. This threshold was the point at which new duet discovery rates approximately levelled off (unpubl. data). Mean individual repertoire sizes from these samples were I phrases: $19.45 \pm 1.148(\text{SE})$, M phrases: $15.5 \pm 0.682(\text{SE})$, F phrases: 16.24 ± 0.784 , Duets: $27.28 \pm 2.24(\text{SE})$.

6.3.a Repertoire sharing between populations: Population repertoires at both La Suerte and El Zota were compiled and compared. The majority of I and M phrases were unique to one population, but some were common to both. F phrases showed roughly equal proportions of unique and shared phrases across the two populations (Fig. 6.1).

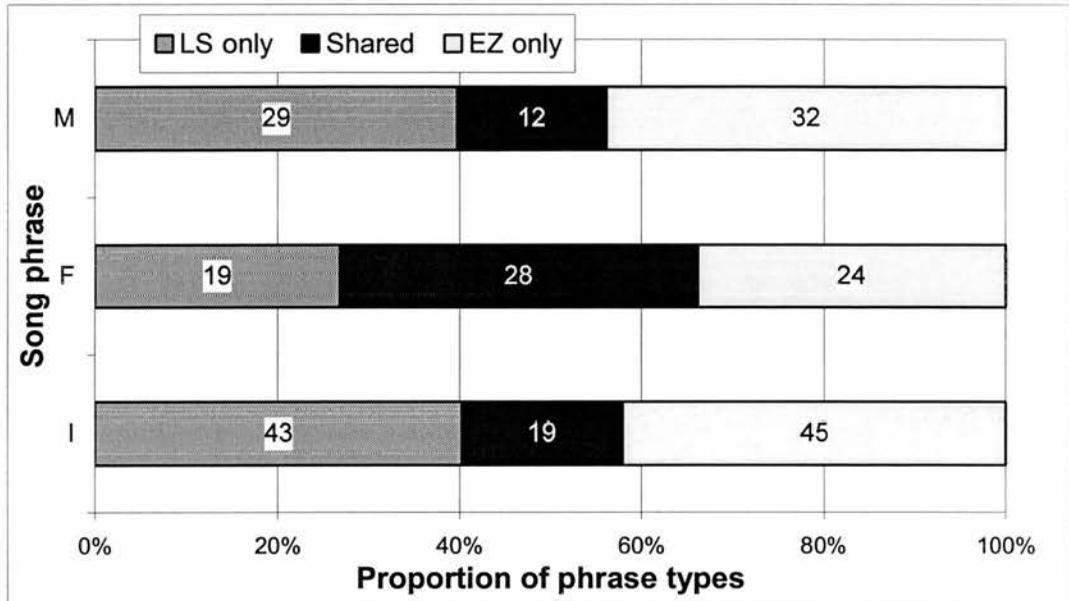


Fig. 6.1: Numbers of phrases (no. in column) in the population repertoires of birds at La Suerte (LS) and El Zota (EZ) that were unique to those populations, or present in both repertoires. Song phrases: **I** introductory phrase, **F** female duet phrase, and **M** male duet phrase.

6.3.b Repertoire sharing between individuals: Wrens at La Suerte showed a greater degree of repertoire overlap and fewer unique phrase types for all three song phrases than wrens at El Zota. This pattern remained when a subset of the La Suerte population (occupying an area referred to as the ‘small pasture’) was analysed to match the sample size for El Zota (analysed through one-way ANOVAs, see Fig. 6.2).

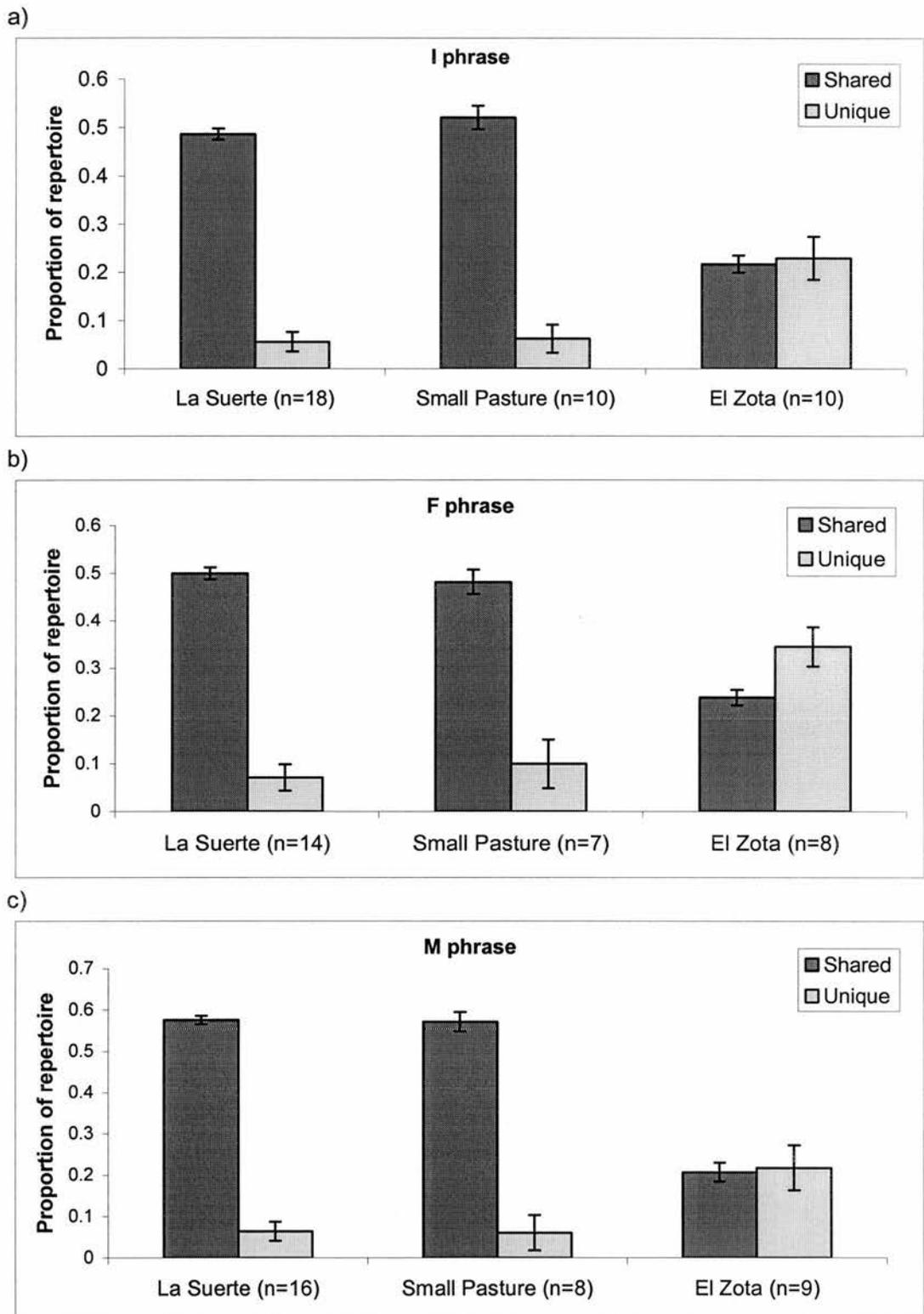


Fig. 6.2: Mean proportions of individual bird repertoires that are shared with other birds or are unique to that bird \pm SE, for La Suerte, a La Suerte subset Small Pasture, and El Zota. a) **I phrase**: Shared $F_{2,473}=14.66$, $p=0.008$, Unique $F_{2,34}=9.75$, $p<0.001$; b) **F phrase**: Shared $F_{2,277}=58.24$, $p<0.001$, Unique $F_{2,26}=15.67$, $p<0.001$; c) **M phrase**: Shared $F_{2,365}=145.42$, $p<0.001$, Unique $F_{2,30}=5.33$, $p=0.010$.

Within the Small Pasture subset, proportions of repertoires that were shared, did not differ significantly between immediate neighbours (with shared borders) and non-neighbours (GLM with pair and neighbour/non-neighbour: $F_{9,79}=1.72$, $p=0.480$, $n=10$ pairs, 90 comparisons). The rest of La Suerte and El Zota were not analysed, as there were not sufficient numbers of shared borders.

6.3.c Duet repertoire sharing: Duet sharing (exact matches of I, F, and M phrases in a duet) with neighbours and non-neighbours were compared with potential degree of sharing. This potential was calculated for each of six pairs from the number of exact duet matches that were possible from the repertoires of two neighbour and two non-neighbour pairs. There was no difference in the degree of duet sharing between non-neighbours (from the same population) and neighbours ($T=0.77$, $p=0.448$, $df=22$). And these proportions did not differ from a random distribution (mean: 0.472 ± 0.049 (SE), $T=0.88$, $p=0.381$, $df=46$).

6.3.d Individual variation in phrase characteristics: Principal Component Analysis was used on measures of phrase types that were shared by several birds. Five phrase types each of I, F and M song phrases were selected, and 3-5 sample recordings each from 4-7 individuals were included in each analysis. The four principal components drawn from the data for each phrase type explained >80% (cumulative) of the variance. All components were then tested against individual bird in Kruskal-Wallis ANOVAs (non-parametric tests were used for all sets for consistency, as a few components were not normal).

14 of the 15 phrase types analysed showed significant inter-individual differences for at least 1 of the principal components, the exception, an M phrase (M260) showed borderline significance for two components (see Table 6.2). Figure 6.3 shows an example of the separation of individuals through two principal components, with representative spectrograms.

Table 6.2: Kruskal-Wallis results for 4 principal components derived from PCAs of parameters of each phrase type, tested for differences between individuals. 1st line per box shows χ^2 value and d.f., 2nd line shows p-value. P-values in bold are significant at $p < 0.01$, those not in bold approach significance.

Phrase	Prin Comp 1	Prin Comp 2	Prin Comp 3	Prin Comp 4
I150		(χ^2)15.24, (df)4 (p) 0.004	15.64, 4 0.004	9.60, 4 0.047
I205		12.78, 6 0.047	17.49, 6 0.008	13.76, 6 0.033
I261	15.50, 3 0.001	17.58, 3 0.0005	12.39, 3 0.006	
I300	16.71, 3 0.0008	13.22, 3 0.004	13.37, 3 0.004	
I360	18.83, 4 0.0008	13.92, 4 0.008		10.53, 4 0.032
F240	15.07, 3 0.002	11.99, 3 0.007	11.14, 3 0.011	
F260	26.36, 5 <0.0001	24.97, 5 0.0001	23.67, 5 0.0003	14.06, 5 0.015
F300	13.05, 3 0.005	12.12, 3 0.007		
F340	11.15, 3 0.010			
F370	14.50, 3 0.002		9.41, 3 0.024	
M100	14.19, 3 0.003	14.86, 3 0.002		
M180	16.42, 3 0.001	11.71, 3 0.009	12.67, 3 0.005	
M260	7.53, 3 0.057		7.49, 3 0.058	
M280	12.05, 4 0.010		9.53, 4 0.049	
M330	13.0, 3 0.005		11.13, 3 0.011	

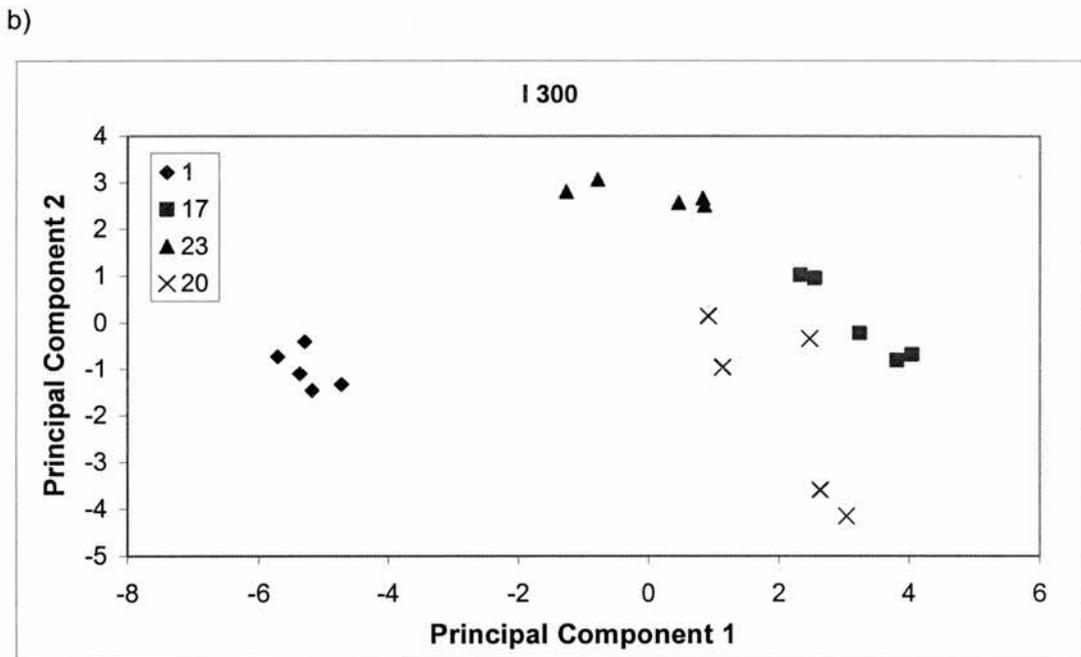
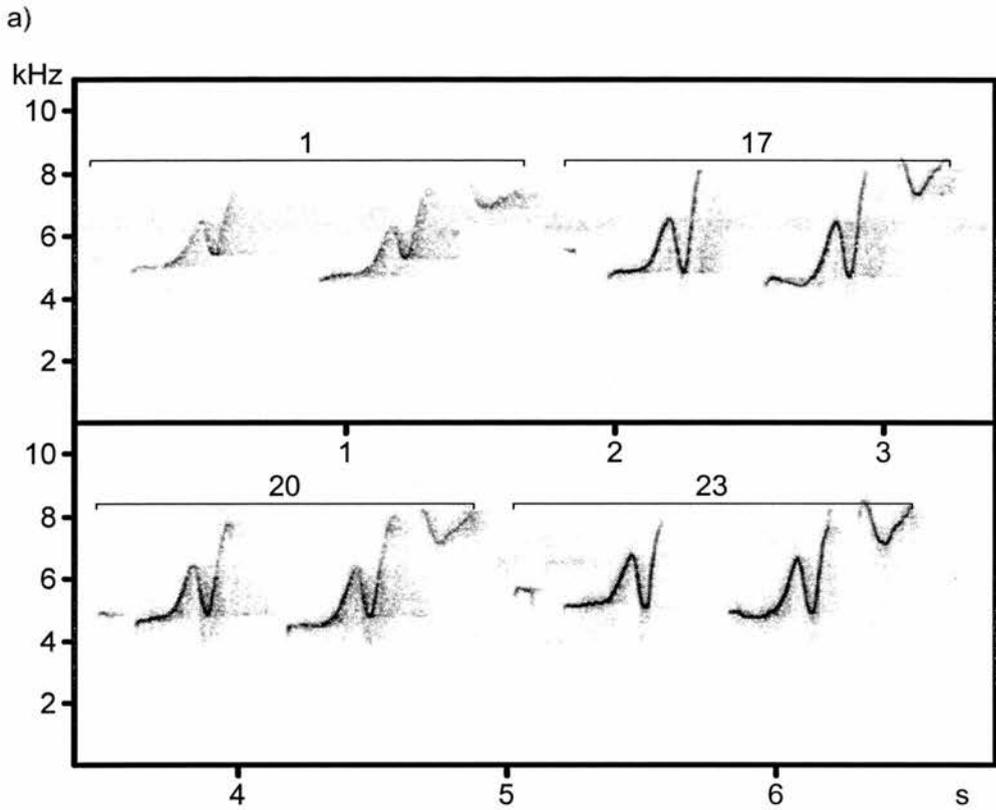


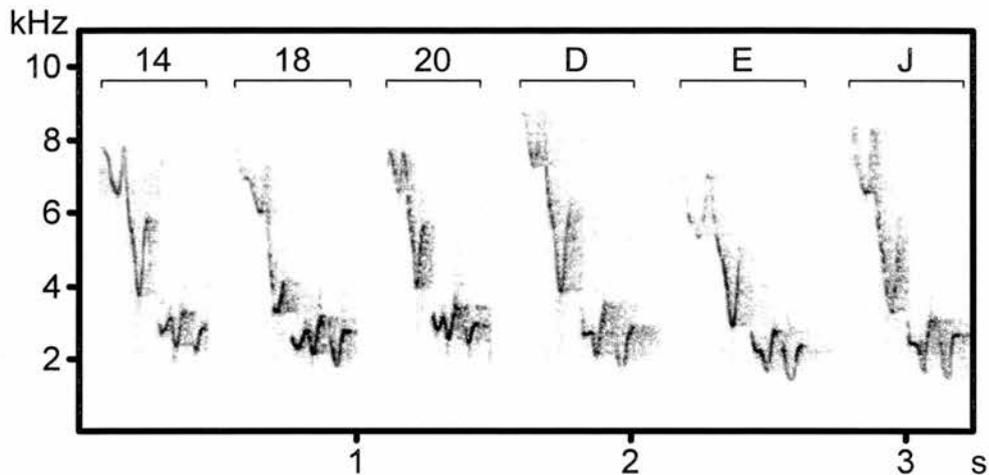
Fig. 6.3: a) Spectrogram showing examples of introductory (I) phrase type I300 from 4 males, and b) Plots of the 1st and 2nd components derived from a PCA of parameters for I300. Each point represents a different recording of the phrase (all from different song bouts). The 4 males presented are: 1, 17, 20 & 23; all from La Suerte.

Only two phrase types (I205 & F260) occurred at both La Suerte and El Zota with sufficient samples for comparative analyses. Both phrases were tested for consistent differences between populations as well as between individuals within populations. Normal distributions allowed GLM models with both individual and population as factors. Table 6.3 shows the model results for each principal component, Figure 6.4, as an example, shows spectrograms of individual phrases of female phrase F260 and plots the separation of both individuals and populations through the 1st and 2nd components.

Table 6.3: GLM results for 4 principal components derived from parameters of each phrase type, tested for differences between individuals and populations (La Suerte and El Zota). Sample and error df are given in brackets for each row, the 1st line per box shows F value, the 2nd line shows p-value.

Phrase	Factor	Prin Comp 1	Prin Comp 2	Prin Comp 3	Prin Comp 4
I205	Individual (df:5,13)		(F) 6.24 (p) 0.004	6.22 0.004	7.34 0.002
	Population (df:5,13)			41.13 <0.0001	
F260	Individual (df:4,24)	(F) 23.86 (p) <0.0001	25.38 <0.0001	22.33 <0.0001	3.60 <0.0001
	Population (df:4,24)	24.87 <0.0001	26.94 <0.0001		

a)



b)

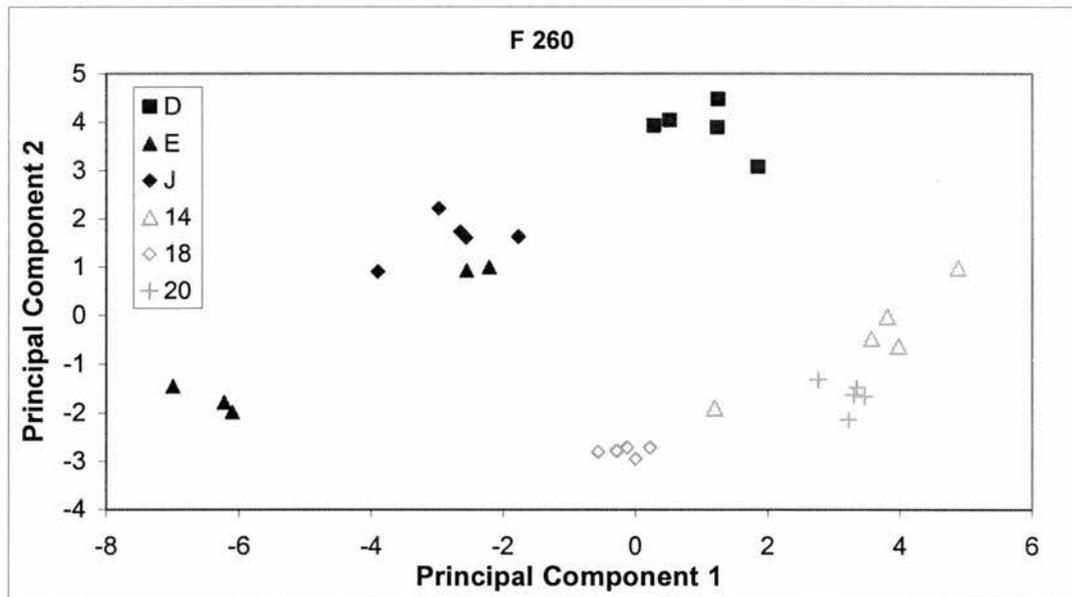


Fig. 6.4: a) Spectrogram examples of F phase F260 from 6 females, and b) plots of the 1st and 2nd components derived from a PCA of phrase parameters for F260. Each point represents a different recording of the phrase (all from different song bouts). The 6 females presented are: D, E & J from El Zota; and 14, 18 & 20 from La Suerte.

The plot in Figure 6.4b suggests that inter-individual variation within phrase types was greater at El Zota than at La Suerte. To test this possibility, within phrase variation was compared between sites. Variability in the performance of a phrase type was measured as the variance of each principal component across all

individuals. Thus four variance values (one for each component) were obtained for each phrase type. The analysis was not possible with the M phrases as there was only one available phrase type from El Zota. However, the variance of I and F phrases in the two populations were compared through two sample t-tests for each component. The variance of the 2nd component differed between the populations, the other components showed no difference. Figure 6.5 shows the difference in population variance for both I and F phrases in Principal Component 2, I phrase results only approach significance.

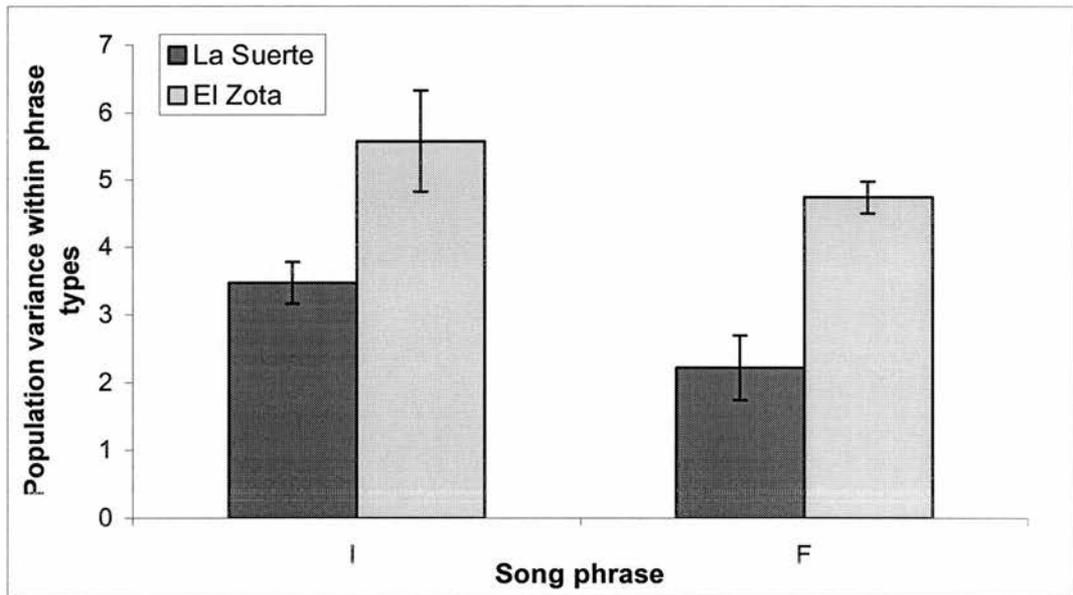


Fig. 6.5: Mean variance within different phrase types at La Suerte and El Zota. Five I and five F phrase types were analysed for each population, error bars show SE. t-tests for differences between populations: I phrases: $t=2.60$, $p=0.06$, $df=4$; F phrases: $t=4.72$, $p=0.009$, $df=4$.

6.3.e Song switching rate: Data from the song switching playback experiment were divided into solo male subjects and paired subjects and analysed through the same GLM model separately. Song switching rate was significantly correlated with I phrase singing rate (no. per min) in solo males (Pearson's correlations. $R=0.421$, $p=0.017$, $df=31$) and with duetting rate (no. per min) in pairs ($R=0.377$, $p=0.0011$, $df=71$), but not with any other response variable (perching height, distance to speaker, distance to mate). Both pairs and solo birds showed greater song switching rates during playback relative to during the subsequent interval (see Fig. 6.6). This

increase in switching rate was proportional to increases in song rate, as phrase switching rate per duet (for pairs) or per I phrase (for solo males) did not change significantly between experimental sections ($p > 0.1$). The results tables for the GLMs of song switching rate and song switching rate per duet (paired subjects) are shown in Table 6.4, as an example of the structure of the model, and of the change in outcome for the two measures of response. There were no changes in song rate, song switching rate or approach behaviour to the two different stimuli. This pattern of greater song switching rate during playback was consistent across all experiments that were carried out, regardless of the stimuli involved (Fig. 6.6).

Table 6.4: ANOVA results tables from a repeated measures GLM run on a) the phrase switching rate response, and b) the song switching rate per duet of pairs in the 'song switching rate' playback experiment. The subject in the models was 'pair', with trial and experimental section as the repeated measures and as effects.

a)

Null model	Degrees of Freedom	Chi-squared value	P value	
<i>Song switching rate</i>	1	7.42	0.0065	
Effect	Numerator d.f.	Denominator d.f.	F value	P value
Trial	1	59	0.20	0.655
Expt. Section	3	59	5.44	0.002

b)

Null model	Degrees of Freedom	Chi-squared value	P value	
<i>Song switches per duet</i>	1	3.22	0.073	
Effect	Numerator d.f.	Denominator d.f.	F value	P value
Trial	1	46	0.41	0.525
Expt. Section	3	46	0.77	0.516

6.3.f Phrase matching to playback: Occurrence of matching I, F, and M phrase types to the playback were counted from two series of playback experiments (*song switching*: non-switching stimulus, and *species recognition*: conspecific stimulus), one playback stimulus being taken from each trial (11 pairs, 26 trials). Probability of phrase matching occurring at random was calculated from the repertoire size of the subject, the number of different phrase types sung in response to playback, and the

number of precise matches available in the subject's repertoire (could match up to 3, if I, F and M were all available). Phrase matching was found to be greater than expected for both La Suerte and El Zota populations (see Fig. 6.7), but the use of La Suerte stimuli at El Zota meant that the potential matches were much lower at El Zota, making the relationship much weaker.

6.3.g Duet matching to playback: Duet type matching in response to playback was calculated from the samples used in the above phrase matching analyses. Potential duet matches were where the subject pair possessed in their repertoires all three (I, F, and M) phrases used in the playback. This meant that the pair, theoretically, had the ability to match the playback duet perfectly. Although potential duet matching averaged 0.13 for 17 trials where duet matches were possible (if the duet match was not possible, no analyses were carried out), only 2 matches occurred from 58 duet types sung (matching rate of 0.03). No analyses were possible due to the high numbers of zero values, and this strongly suggests that duet type matching did not occur at a greater than predicted rate.

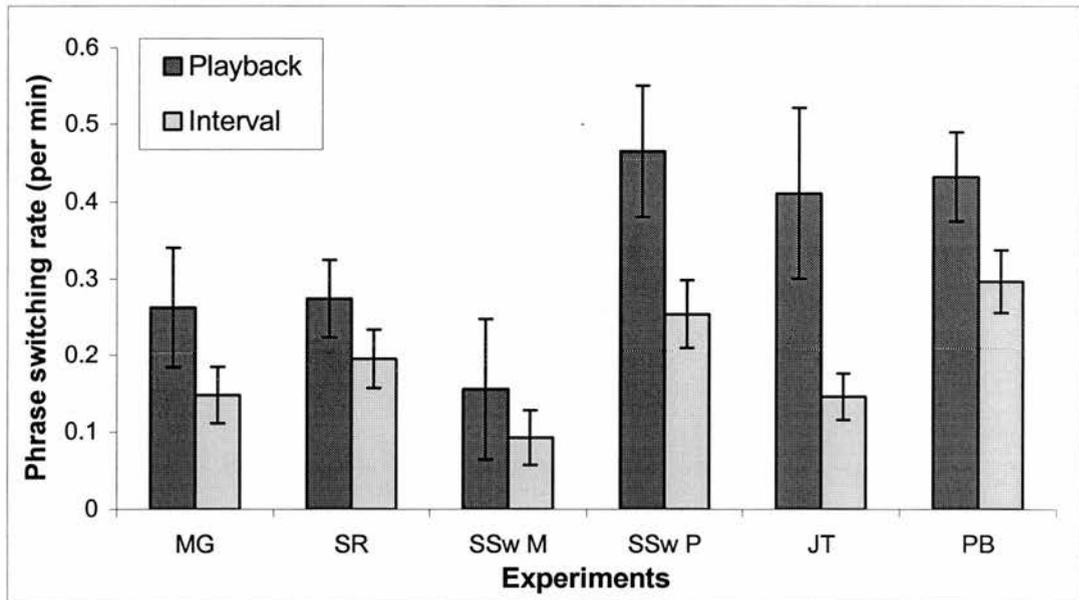


Fig. 6.6: Mean phrase switching rate (no. per minute) during playback of stimuli and silent intervals for 5 experiments. $N = 6, 14, 9, 4, 5$ and 15 respectively, error bars show SE. *Mate guarding*: $F_{1,49.8}=23.05$, $p<0.0001$; *Species recognition*: $F_{1,61.8}=14.03$, $p=0.0004$; *Song switching, pairs*: $F_{3,59}=5.44$, $p=0.002$; *Song switching, males*: $F_{3,21}=22.81$, $p<0.0001$; *Joint territory defence*: $T = -3.46$, $p=0.004$, $df=14$; *Pair bond strength*: $F_{7,184}=4.39$, $p=0.0002$.

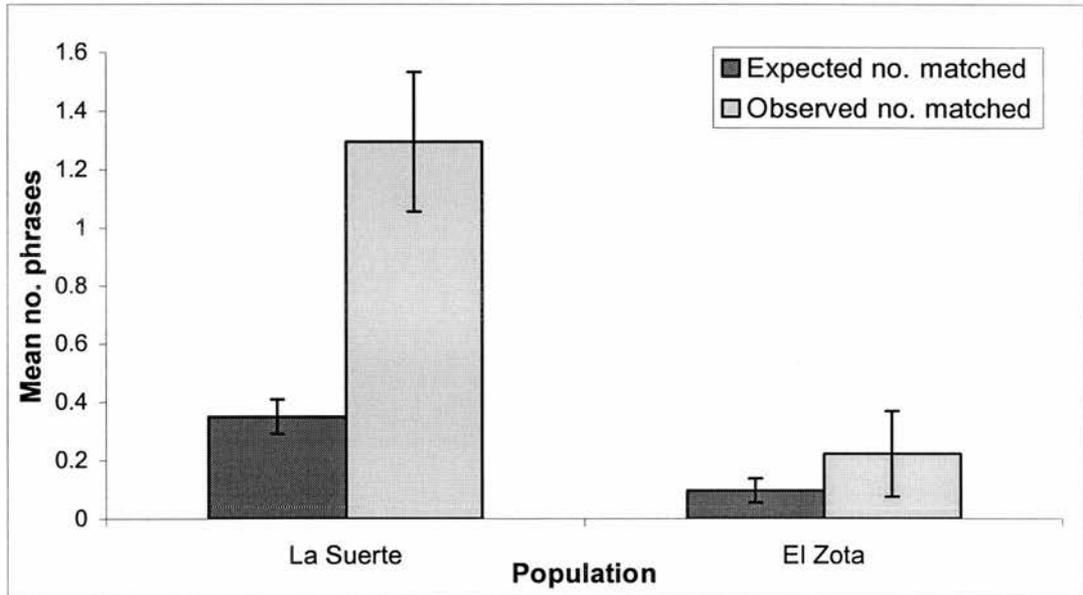


Fig. 6.7: Mean numbers of phrases (maximum of 3: 1x I, F and M per trial) sung by the subject pair that matched playback phrase types, against the mean number of matched phrases predicted to occur by chance. La Suerte (n=7): $G=54.12$, $p<0.001$, $df=12$, El Zota (n=4): $G=4.17$, $p=0.03$, $df=1$.

6.4 Discussion

6.4.a Repertoire sharing: This study showed clear geographic differences in the repertoires of two populations within a subspecies zone. Although the data would benefit from the addition of more sites, there seems to be a difference in degree of geographic variation in repertoires between males and females, with 17.7% and 16.4% of male I and M phrase types and 39.4% of female F phrases being common to the two populations. Beyond simple differences in population repertoires, the two populations differed considerably in the inter-individual repertoire patterns. Birds at La Suerte shared a large proportion of their repertoires with other individuals in the population, and had few totally unique phrase types. At El Zota, the proportion of phrases shared was lower and birds had many more unique phrases. This pattern was the same for I, F and M song phrases.

Characteristics of shared phrases were shown to be individually distinct for both sexes and both populations, with birds being individually identifiable from their song performance, independent of their repertoire content. This inter-individual variation

was greater within the El Zota population than at La Suerte. Birds at La Suerte therefore, not only shared more phrase types, but also more closely matched the structure of those phrases with their conspecifics than did birds at El Zota.

These very marked differences between the two populations may be linked to ecological factors. Much of the land at La Suerte consisted of old pasture and scrub with a network of marshes, all of which was suitable habitat, and thus most territories were bordered by several others. El Zota, by contrast, consisted mostly of secondary forest, with isolated marshes. These marshes usually contained one pair, and hence most pairs had no direct neighbours. The imitation hypothesis for geographic variation in repertoires (Cunningham *et al.*, 1987; Nelson *et al.*, 2001) predicts very close similarity in both repertoire and phrase characteristics between direct neighbours, and less similarity with greater territory separation. The selective attrition hypothesis (Marler and Peters, 1982; Nelson, 2000) predicts a weaker but matching pattern in repertoire sharing, but without the trend in phrase characteristics. This study presents evidence of lower repertoire sharing, and also greater variation in characteristics of shared phrases when territories are isolated, which appears to match the imitation hypothesis. However, in contrast to other studies (Latruffe *et al.*, 2000; Griessmann and Naguib, 2002), within La Suerte there was no difference in the proportion of repertoires shared with direct neighbours or with non-neighbouring birds. This suggests that birds were not learning their repertoire exclusively from their near neighbours, but possibly were learning from a wider range of local conspecifics. The territories at La Suerte being in such close proximity, it is not unlikely that birds would be able to learn songs from a range of individuals both before and after settling. At El Zota, the greater spacing between territories appears to have led to less sharing of phrase types and higher numbers of unique phrases. This may be attributable to higher innovation in song development, or greater dispersal distances meaning that more song types were learnt from birds beyond the study area. Those phrases that were shared showed much greater inter-individual variation than seen at La Suerte, which suggests that without the constant reinforcement of neighbour song, learning is less accurate, or characteristics of a phrase drift after learning.

The above patterns in repertoire sharing involved individual repertoires of I, F and M phrases. In a study of another duetting wren *T. nigricapillus*, Levin (1996a) found no evidence of changes in an individual's repertoire after mate loss or re-mating, suggesting that individual repertoires are developed independently of the mate. Repertoires of duets, however, involve both members of the pair developing the ability to consistently match certain phrase types together (i.e. $I_1F_1M_1$ and $I_2F_2M_2$ etc, see Chapter 3). To share a duet type with another pair would first involve sharing the I, F and M phrases used, and would then involve both pairs singing that exact combination in a duet. The proportion of potentially shared duets (where both pairs had the same I, F and M constituent phrase types) that were actually shared, did not differ between direct neighbours and non-neighbours in the same population. More surprising was that this duet matching occurred at a random rate. This suggests that there was no significance of duet types in inter-pair interactions, as there was no avoidance of, or tendency to duet share with other pairs. Development of the content of duet repertoires must therefore be a purely intra-pair behaviour, with inter-pair signalling being based on the content of individual repertoires rather than that of the pair.

6.4 b Aggressive signalling: Both males and females in the study possessed large repertoires of phrase types, and pairs demonstrated eventual variety in their singing both during playbacks and during non-experimental observations. A switch in song type was usually led by the male changing his I phrase type (this was also the way solo males switched type), followed by a switch in the F and M types. Occasionally the female would elicit a switch by singing a new F phrase type in response to the 'old' male I, the male would then usually respond with a new M to form a new duet, continuing with the new I phrase after the duet finished. Thus although the majority of phrase type switches were initiated by the male, switching could be initiated by either member of the pair.

If song switching rate in itself were indicative of the aggression level of a pair, pairs were predicted to respond more strongly to a stimulus with two song switches in six minutes than a stimulus with no switches in six minutes (with the same song rate). As there was no difference in song rates, switching rates or approach to the two stimuli for either pairs or solo males, it seems that the higher song switching rate was

not perceived as a greater or lesser threat. However, despite this result, subjects consistently increased their song switching rate in response to all playback stimuli from five different sets of experiments. It seems then, that although they did not distinguish between different rates of song switching, the birds did use an increase in song switching rate in their own response to simulated territorial intrusion. This suggests that rather than being a specific signal of aggression, an increase in song switching may serve to strengthen the song signal during an inter-pair interaction. This fits with the predictions for an anti-habituation function (Hartshorne, 1956), whereby changing song types prevents receiver habituation and maintains the level of threat represented by the singing pair. Also supporting this idea is that, despite not correlating with any other measured response, song switching rate was correlated with I phrase singing rate in solo males and duetting rate in pairs. In contrast, in an observational study carried out in 2001, neither I phrase singing rate nor duetting rate were correlated with song switching behaviour when no inter-pair interactions were occurring (I phrases: $R=0.083$, $p=0.113$, $n=922$; duets: $R=0.001$, $p=0.988$, $n=210$; 8 pairs). If song switching functioned to prevent muscle fatigue as suggested in the anti-exhaustion hypothesis (Lambrechts and Dhondt, 1988), it would be correlated to song rate even when there were no receivers outwith the pair. Thus it seems most likely that an increase in rates of song switching in this species is used during territorial interactions to prevent receiver habituation from the increased song rate and maintain the strength of the song signal.

If the apparent link between territory proximity and repertoire similarity presented earlier is due to song copying, there may be an evolutionary preference for copying when tutors are accessible, over improvisation. This preference would suggest a function for song sharing with neighbours and conspecifics. Sharing song types with other birds allows both repertoire matching and song type matching during inter-pair interactions. The playbacks in this study all consisted of stranger songs, so subject birds should not have had any knowledge of the repertoires of the playback birds. This prevented testing for repertoire matching behaviour. Exact duet matching was extremely rare in the experiments, which is not surprising considering the lack of any tendency to share duet types with neighbours. In contrast, the strong tendency for individual repertoire sharing within a population fits with the experimental proof that both male and female wrens phrase type matched to playbacks more than would be

expected by chance. This supports previous studies that have shown an aggressive function for phrase type matching in male-male territorial interactions (Krebs *et al.*, 1981; Falls *et al.*, 1982; Falls, 1985; Beecher *et al.*, 2000a; Burt *et al.*, 2001).

Both repertoire sharing and phrase type matching were limited to the individual phrase, rather than pair duet repertoires. This fact implies an intra-sexual targeting of aggressive signals, with the duet song being used by females to type matching female competitors and by males to type matching males. This is the first study that has shown the use of phrase type matching in response to playback in a duetting species. Several studies of duetting species or of species that display female song, have shown that territorial aggression tends to be aimed at members of the same sex, as it is these that may represent the greater threat to an individual's position on the territory (Beletsky, 1983; Levin, 1996b; Whittingham *et al.*, 1997; Elekonich, 2000; Hall, 2000; Seddon *et al.*, 2002). This study provides evidence for a segregation of intra-sexual functions for repertoires, and intra-pair development of duets.

6.4.c Conclusions: This study has presented evidence of geographic variation in repertoires of the canebrake wren, and also an apparent effect of habitat structure on the patterns of repertoire development. Where birds lived in close proximity to other pairs, songs appeared to have been learned by imitation from the local population. Where birds often had no neighbours, they showed greater individuality and less precise learning of shared phrases. Repertoire sharing may be used in aggressive interactions in this species, as phrase type matching was involved in response to simulated intrusions. Increased rates of switching song types did not however, appear to be a direct indicator of aggression, but rather a mechanism for increasing the persistence of other song signals such as song rates. The use of repertoire sharing and type matching was limited to intra-sexual contexts, and it seems that duets as an entire signal were not used in inter-pair interactions, and duet repertoire content may function largely within the pair.

Chapter 7: Multiple functions to duet singing: hidden conflicts and apparent cooperation.

7.1 Introduction

The performance of duet singing has been reported in over 200 species of birds (Farabaugh, 1982), and appears to be associated with both sexual monomorphy and social monogamy, as well as long term territoriality (Payne, 1971; Farabaugh, 1982; Langmore, 1998). A duet song usually refers to a joint song performance by a mated pair, although two-bird duets are also described within chorus singing social groups (Price, 1999; Seddon, 2002). A very wide range of duet types has been reported, from overlapping songs where mates have similar songs (Payne, 1971; Huxley and Wilkinson, 1979), to a very simple call and response as heard in some owl species (Klatt and Ritchison, 1993; Appleby *et al.*, 1999), and to the most complex of antiphonal duets, where the call and response go through several cycles and males and females have separate repertoires (Grimes, 1966; Tingay, 1974; Arrowood, 1988).

Adapting a song performance to fit in with that of a mate, or even simply listening for a mate's song to allow basic temporal synchrony both incur a greater investment than that involved in solo singing. For duetting to have arisen, especially to the extreme degree of complexity seen in some species, benefits of duetting must differ from those gained by solo singing in both sexes. There has been much debate over why co-ordinated duet singing has arisen in several very different genera of birds as well as other organisms such as primates (Serpell, 1981; Haimoff, 1986). Over the years many theories have been presented (see reviews in von Helversen, 1980; Serpell, 1981; Farabaugh, 1982), and three main functions have emerged, which are that duets are used to signal joint territory defence (Seibt and Wickler, 1977), to mate-guard (Wickler and Seibt, 1980; Sonnenschein and Reyer, 1983), and to aid in the formation and maintenance of a pair bond (Thorpe and North, 1965; Thorpe, 1967). Although evidence has been presented for all three hypotheses, there have been few conclusive studies. This stems from a combination of a paucity of thorough experiments, small sample sizes, and a lack of consensus about how to test for, and

exclude interactions between, the different functions (Serpell, 1981; Wickler and Seibt, 1982; Whittingham *et al.*, 1995; Hall, 2000).

The three main functions mentioned above could arise through various means, each predicting slightly different uses of duets, and none of which are mutually exclusive. I use my own terms to define the different hypotheses as not all have previously been described in detail. There are two mechanisms for the use of duets in joint territory defence (Hall, 2000). The first, 'pure territory defence' assumes that duets are used because two birds are required to defend a territory, and so the song simply signals the presence of both members of the pair. This hypothesis predicts that duet rates will increase in response to territory threat, and that duets are a greater threat than solo song. The second hypothesis, 'intra-sexual defence' includes an aspect of mate-guarding, in that birds use the duet to exclude competitors of the same sex, thereby guarding not just their place on the territory, but also their mated status. This theory, contrary to the first, predicts a difference in the response of males and females, with each participating more in duets in response to same-sex threat. In principle, pure territory defence requires only a very basic duet where the two parts are easily distinguishable, although this will be discussed again with respect to pair bond functions later. Again in principle, the intra-sexual defence theory does not necessarily require duets, and could be fulfilled by solo song of both sexes, so is unlikely to be the sole function to duetting behaviours.

Whereas intra-sexual defence involves both males and females, the term mate-guarding is applied most often to paternity guarding by males (Birkhead and Møller, 1992). Duets could be used to maintain contact with a mate when not in visual contact (Thorpe, 1963), or to force their attentiveness (Smith, 1994); both thereby monitoring the mate's movements and ensuring against cuckoldry. 'Contact maintenance' theory predicts an increase in duet singing with increased separation from the mate, whereas 'forcing attentiveness' assumes that the second bird is replying to prevent the first solo singing as solo song would otherwise attract potential mates and therefore increase the risk of cuckoldry. Again, neither of these theories require the development of highly complex duets, although they may select for faster response times if a faster response suggests being closer and more attentive (Smith, 1994). In both birds and primate species where males mate-guard, this

behaviour has been shown to incur an energetic cost of decreased foraging time (i.e. Alberts *et al.*, 1996; Komdeur, 2001). If duets do indeed represent a form of mate-guarding by the male, then the male's motivation to duet should be elevated when he is most at risk of losing paternity, i.e. when his mate is fertile (Møller, 1991). I refer to this as 'paternity guarding', as opposed to the less specific mate- and territory guarding involved in intra-sexual defence.

The use of duets to form and maintain pair bonds is possibly the most intuitively attractive theory of duet evolution due to the apparently cooperative nature of the behaviour. However, convincing mechanisms for this function, and supporting evidence, are both lacking. The only mechanism so far presented for the role of duets in pair bond formation was put forward by Wickler (1980), when he proposed that a duet learning period at the onset of a pair bond involves a non-transferrable investment in that pair. This would introduce a cost to desertion (having to re-invest in a new pair bond) and thereby ensure the maintenance of the bond. This 'coyness model' predicts that newly formed pairs will show a lower duetting ability than established pairs, and that ability will improve with time after pairing. It seems logical to predict that complex duets must involve a learning period, particularly in the ability to coordinate the timing of responses, and yet supporting evidence for Wickler's theory has not been forthcoming (Farabaugh, 1983; Levin, 1996a). A more simple prediction, if duets are important in pair bond formation, is that duet singing will be much more frequent at this time independent of its content (Arrowood, 1988, referred to here as 'elevated duetting').

When different pairs show a range of abilities in duet singing (possibly from learning), then a pair's performance could contain information on their pair bond strength or fitness. This advertisement of pair quality may be important in competitive interactions with other pairs, in the same way that male territorial song has been shown to reflect relative fitness (Buchanan *et al.*, 1999; Forstmeier *et al.*, 2002). An interaction between joint territorial defence and pair bond maintenance could have driven the evolution of complex duets, and leads to two predictions. First, that better quality, more threatening duets would elicit a greater response than poor duets. Second, if better duets represent a stronger pair bond, then newly formed pairs

would sing poorer duets; this prediction fits in with the ‘coyness model’ prediction of a learning period in duet performance of a pair.

This study investigated the hypotheses outlined above through a combination of playback experiments and observations, but some behaviour patterns relating to the predictions have already been described in previous chapters (Chapters 3 and 5). First, pairs of the canebrake wren (*Thryothorus modestus zeledoni*) tended to stay very close together at all times (mean distance apart: $4.57\text{m} \pm 0.087\text{m}(\text{SE})$, $n=8$ pairs, 2606 samples), and duetting rates were not correlated with distance between mates ($p>0.2$). Thus, with mates usually in visual contact, and not increasing their duetting with separation, it is unlikely that duets functioned purely for maintenance of contact. Earlier work has shown that duetting increased in response to simulated and real territorial intrusions, which is suggestive of a use in territorial defence, a theory that will be tested here. Newly formed pairs in the observational study in 2001 did not attempt to breed in their first season together, but were seen breeding in the following year. This delay in the onset of reproductive behaviour is suggestive of a learning period, or at least an extended period of pair bond formation. The detailed study of duetting behaviour in this species has shown that there are several possible aspects of the performance that may require learning, beyond simple development of timing as has been studied in other work (Levin, 1996a). Pairs might improve in both the length of duets (number of FM cycles), and their accuracy (whether they fit the I(FM) n structure without missing/added phrases). As demonstrated in chapter 3, pairs consistently matched certain F and M phrases together to form a set repertoire of duets, and this duet consistency may also require learning and adaptation to a new mate. Finally, we have already seen that contrary to a theory of targeted mate-guarding (Møller, 1991), males did not increase their song rates (and thereby their duet initiation rates) when their mates were fertile. Thus they did not invest more time in broadcast singing to exclude competitors when they were vulnerable to paternity loss.

Following on from data from earlier chapters, the three hypotheses (joint territory defence, mate-guarding and pair bonding) were tested through various predictions of responses to different stimuli. **1)** Pure territory defence: Duet playbacks will elicit a stronger response than solo song playbacks. **2)** Pure territory defence: Duet playback

will represent a greater threat to solo territory-holding birds than to paired birds (tested through both removal experiments and comparison of paired and unpaired birds). **3) Intra-sexual defence:** Members of pairs will respond more strongly to same-sex solo song playback than to solo songs of the other sex. **4) Paternity guarding:** Males will respond more strongly to male solo song playback when their mate is fertile than in later breeding stages (post-fertile). **5) Pair bond formation (coyness model):** There will be a difference in one or more aspects of duetting between new and established pairs. **6) Pair bond formation (elevated duetting):** Duetting behaviour will be increased in newly formed pairs relative to established pairs. **7) Pair bond strength:** Playback of better quality duets (more precise timing, and more FM cycles) will elicit a greater response than that of poor quality duets.

7.2 *Methods*

Experiments were carried out at both La Suerte and El Zota stations in 2002, involving 16 pairs and 4 (territory holding) solo males at La Suerte, and 9 pairs and 1 solo male at El Zota. Work was mostly carried out between May and August 2002, with a subset of experiments carried out from October to December 2002. Some data are also presented from observational studies carried out in 2001 at La Suerte.

7.2.a Observations: Data from observational studies were used to test predictions 5 and 6 (see above). Eight pairs were used as subjects from March to August 2001 and were each visited every 2 days on a rotating observation timetable involving 3 morning periods between 0500 – 1000 hrs and occasional afternoon periods between 1500 – 1800 hrs, covering the most active parts of the day. Each observation period lasted 60 – 90 mins and the information gathered is outlined in Table 3.1 (Chapter 3, pg 3.3).

7.2.b Playback design: Four experiments were carried out with different sets of stimuli to test hypotheses 1, 2, 3, 4 and 7 (see above). Stimuli were constructed using Avisoft SASLab v5.1 (R. Specht, 2002) to be constant in amplitude, length and timing of the songs (both duets and solos). They were allocated to subject pairs in a modified randomised block design, where the sequence of the stimuli was randomly assigned in the 1st of two or four trials on each subject pair, and reversed/re-

randomised for the 2nd and later trials. To further avoid pseudo-replication, the source recordings that the stimuli were constructed from were also randomised, never used more than twice overall, and no subjects ever heard the same recording twice. Source recordings were taken from 12 pairs and were non-neighbour to the subject pair to avoid confounding effects of familiarity. In the mate-guarding experiment, subjects heard different stimuli from the same original pair in all trials, to prevent effects of different source pair quality (each subject had a different source pair). The stimulus types, timing, trial numbers and sample sizes for each of the four series of experiments are given in Table 7.1. The mate-guarding experiments required that subjects were identified prior to breeding, so that trials could be carried out during the fertile stage (nest-building & laying: Birkhead and Møller, 1992), and 10 days later during incubation (i.e. when post-fertile).

Playbacks were carried out using a portable Panasonic CD player (SL-SX228), 40W amplifier (RS, T1140), and Visaton 5" passive full range speaker. The speaker was positioned approximately 10m within the subject's territory boundary and facing into the territory centre. Playback site was kept the same across replicates to control for perch distances affecting approach behaviour. Playback amplitude was controlled in all experiments by occasional testing with a sound pressure level meter (Beha 93411), with the volume being set to a level equal to that measured from natural wren song. Continuous sound recordings were taken throughout all trials, using Sennheiser ME-66 gun microphones and Marantz CP430 tape recorders (chrome tapes). These were positioned about 5m from the speaker, again facing into the territory. Variables measured were as outlined in Table 5.1 (Chapter 5, pg 5.5). Recordings from the experiments were used to test prediction 5 (above) in combination with data from the observational study.

Duet descriptions follow previous chapters, with the male introductory phrase **I**, and female **F** and male **M** cyclic duet phrases forming a duet in the structure I(FM)n.

Table 7.1: The stimuli and set up for the 4 playback experiments. The Stimuli column shows the song structure used, and for the pair-bond strength trials, the speed of the duet is indicated with the inter-phrase interval in seconds (I-F, F-M and M-F intervals; in all other expts, inter-phrase intervals were 0.05s). Stimuli and trial duration shows the time in minutes for each stimulus, and for an entire trial, including 10 minute silent intervals separating and following each stimulus.

Experiment	Stimuli	Stimuli & trial duration	No. Subjects	No. trials per subject	Time of day
Pair-Bond Strength	1. Long: I(FM) ₈ , 0.05s 2. Short: I(FM) ₂ , 0.05s 3. Fast: I(FM) ₅ , 0.015s 4. Slow: I(FM) ₅ , 0.1s	5min each 40 min trial	15 pairs	2	5.30-7.00am
Mate-Guarding	1. Male solo I(M) ₅ 2. Female solo: F ₅	6min each 32min trial	6 pairs	2 fertile 2 post-fertile	3.30- 5.30pm
Joint Territory Defence	1. Male solo: I(M) ₅ 2. Female solo: F ₅ 3. Duet: I(FM) ₅	5min each 35min trial	5 pairs	1	5.30-7.00am
Solo & Joint Territory Defence	1. 3 duet types: I(FM) ₅ 2. 1 duet type: I(FM) ₅	5min each 20min trial	8 pairs, 5 males	2	7.00-8.30am

7.2.c Data analysis: Observations: More than 228hrs of per-minute samples were gathered from the eight pairs from March to August 2001. Only two males underwent re-mating during the study, so t-tests looked at changes in behaviour between original mate and re-mating within these two males. A sufficiently large sample of song recordings was obtained from only one of these males (pair 12a & b: original and new mate respectively). Duet songs were analysed in Avisoft, and intervals between phrases I-F, F-M and M-F were measured from duets where the birds were less than 1m apart (1 each of the three intervals measured from each duet, one duet sampled per song type bout). These measurements of the precision of the duet timing were then analysed through t-tests, comparing pairings 12a and 12b.

Experiments: Responses were compiled as means for each experimental section (Stimulus 1 playback, subsequent interval, stimulus 2 playback & subsequent interval etc.), for each subject pair and trial. These response data were tested through a repeated measures GLM model (residuals fitting normality) that was designed to overcome the effects of variation between days and pairs, allowing the data were tested for relative differences within each trial. Thus the model subject was *pair*, with *trial* and *experimental section* being the repeated measures, and these last two also acting as the effects in the model. The factor *breeding stage* replaced *trial* in the model for the mate-guarding playback to test for differences in responses during fertile and post-fertile trials.

The response factors examined in the experiments depended on the experiment (i.e.: female and male distance were highly correlated and only analysed separately in the mate-guarding experiment), but were generally the following: male distance to speaker, male I calling rate, duet rate, duet length, and song switching rate (switch in either I, F or M phrase type). Additionally female initiation of duets, rate of irregular duets (those that deviate from an I(FM)*n* structure), and male height were analysed in the mate-guarding, pair bond, and solo/joint territory defence experiments respectively. Wherever multiple variables are considered (including *post-hoc* tests), the critical probability value was taken to be $p_{crit}=0.01$. For tests involving single comparisons, $p_{crit}=0.05$ and correction factors were always applied. Wherever results approached significance at $p=0.01$ (i.e.: were between $0.05 > p > 0.01$) the data are discussed. Any non-significant result where the statistics are not presented was therefore greater than $p=0.05$ (and usually $p>0.1$).

Recordings made during the pair-bond strength experiment were analysed, and the precision of the duets measured with inbuilt cursors in Avisoft (I-F, F-M and M-F intervals, one set per duet, and one duet per song type bout). The intervals from duets sung in response to the different stimuli were analysed in another GLM model (with *pair*, *experimental section* and *trial* as independent factors). Recordings from all experiments were used to examine consistency of FM combinations in duet repertoires of different pairs. See Chapter 3 (pg 3.10) for details of methods, but briefly, the frequency of occurrence of F and M phrase type combinations was

compared with a predicted Poisson distribution and with the basic rule that where the mean (μ) = variance (σ), FM combinations occur randomly, and where $\mu < \sigma$, the same FM combinations consistently occur. In other words, where σ/μ exceeds 1, the occurrence of FM combinations is clustered rather than random, and the greater the σ/μ value, the stronger the clustering and the more consistent the FM phrase matching. Some pairs at La Suerte were known to have been together for at least two seasons and others to be newly formed in 2002. Duet consistency between these pairs was compared from recordings made in 2002.

7.3 Results

7.3.a Joint territory defence: Removal experiments: Removal experiments planned for the winter of 2002 were stopped part way through, as the birds appeared to be unable to tolerate the isolation of being removed for 24 hours. This was even with sheltered, undisturbed aviaries, and the birds learning to take the provided food within 2 hours of captivity. One of the removals also resulted in re-pairing of the remaining bird within the 24hr period, which rendered playback trials pointless. The resulting data (5 pre-removal trials, 5 pairs) were insufficient to permit analysis. However, rate of switching song types and rate of Introductory phrase singing both showed trends towards higher values in response to duet stimuli than to solo song of either sex (Fig. 7.1).

7.3.b Joint territory defence: Solo and Joint defence: Solo territory holding males and paired subjects were exposed to playback of duet stimuli, and the responses of males in the two classes compared. Effects on male song rates are impossible to dissociate from the influences of female presence, thus song type switching rate, distance to speaker, and height above ground were the only variables available for comparison. All three response variables showed significant differences depending on mated status, with solo males song switching at a lower rate, staying further from the speaker, and perching lower in the vegetation (Fig. 7.2).

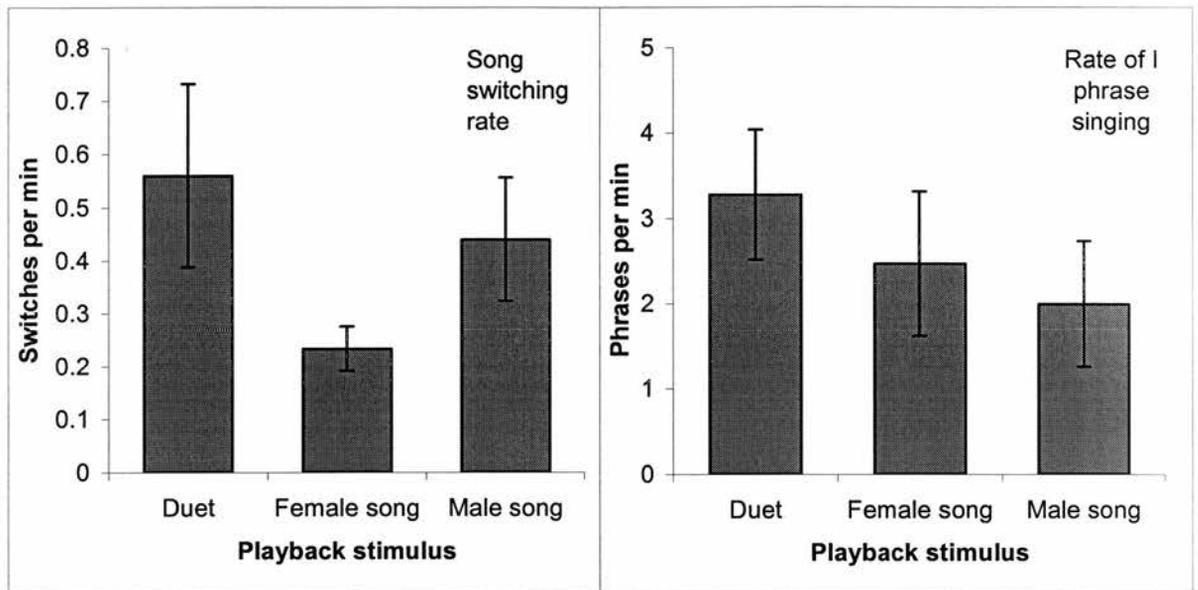


Fig. 7.1: The mean rates (\pm SE) of switching song types, and of introductory (I) phrase singing (per min), in response to duets, female solo and male solo song stimuli, $n=5$ pairs.

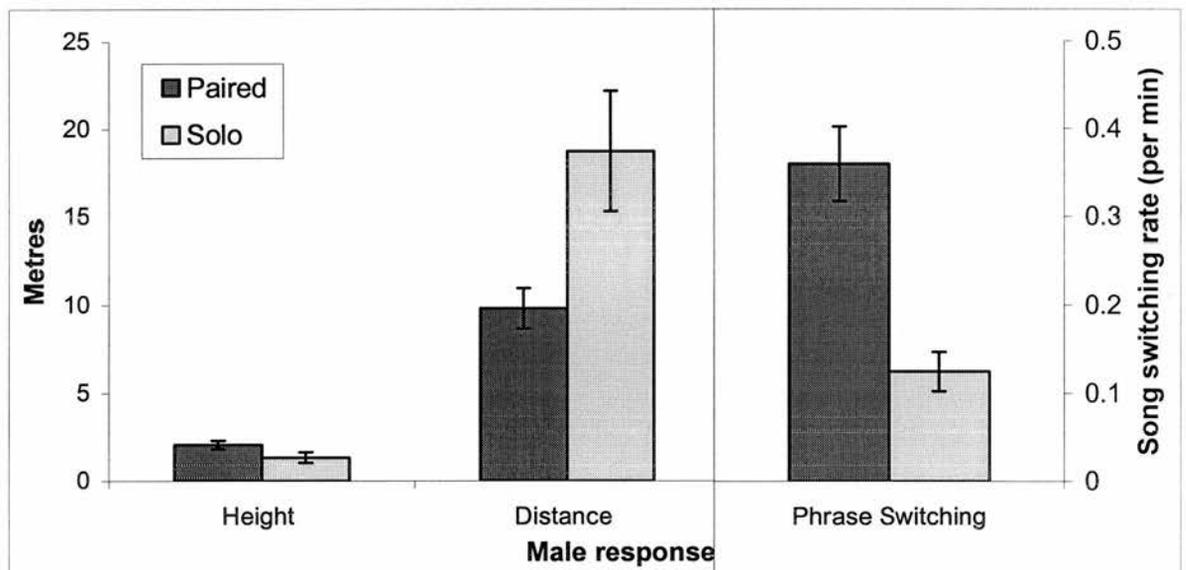


Fig. 7.2: Mean (\pm SE) responses of paired ($n=8$) and solo ($n=5$) males to duet playback, in perching height (m), distance from speaker (m), and phrase type switching rate (per min). Two sample t-tests on means per male, $df=11$: Height: $t=2.34$, $p=0.039$; Distance: $t=2.22$, $p=0.047$; Phrase switching: $t=2.55$, $p=0.027$.

7.3.c Mate-guarding: Sex-specific defence: Independent song rates for each sex were used as indicators of response in the repeated measures GLM described in section 7.2.c. These response variables were male I calling rate, and female initiation

(F(FM)_n) or ending of duets (I(FM)_nF), both of which indicate higher female motivation. Male I calling rate was higher in response to male solo song than to female song playback (Fig. 7.3), see Table 7.2 for the ANOVA table from this test, showing the effects of experimental section and breeding stage on male responsiveness to playback. Female initiation (approaching significance) and ending of duets occurred more often in response to female solo song than to male song (see Fig. 7.4). Approach distances and distance between mates did not change with stimulus.

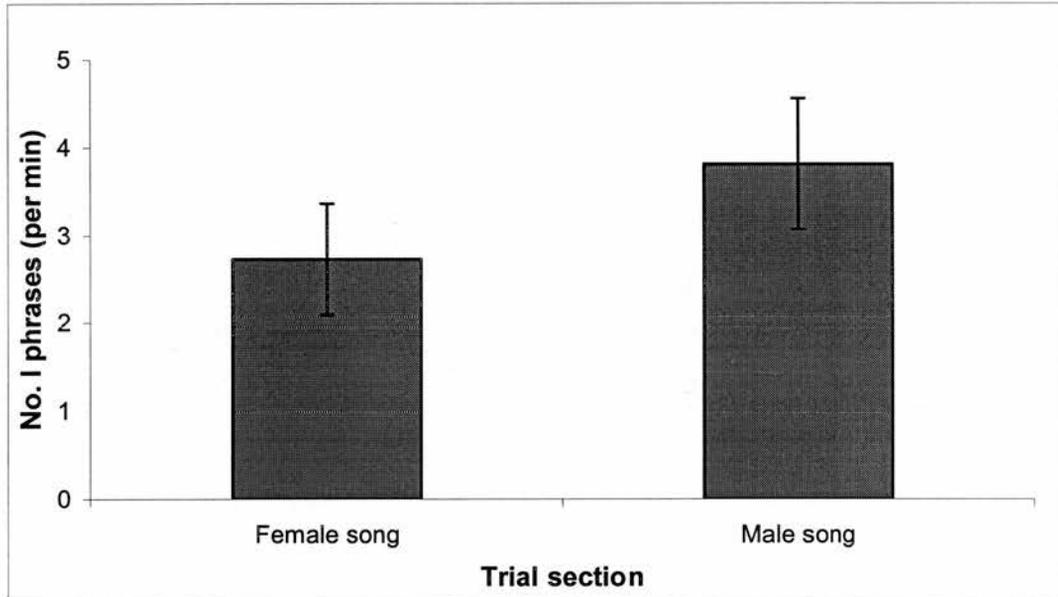


Fig. 7.3: Mean I calling rate for male subjects in response to Female solo song and Male solo song stimuli, bars show SE, $F_{3,38} = 4.60$, $p = 0.008$, $n = 6$ pairs.

Table 7.2: The results from a repeated measures GLM for the effects on male Introductory calling rate in response to playback of male and female solo song (experimental section), during the mate's fertile and post-fertile periods (breeding stage). Pair was the subject in the model with experimental section and breeding stage as both repeated measures and effects.

Null model	Degrees of Freedom	Chi-squared value	P value	
	1	7.29	0.006	
Effect	Numerator d.f.	Denominator d.f.	F value	P value
Expt. Section	3	38	4.60	0.008
Breeding stage	1	38	5.95	0.019

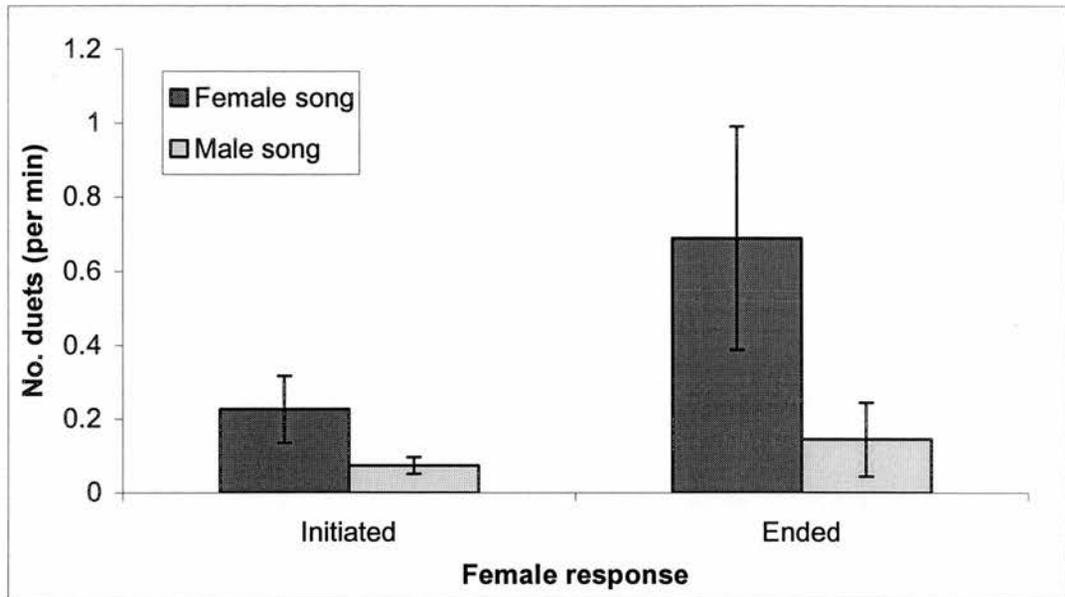


Fig. 7.4: Mean rates of female initiation and ending of duets in response to female solo song and male solo song stimuli, bars show SE. Initiation: $F_{3,14} = 4.19$, $p = 0.026$; Ending: $F_{3,14} = 10.76$, $p = 0.005$, $n = 6$ pairs.

7.3.d Mate-guarding: Breeding stage effects: Male response according to their mates breeding state was tested through comparison of responses during their mates fertile and post-fertile periods. I phrase singing rates were higher during the early breeding stage trials than during the female incubation period, and males tended to stay further away from the speaker during early breeding stage trials than later (Fig. 7.5).

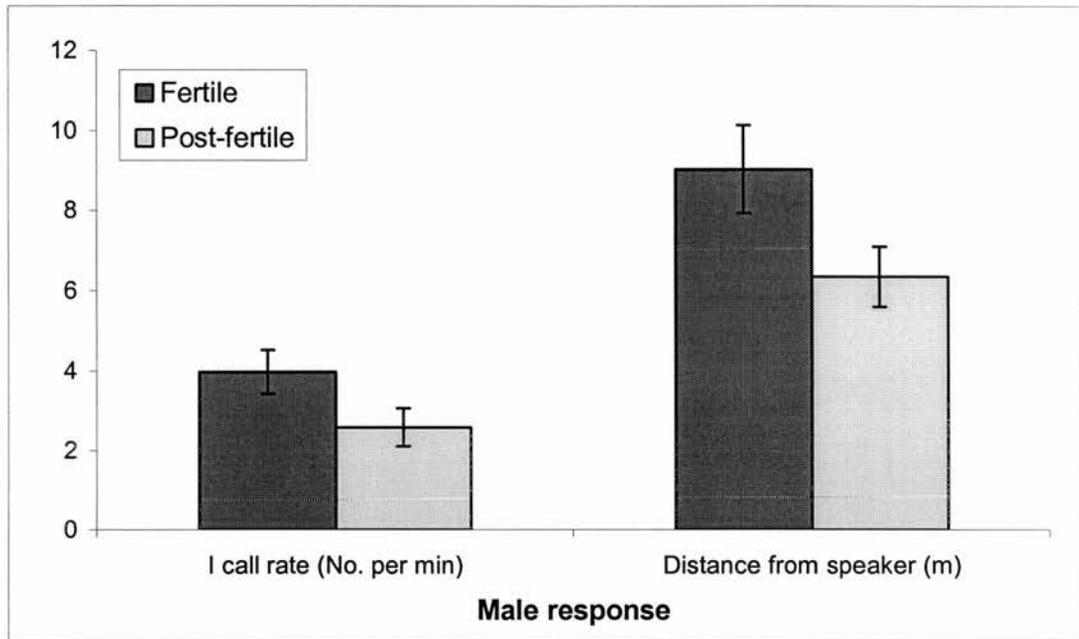


Fig. 7.5: Mean Introductory calling rate and mean distance from speaker of male subjects in response to playbacks during early breeding and mid breeding stages, bars show SE. I calls: $F_{1,38}=5.95$, $p=0.019$; Distance: $F_{1,83}=5.41$, $p=0.022$, $n=6$ pairs.

7.3.e Pair-bond formation: In 2001, the females of two pairs were lost to predation and the males re-mated with new females (see Chapter 4 for more detail). ANOVA analyses of observational data show that the newly formed pairs did not differ from the previous pairing in male to female distance, duet length or rate, rate of irregular duet performance, rate of female initiation of duets, or rate of phrase type switching. There was an apparently higher rate of I phrase singing in the re-mated pairs than in the established pairs (pre-breeding levels). However, on closer inspection of the *post-hoc* results, the trend was only present for male 6, and was not strong, and thus its validity is very doubtful. Duets analysed from pairs 12a and 12b were compared through t-tests for each interval (I-F, F-M and M-F). There were no differences in duet interval duration in the original pair and the newly formed pair for any of the intervals ($df=66$, $p>0.2$ for all three tests).

Duet type consistency was compared across eight pairs from La Suerte. Four were new pairs in April 2002, and thus had been together less than 7 months (last checked August 2001), and one was pair 12b mentioned above which formed in May 2001, and thus had been paired for 1 year. Three more pairs had been together since a pilot

study in August 2000, and thus for at least 2 years. Comparing the degree of duet consistency (σ/μ , see section 7.2.c) in a 1-way ANOVA, 2+ year pairs had significantly greater consistency in their FM phrase matching than new pairs (Fig. 7.6). As only one 1yr pair was included, this could not be tested for any differences to the other classes. However, Figure 7.6 shows very little variation within the other classes and the 1yr pair falls in between the two extremes, suggesting that there may be an increasing phrase matching precision over at least 2 years.

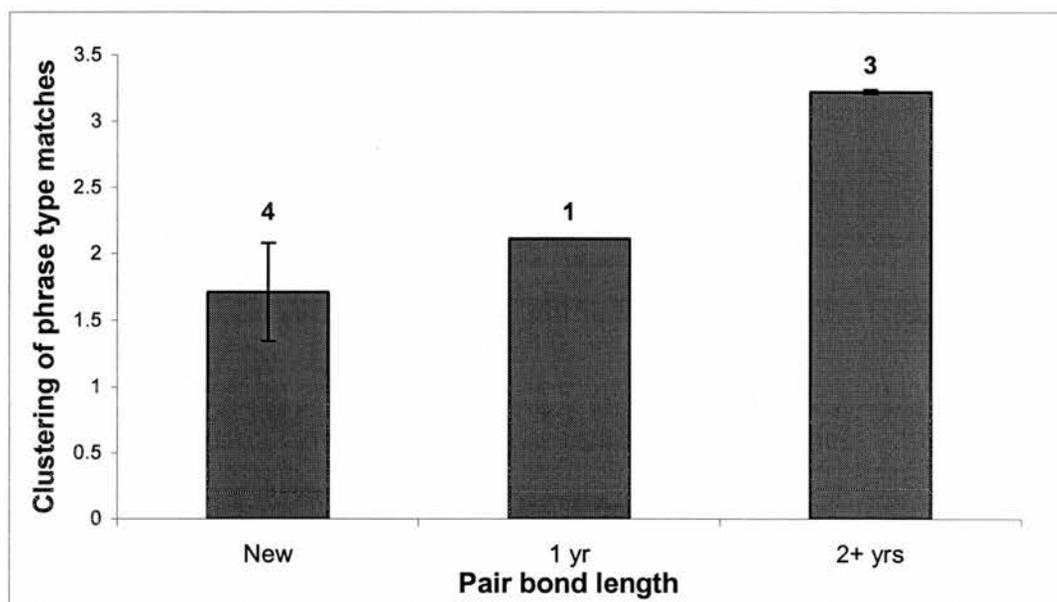


Fig. 7.6: From the distribution of F and M phrase type combinations occurring within pair duet repertoires (in 2002, see Chapter 3), these data show the degree of clustering in FM combinations as the mean/variance (σ/μ) for pairs with different known pair bond lengths. Greater σ/μ values represent more consistent FM phrase matching. All pairs are from La Suerte, numbers above columns show the number of pairs in each class. Pair bonds of 2+ seasons, had significantly greater σ/μ values than new pairs: 1 way ANOVA (with *post-hocs*) $F_{2,5}=6.14$, $p=0.045$.

7.3.f Pair-bond strength: In the repeated measures GLM analysis already described, none of the behavioural variables measured showed any change in response to the playback of stimuli containing long, short, fast and slow duets ($p>0.1$ for all GLMs). However, measurements of subject duet speed (duration of inter-bird intervals: F-M & M-F within the duet) as taken from recordings, were tested through a GLM (with *pair*, *experimental section*, *pair*experimental section*, *F phrase*

duration, *M phrase duration*, and *male-female distance* as factors). This analysis showed that the birds were singing faster duets in response to slow and short duet stimuli, and slower duets in response to fast and long duet stimuli (Fig. 7.7). This variation is independent of male to female distance and distance to speakers, as these did not change in response to the different stimuli in the previous repeated measures GLM (male-female distance was also not a significant influence on F-M intervals in this GLM, although it did affect M-F intervals). The ANOVA results shown in Table 7.3 demonstrate the strength of the model in explaining a relatively large proportion of the variation in both F-M and M-F intervals. They also show that the effect of experimental section remains strong after accounting for effects of the subject pair, the duration of the female and male phrases, and the distance between the birds at the time of singing.

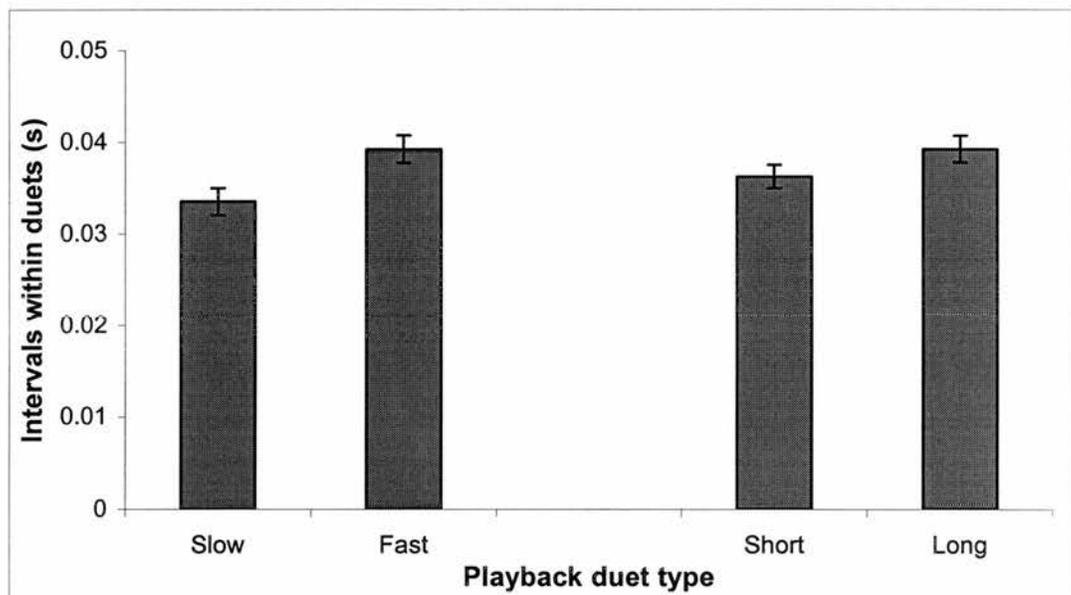


Figure 7.7: Mean duration of intervals between female and male phrases (F-M and M-F intervals combined) within duets sung in response to different stimuli, bars show SE, $n=2228$ duets measured from 15 pairs. GLM results for *pair*experimental section*: Female-Male interval: $F_{85,981}=2.74$, $p<0.001$; Male-Female interval: $F_{85,956}=2.59$, $p<0.001$, *post-hocs*: slow<fast, short<long.

Table 7.3: ANOVA tables showing the GLM results for a) F-M interval duration, and b) M-F interval duration. The tables show the strength of the model overall (R-squared value) as well as the relative importance of the six factors.

a)

Source	Degrees of Freedom	Sum of Squares	Mean Square	F value	P value
Model	110	0.373	0.003	7.75	<0.0001
Error	981	0.429	0.001		
Corrected total	1091	0.802			
Pair	15	0.173	0.012	26.35	<0.0001
Expt. section	7	0.004	0.001	1.46	0.178
Pair*Expt. section	85	0.102	0.001	2.74	<0.0001
F duration	1	0.018	0.018	40.69	<0.0001
M duration	1	0.003	0.003	6.83	0.009
F-M distance	1	0.0002	0.0002	0.62	0.432
R-squared	0.465				

b)

Source	Degrees of Freedom	Sum of Squares	Mean Square	F value	P value
Model	110	1.362	0.012	27.62	<0.0001
Error	956	0.429	0.001		
Corrected total	1066	1.791			
Pair	15	0.761	0.051	113.2	<0.0001
Expt. section	7	0.012	0.002	3.90	0.0003
Pair*Expt. section	85	0.099	0.001	2.59	<0.0001
F duration	1	0.001	0.002	3.36	0.0672
M duration	1	0.125	0.125	278.1	<0.0001
F-M distance	1	0.007	0.007	15.09	0.0001
R-squared	0.761				

7.4 Discussion

7.4.a Joint territory defence: It was unfortunate that removal experiments were not successful, as such a test of joint territory defence, comparing responses to solos and duets has not yet been carried out on duetting birds. Some removal studies report holding the removed bird for 2-4 hours, during which the post-removal playback is carried out (i.e. Beasley, 1996). This would not have been a feasible procedure with the canebrake wrens however, as they were mist-netted by luring with a CD of conspecific song (Ross and Whitney, 1996). The combination of the stress of the simulated intrusion and of handling would inevitably affect the non-removed bird in the period immediately following removal, biasing any experiment carried out in that time. Despite the failure of this experiment, results from the few pre-removal trials were presented, along with those from other tests, and these provide good evidence for the joint territory defence hypothesis.

Both predictions from the pure territory defence theory were supported in this study: duet playback appeared to elicit a greater response than solo song in the pre-removal trials, and duets were much more threatening to solo males than paired birds. Responses to duet playback were totally different for solo territory holding males and males accompanied by their mate. Staying lower down in the vegetation (less conspicuous), and further away from the speaker, the solo males were much less willing to respond aggressively to the intrusion into their territory. Phrase switching rates have been shown to be used in aggressive interactions in this species (Chapter 6), and solo males phrase switched significantly less than paired males, again suggesting an inability or unwillingness to enter an escalated conflict. Although song rates could not be analysed due to their dependence on female presence, solo males sang very little and would often approach the speaker silently before retreating to sing from some distance away.

Although these data strongly suggest that duets are used in territorial defence and represent a greater threat than solo song, this conclusion is apparently refuted by the simple fact that there were solo males in the study population who maintained territories through at least one breeding season. Levin (1996a) carried out permanent removals of a related duetting species, the bay wren (*T. nigricapillus*), and when

none of the remaining solo birds lost their territories, she concluded that joint territory defence was not necessary in that species. Experimental tests of relative responses to duets and solo songs would have been very useful in strengthening these conclusions. Given the fact that there were several territories at La Suerte that were occupied in 2001 and vacant in 2002, and that there were no solo males at the start of the 2001 season (Chapter 2), it seems that competition for territories may have been low in the La Suerte population in 2002. This was possibly due to high adult mortality or dispersal following habitat change (see Chapter 2). El Zota, by contrast only had one solo male in a very poor pasture territory, rather than the marshes that all pairs occupied, suggesting a more saturated population. The presence of solo males on territories, therefore, does not necessarily exclude the possibility that duets are a requirement for territory defence in more stable populations.

7.4.b Mate guarding: Studies on the magpie lark (*Grallina cyanoleuca*) showed that these birds use duets in territory defence without any strong female intra-sexual responses, but with definite male intra-sexual aggression (Hall, 2000). Hall suggested that the pair's joint territory defence consisted of the female defending the territory, and the male focussing his defence on the pair bond. Conversely, Levin's playback study on the bay wren (1996b) showed sex-specificity in responses to playback by both males and females. This study on a congener fits with the results presented here from the canebrake wren, where both males and females increased their singing behaviour in response to same-sex playback relative to song of the other sex. This is despite the pair still moving and singing very much as a unit: distances to speaker did not differ for males or females and neither distance to the mate nor duet rates changed in response to either stimulus. Thus within the cohesive joint defence of a territory, both males and females were more motivated to duet when the intruder was of their own sex, and hence when their own place on the territory was more threatened. In several species where the female performs solo songs, studies have demonstrated an intra-sexual aggressive function (Beletsky, 1983; Arcese *et al.*, 1988). The additional need in some species for cooperative territory defence may drive the development of an intra-sexual aspect to duet communication (Whittingham *et al.*, 1997). This is supported by results in Chapter 6, where repertoire sharing patterns and aggressive signalling through phrase type matching

are both specifically intra-sexual rather than inter-pair (i.e. within the duet, birds type match the phrase of the same sex opponent, but pairs do not type match whole duets).

The second mate-guarding hypothesis investigated whether males targeted their mate guarding by increasing their response to intrusions when their mate was fertile. Although many of the response variables were not independent of the change in female behaviour (incubation in the post-fertile trials), the one independent male response, I phrase singing rate, was significantly higher when the female was fertile than in post-fertile states. This contrasts with earlier results from observations (Chapter 3) which showed that males did not sing more when their mates were fertile. It appears that although they do not increase their use of song in daily territorial defence, males become more aggressive in their response to intrusions when they are at risk of losing paternity. The constant close proximity of the pair possibly means that males do not normally need to change their song behaviour to prevent cuckoldry. However, when the pair bond is directly threatened by an intrusion, males will respond more during the period of greatest risk to paternity. The slight trend for males to stay further from the speaker in fertile period trials may represent an interaction with female presence: whenever females were present the pair stayed close together, whereas in later trials females sometimes stayed on the nest for some of the trial, during which time the male could approach without being affected by the female's behaviour. The contrast between results from observations and experiments highlights the problems with using only one method to test results. In an observational study, Hall and Magrath (2000) concluded that, as in the canebrake wren, magpie larks mate guarded through physical proximity rather than duets. However, male intra-sexual responses to song revealed in playback experiments to magpie larks (Hall, 2000) suggests that mate guarding may still be a function for duets in that species.

7.4.c Pair bond formation and maintenance: Previous studies that investigated the existence of learning to coordinate as a requirement of duet singing and thereby of pair bond formation, have considered the temporal precision of the performance (Wickler, 1980; Farabaugh, 1983; Levin, 1996a), or the adaptation of individual repertoires in response to a new mate (Levin, 1996a). This study has examined several aspects of duet performance including temporal precision, but also covering

length and accuracy of duets and the consistency of the pair in their duet phrase matching. This type of study would benefit greatly from a more long-term approach, following the duetting behaviour of many pairs of birds with known pair bond durations and individual ages. The data in this study are limited by sample sizes and by the fact that the comparisons are between different pairs. They have, however, partly controlled for effects of individual age on singing ability as some new pairs were completely new territory holders, and others had one member who had been resident with a previous mate, but had re-mated following divorce or predation.

Surprisingly, considering the very rapid speed of the duets, canebrake wren pairs showed no evidence of learning in duet timing, length or accuracy of structure. New pairs stayed as close together as established pairs, and newly mated females did not show a higher rate of initiating duets. In another study, bay wrens likewise did not show any evidence of learning in the timing of their duets, nor did they shift their repertoires when re-mated (Levin, 1996a). From these results, Levin concluded that there was no evidence of learning in duet behaviour during pair formation. This result seemed to fit with observations made by Farabaugh (1983) on another congener, the buff-breasted wren (*T. leucotis*), where very rapid duets were performed within minutes of new females arriving on a male's territory. However, these studies did not look at the duet type consistency of new and established pairs (indeed, whether such phrase matching occurs has not been precisely documented in these species). The canebrake wren shows a variation between pairs in their ability to consistently match F and M phrases to form a set duet repertoire. This variation, where pair bond durations were known, was strongly linked to the time that the pair had been together. Thus newly paired birds have to learn which phrases from their own repertoire to match with each phrase from that of their mate. It is possible that this learning process was prolonged, in which case there is not simply an initial investment in the pair bond, but a continued accrual of benefit to remaining with the same mate.

This study is possibly the first evidence suggesting that a variation on Wickler's (1980) coyness hypothesis does apply to antiphonal duet singing. Theoretically, a behaviour that expresses information on pair bond duration may function as a pair fitness signal in territory defence. In the case of duet repertoire consistency, however,

other birds would have to be familiar with the entire repertoire of the pair to assess how well they were maintaining the duet types. This would prevent the use of such a signal in interactions with newly arrived birds or unknown intruders, and would also require birds to invest time in learning the repertoires of neighbouring pairs. It seems likely therefore, that any function to signalling pair bond investment through improving the duet consistency, would act within the pair.

Contrary to the evidence of learning in newly formed pairs, canebrake wrens did not show any evidence of an increase in duetting rates following pair bond formation. This may be because the scale of analysis was not fine enough, as behaviour was not compared at different stages just after pair formation. If duetting rates were elevated on just the first few days after pairing, this would not have been detected by the analyses in this study. I did not attempt to break my data down in this way, as the small sample sizes would have rendered any conclusions unsupportable. Levin (1996a) did find some evidence of an increase in bay wren male song rates with re-mating as opposed to rates with the previous mate, although she too was hampered by small sample sizes.

Perhaps the most intriguing result from this study was that canebrake wrens showed no difference in approach or song rate responses to short, long, fast or slow duets; and yet showed a marked and inverse change in duet speed in response to the different stimuli. The absence of any difference in the usual indicators of aggression (duet rates, male I phrase singing rates, approach distances, and phrase switching rates) was not surprising given the finding that duet speed and duet length are not aspects that require any learning, and thus are unlikely to differ in the level of threat they represent. In the light of this, it is even more perplexing that the subjects were altering the speed of the duets they sang in response to the stimuli, and were altering them in a seemingly counter-intuitive fashion. If fast and long duets were the greater threat for a reason other than learning, the subjects should increase the speed of their duets in response to those stimuli. However, they did exactly the opposite. It seemed possible the initial prediction that the established pair would be the greater threat was incorrect, and that in fact new pairs were more likely to attempt to establish a territory. In this case perhaps birds should respond more aggressively to new pairs than to old, and this was what was occurring in the experiment. However two points

gainsay this: that slow and short duets did not indicate newly formed pairs, and that all playbacks were of stranger pairs, and so all should have appeared to be a threat to the territory.

It is very interesting to note that the precision of duet performance in these wrens was evidently flexible, and partly independent of the distance between mates. Previously, researchers have assumed that duets are always performed at an optimal rate, delimited only by distance to mate, auditory reaction times, and learned ability (Thorpe, 1963; Wickler, 1980; Levin, 1996a). This study raises the possibility that the precision with which a pair duets is not the presumed physiological optimum, but rather a context dependent behaviour under the specific control of the birds. This not only adds another dimension to investigations into learning, but also introduces an entirely new aspect of behaviour that may perform a function in duet singing.

7.4.d Conclusions: In the light of a gradual development of theories for the evolution of duetting, it was possible in this study to comprehensively test the major hypotheses and their interactions in one species. Some of the data would benefit from longer studies, but sufficient evidence is presented to propose that duetting in the canebrake wren performs several functions. Duets as a cooperative joint signal appear to be very significant in the defence of territories, and yet within this joint signal, intra-sexual competition is shown to play a major part. This intra-sexual competition is greatest for males, and males mate guard through song much more when they are at risk of losing paternity.

Despite the remarkable speed and precision of the wren's antiphonal duets, the performance of duets in this species does not appear to require learning during pair-bond formation. However, the pairs' ability to produce a consistent duet repertoire is learned, and this duet phrase matching may well require a very prolonged learning period above that predicted for an investment in pair bond formation. It appears that simple duet performance does not contain information on pair bond strength; however, duet type consistency may do so. Data are also presented that demonstrate a surprising flexibility in the speed at which pairs can produce their duets, an aspect of behaviour that cannot as yet be fully explained. This study has highlighted the need for combined observational and experimental studies to test all the ramifications

of different hypotheses for duet function. It has also reinforced the growing realisation that the evolution of duetting has not been driven by simply one selective force, but by the integration of several, both cooperative and selfish.

Chapter 8: Discussion

This thesis has presented data gained from over a year of fieldwork on the canebrake wren in the Caribbean lowlands of Costa Rica. The fieldwork focussed primarily on behavioural observations and playback experiments, but also encompassed territory mapping, habitat and insect surveys, blood sampling and nest surveys. At two different sites in the region of Limón, 35 territories were studied, some of which were only present in one year, and some of which were held by solitary males. Previous knowledge of the canebrake wren, *T. modestus zeledoni*, and of the plain wren, *T. m. modestus* was sparse. Early descriptions by Skutch (1940) gave a few observations of nests and behaviour of the plain wren, but basic knowledge was still lacking, as illustrated by the fact that the current field guide to Costa Rican avifauna has the duet parts incorrectly ascribed to the two sexes (Skutch and Stiles, 1989). A pilot study on the canebrake wren prior to this thesis utilised genetic sexing to determine the roles of each sex in the duet (Mann *et al.*, 2003). This was followed by observational work in the first field season that provided the first descriptions of the wrens' repertoires and phrase matching behaviours, as well as new observations on their breeding and nesting (Mann *et al.*, 2003; Marshall-Ball and Slater, 2003).

This chapter brings together the various strands of this thesis, tying together conclusions and summarising the main findings. I shall discuss the more general data on duet use and effects of breeding on duetting, before going on to the more complex theories and arguments concerning repertoire sharing and aggressive signalling in song. To present conclusions from this study about the functions that may have led to duet evolution, I will return to the first review of duetting (von Helversen, 1980). As in the introduction (Chapter 1), I shall discuss the theories he presented, but here in the light of my own results, and I shall explore how my conclusions fit with results from other duetting species.

8.1 *Patterns of duet use*

It is not surprising that there were considerable differences in repertoire size and in duetting rates between different pairs (Chapter 3) as, although not well documented

in duetting species, such patterns of inter-individual variation in singing behaviour is commonly reported in species with solo male song (e.g. Doutrelant *et al.*, 2000; Nowicki *et al.*, 2002). The fact that the duet involves two birds may increase the scope for variability, as the motivation levels of each mate are likely to affect the performance differently. From both observations and experiments, different aspects of song emerged as being representative of the motivation of each sex, with males altering their introductory (I) phrase singing rate, and females changing their duet initiation rate or continuing the duet cycle for longer. Song behaviour was very variable within individuals but, despite this, the wrens showed consistent inter-pair differences in both song and activity patterns

In this thesis (Chapter 3) and in an earlier paper (Mann *et al.*, 2003), I developed a way of quantifying the duet phrase matching consistency of canebrake wrens, by comparing the occurrence of F and M (female and male duet phrases) combinations against predicted random associations. This aspect of duetting had not previously been quantified, and the strong tendency for pairs of this species to consistently match F and M phrase types to form a set duet repertoire, suggested that this was an important behaviour. In later analyses (Chapter 7) it appeared that a pair's ability to phrase match developed over time, possibly over a very extended period. This was an unexpected result given that the impressive speed of the duets required no learning on pair-bond formation; its implications for duet functions are discussed later in this chapter.

Perhaps the predominant trend seen in individual song production was that males spent much more time singing than females did, thus the majority of song was solo male I phrases rather than duets (Chapter 3). This imbalance in singing investment contrasts with the similar repertoire sizes and phrase complexity for each sex, so that although song is as highly developed in females as it is in males, males are still responsible for the majority of a pair's song. Linking neatly with the male's greater investment in singing was the observation that although mates foraged in very close proximity to one another, males always foraged higher in the vegetation than females did (Chapter 3). It seems likely that a male's greater role in defence of the territory and pair bond required that he remain higher in the vegetation, allowing both better

song broadcast and better monitoring of the female (Morse, 1968; Busby and Sealy, 1979).

8.2 *Duetting activity and breeding state*

Several patterns emerged from an investigation into changes in duetting behaviour with breeding state (Chapter 4). Some analyses were hampered by small numbers of pairs, but methods allowed for these problems where possible. Despite the low sample size, there was clear evidence of greater male song rate and singing height when males were unmated, a pattern that ceased after re-mating. This suggests that male solo song attracts mates by signalling availability and possibly also territory ownership. This pattern of greater male song rates in un-mated males has been well documented in other bird species (e.g. Staicer, 1996; Gil *et al.*, 1999; Amrhein *et al.*, 2002). It also fits with the pattern seen in removal experiments by Levin (1996a) on the bay wren (*T. nigricapillus*), a duetting congener; where males, but not females, increased singing rates following the removal of their mate.

Sonnenschein and Reyer (1983) reported that males of the slate-coloured boubou (*Laniarius funebris*) did not change their song rates through the breeding season, but that females sang less during parental care periods. This matches the behaviour of the canebrake wrens in this study (Chapter 4), and suggests that females in both species must make a trade-off between parental care and song investment whereas males do not need to do so. Although there may be a slightly greater female role in parental care in some duetting species (Sonnenschein and Reyer, 1983, pers. obs.), the imbalance is neither large nor general (Hall, 1999). The lower female song during parental care may, therefore, reflect higher energetic requirements than the male following laying and incubation, rather than a greater investment in parental care.

Contrary to the proposal by Møller (1991a) that males may mate-guard through increased song rates during their mate's fertile period, this study demonstrated that, if anything, male song declined slightly when their mate became fertile (Chapter 4). This fits with the emerging consensus, in both solo singing and duetting species, against this 'fertility announcement hypothesis' as a mechanism for mate-guarding (Krokene *et al.*, 1996; Rodrigues, 1996; Gil *et al.*, 1999; Hall and Magrath, 2000).

By contrast, the canebrake wrens showed a change in song which suggests that females signalled their fertility to their mate (Chapter 4). This behaviour may serve to coordinate reproductive behaviour and provide paternity assurance to the male. In the canebrake wren, the high level of male post-hatching parental care is likely to depend on the male's willingness to invest in the brood. If this is the case, providing paternity assurance to her mate may be the female's way of ensuring adequate paternal care later on (Iwasa and Harada, 1988; Møller, 1991b; Markman *et al.*, 1995). The occurrence of calls and songs that solicit matings has been demonstrated in promiscuous systems in both birds and primates (Wiley *et al.*, 1991; Langmore *et al.*, 1996; Semple and McComb, 2000), but to my knowledge, such a function for female song has not been previously suggested in a monogamous species. This may be because few studies have specifically looked for changes in female song rate with breeding state. However, the two previous studies that have followed breeding and song activity in duetting birds reported no equivalent changes in behaviour (Sonnenschein and Reyer, 1983; Hall and Magrath, 2000).

These analyses present evidence that, whatever the evolutionary function of duets, changes in duet use can carry information on reproductive state. Distinctive patterns of duet use in the canebrake wren appeared to contain information on the male's mated status, the female's fertility, nest predation events, and the presence of independent juveniles with their parents. Whether this information is used by other birds is a possibility that would be very difficult to test experimentally. However, since the brief study on shrikes by Sonnenschein and Reyer (1983), this is the only research that has demonstrated that specific patterns of duetting activity may transfer information about the status of the individuals and of the pair.

8.3 *Species recognition & territoriality*

The playback experiment in Chapter 5, comparing the response to canebrake and black-throated wren song, was initially planned simply to provide a comparative test of whether the canebrake wren playback was recognised as that of a conspecific. However, as well as showing an unsurprising recognition of their own species, a trend also emerged for a difference in male response to black-throated wren song between the two study sites which correlated with niche overlap. I found this result

very interesting, and planned to develop the hypotheses for inter-specific aggression and niche overlap through wider experiments. Various problems in the field prevented expansion of the experiments, and the available data simply identify an untapped area of study into duetting species. Although research into duetting has largely ignored inter-specific interactions, the area where the work in this thesis was carried out, for example, included populations of five species of *Thryothorus* wrens. These species displayed varying spatial overlap and varying degrees of duetting. There is considerable scope in this field for further work into the extent of inter-specific recognition and territoriality, and the functions for this behaviour. Duetting is thought to have arisen partly due to intense territorial competition within a species, and partly from the need for cooperative defence by both members of a pair. Duetting species which compete for similar resources may duet to signal the presence of two residents to other species as well as to their own and, if so, individuals might not only be able to recognise songs of other species, but might distinguish between their solo songs and duets.

8.4 *Repertoire sharing and aggressive signals*

A thorough study of geographic variation in population repertoires would have required data from several locations, which was beyond the scope of this study. However, the two wren populations studied did show marked differences, not just in repertoire content but also in inter-individual variation (Chapter 6). The incomplete overlap in phrase repertoire content between the two populations suggests that studies on a larger scale would find marked geographic differences in the song types used, as reported in some solo singing species (e.g. Tracy and Baker, 1999; Irwin, 2000). The results from the canebrake wren also match a small scale study on a non-duetting congener, the banded wren (*T. pleurostictus*), where population repertoires differed partially over as little as an 8km separation (Molles and Vehrencamp, 1999).

Habitat differences between La Suerte and El Zota led to one population being very dense, with pairs often having several direct neighbours, and the other being much more scattered, with territories only occasionally abutting. This difference in population structure is likely to have caused the observed differences in inter-individual variation (Chapter 6). Birds at El Zota, the more dispersed population,

shared fewer phrase types with other birds in the area and, within shared phrase types, sang less similar versions of the same phrase. The greater exposure to neighbouring pairs at La Suerte appears to have led to a high degree of repertoire sharing between birds, and more precise copying of shared phrases. This effect of population density on shared phrase similarity suggests that wrens learned phrases by imitating other adults in the population (Cunningham *et al.*, 1987; Nelson *et al.*, 2001). This pattern was not present when comparing similarity to neighbours and non-neighbours within the same population. Canebrake wrens must therefore learn from nearby birds in general rather than exclusively from immediate neighbours. This is contrary to some studies on imitative learning in male solo song (Latruffe *et al.*, 2000; Griessmann and Naguib, 2002), where learning appeared to be much more exclusively from direct neighbours only. The pattern seen in the canebrake wren matches predictions from previous studies, that birds in more sedentary, long-term territorial populations are more likely to learn by imitation after settling on a territory (Kroodsma *et al.*, 1999; Nelson *et al.*, 2001). The most interesting result here is that even within two very sedentary stable populations, the simple difference in territory isolation may have been the cause of very different levels of inter-individual variation and repertoire sharing.

The patterns described above were all found when analysing individual repertoires, i.e. male repertoires of I and M phrases, and female repertoires of F phrases. Going on to look at duet sharing between neighbours and non-neighbours, the results were very different (Chapter 6). In contrast to the strong degree of sharing individual phrases with members of the same population, pairs of wrens shared duet types with neighbours neither more nor less than was predicted to occur randomly. This suggested that sharing phrase types with other birds may perform some function, but that there was less likelihood of a function to sharing duet types with other pairs. The division here appeared to be one of interactions that were intra-sexual versus those that were between pairs. Thus although phrase type sharing appeared to be an intra-sexual behaviour, there was no inter-pair interaction involving shared duet types, so the formation of duet repertoires may have been driven by purely intra-pair functions.

The results on the extent of repertoire sharing of individual phrases and duet types paved the way for further investigation into the functions of sharing. Previous work in this field had all been carried out on male song in non-duetting species, and most notably on the song sparrow (*Melospiza melodia*) where repertoire sharing was suggested as a means of allowing graded signals of aggression between neighbours, through repertoire matching and song type matching during counter-singing (Nielsen and Vehrencamp, 1995; Burt *et al.*, 2001; Burt *et al.*, 2002). With both sexes of the canebrake wren showing strong phrase type sharing, the potential existed for phrase type matching to be used during counter-singing. Playback experiments using stranger song showed a clear tendency in both males and females to match the phrase types being used by the simulated intruders, supporting the prediction that this behaviour is an aggressive signal (Chapter 6). Fitting in with the earlier observation that duet type sharing did not seem to function between pairs, there was no evidence of any attempt by subject pairs to match the entire duet type of the playback. Thus again, the aggressive signalling was directed by each individual to the same-sex competitor, rather than directed by the pair to the opposing pair.

Although canebrake wrens did increase their song switching rates during response to playbacks, the pattern of the behaviour better fitted the anti-habituation hypothesis (Hartshorne, 1956), rather than predictions based on a specifically aggressive function for song switching (Chapter 6). Thus the increased speed of switching song types during aggressive interactions may have served to prevent receiver habituation, and thereby maintain the strength and threat level of the pair's song, rather than itself being indicative of aggression levels.

The quantitative study of aggressive signalling in male song is a relatively new field and, although repertoire sizes and geographic variation have been defined for many passerine species, few studies have investigated the functions that repertoire sharing may perform (e.g. Nielsen and Vehrencamp, 1995; Beecher *et al.*, 1996; Beecher *et al.*, 2000; Vehrencamp, 2001; Otter *et al.*, 2002). As there has been so little work on species that possess large duet repertoires, it is not surprising that no study has previously presented any evidence for either geographic variation, repertoire sharing patterns, or the use of song matching or song switching in aggressive interactions in a duetting species. The fact that the use of aggressive signals appeared to be

exclusively intra-sexual is intriguing considering the highly coordinated nature of the duet performance. This joint performance, and combined aggressive response, concealed the fact that each bird was directing its aggression solely at its same-sex competitor rather than at the intruding pair as a whole. This behaviour has implications for the evolutionary function of duetting that will be discussed below.

8.5 *Functions for duetting*

All of the aspects of behaviour that have so far been discussed are expressed through changes in duet use by the pair, or characteristics of the pair's duetting performance. Although these are very important in understanding the roles that duets play in the behaviour of the canebrake wren, they do not determine what fundamental advantage to duetting led to its evolution. In the introduction to this thesis (Chapter 1), I presented nine theories collated in the first review of duetting (von Helversen, 1980). In discussing the evidence for all nine theories, it appeared that most could be readily discounted as functions that could have led to the evolution of duetting, although all nine may function in more minor ways. We are thus left with four of the original nine that, either for lack of a consensus or lack of any investigation, remain possible candidates, these are:

- a) Transfer of information
- b) Synchronisation of reproductive behaviour
- c) Pair bond strengthening (including mate guarding)
- d) Territorial defence

Transfer of information is a very general concept, and its occurrence is confirmed by the evidence that synchronisation of reproduction appeared to be involved in the performance of duets in the canebrake wren. As has been discussed above, changes in duet use appear to be indicative of both the breeding state of the pair and also of aggression levels during interactions with intruders. Although the idea that duets are capable of such flexibility and diversity of signalling is new and fascinating, these are not functions that apply exclusively to duetting. The signalling of information on individual state that has been demonstrated in duetting, could potentially also be achieved by solo songs. Thus we are left with three major theories for fundamental functions to duetting: joint territory defence, and the now distinct functions of pair bond formation, and mate guarding.

The canebrake wrens in this study clearly used duetting in a coordinated defence of their territories. Not only did pairs react very aggressively, and in very tight synchrony (both of movements and song) to intrusions, but duet song was also perceived as a much greater threat than solo songs (Chapter 7). This fits with results from playback experiments on the magpie-lark (*Grallina cyanoleuca*) in Australia (Hall, 2000), and matches qualitative observations from many other duetting species (see Payne, 1971; Farabaugh, 1982).

In apparent contrast to the above conclusion, both phrase type matching and other aggressive responses were found to be partly sex-specific. Each member of the pair, within the coordinated territory defence, would therefore be song type matching with their same-sex opponent (Chapter 6) and would also react more aggressively to same-sex solo song than to opposite sex playback (Chapter 7). This intra-sexual aggression suggests that the use of duets during aggressive interactions is linked to mate guarding and not purely territory defence. Outwith intrusion events, neither males nor females showed any evidence of mate guarding (Chapter 4), but under the direct threat of an intrusion both were more aggressive to the intruder who represented the greater threat to their own mated status, i.e. the same-sex intruder. Intra-sexual aggression was reported by Hall (2000) to occur only in male magpie-larks but, as in this study, occurred in both sexes in the bay wren (Levin, 1996b). Although Levin concluded that joint territory defence was not a function of the duets of bay wrens, she did not test for the relative threat of duets over solo song. Hence, as demonstrated in this study, the fact that sex-specific aggression occurred, and that single individuals could hold territories, does not exclude the importance of duets in territory defence.

The use of sex-specific aggression within duet singing suggests that both sexes use duet songs to defend their position on the territory and with the mate. It is possible that this behaviour does not indicate mate guarding, however, but rather a partitioning of territory defence by sex, so that defence is one-on-one and aggressive signals such as song type matching, which could not occur across sexes, are possible. This is a possibility that has dogged previous attempts to test for mate guarding in duet song. However, the role of duets in mate guarding was supported in this study,

by the male wrens' increased responsiveness to playback when most at risk of paternity loss, i.e. when the mate was fertile (Chapter 7). This provides a clear link with protection of paternity without the possible complication of whether defence is partitioned according to sex.

The most difficult hypothesis to interpret in this study proved to be that of duet use in the formation and maintenance of pair bonds (Chapter 7). The prediction that achieving well-coordinated duet singing would involve a learning period (Wickler, 1980), was upheld only in the pair's ability to duet phrase match consistently. Surprisingly, canebrake wrens appeared to be able to produce their very rapid tightly coordinated duets without the need to practice together. Wickler's coyness model refers only to a pre-breeding learning period that serves as an investment in the pair bond. The canebrake wrens in this study showed evidence suggesting a learning period in duet type consistency that extended at least two years after pair formation. Thus, although this is the first evidence in birds for the existence of a duet learning period (see Levin, 1996a; Geissmann, 1999), it goes further than Wickler's predictions and suggests a continuous investment in the pair bond beyond simply a pre-breeding commitment.

One of the assumptions of duets functioning in pair bonding is that the bond strength is correlated with duetting activity (Geissmann and Orgeldinger, 2000), and thus that any learning period provides the means for a signal of pair bond strength. In the canebrake wren, the absence of a learning period in anything other than duet consistency, and the absence of changes in aggressive response to different 'quality' duet playbacks, strongly suggest that pair bond strength was not advertised through duet performance (Chapter 7). It is highly unlikely that any bird outside a pair would be able to rapidly quantify that pair's duet type consistency, therefore the extent to which this is achieved is extremely unlikely to function as an external signal. If learning to consistently phrase match in duets serves as an ongoing reinforcement of the pair bond between mates, this must be purely to signal the pair's commitment to each other, and not to advertise fitness or bond strength to competitors.

The other unexplained result from the pair bond playback experiments was the apparent flexibility in the timing of duets (Chapter 7). It has previously been

assumed that the speed of duet singing is always performed at the physical maximum, limited only by auditory reaction time, distance between mates, and learned ability. By contrast, the speed of duets in the canebrake wrens appeared to be partly determined by the pair, independent of physical limitations. These results contradict the previous assumptions, and raise the question of what function this flexibility may serve. It is possible that further investigation would be able to uncover a link between the flexibility of duet timing and threat signalling or pair bond strength, perhaps stronger pair bonds allow more control over the timing of the duet, or perhaps more precise matching (or contrasting) of the speed of competitor duets shows greater duetting ability than simply singing faster or longer.

8.6 *Summary of duet functions*

This thesis has investigated many aspects of basic song production in the canebrake wren, some of them for the first time in a duetting species. As with solo song in other species, duets of the canebrake wren contained information on individual identity, through both unique repertoires and individual variation in song characteristics. The observed variation in rates of duetting and duet structure provide a basis for carrying information on a wide variety of states, and evidence suggests that this information can range from female fertility to aggression levels. A pattern suggestive of song learning from nearby conspecifics led to repertoire sharing that may be dependent on habitat structure, and to geographic variation in phrase types used and phrase structure.

Duets played a very important role in the joint defence of canebrake wren territories, and I would be inclined to agree with von Helversen (1980) that this function was the most likely initial selective force for the evolution of duetting. However, within the cooperative nature of this joint defence, each sex was also found to use their part of the duet to defend the pair bond against same-sex intruders. The integration of mate guarding into duet singing may have led to increasing the speed and length of the canebrake wren duet, by selecting for closer coordination of the reply and continued cycles to enforce the strength of the 'mated' signal. Although duets do not seem to advertise pair bond strength to other birds, an increasing consistency in duet phrase matching appeared to involve a quite covert learning process. This behaviour could

serve as a way of continually reaffirming the pair bond, as is demonstrated in allo-grooming in gibbons (Geissmann and Orgeldinger, 2000). In a species where the pair bond is critical to breeding success, this may have led to the evolution of more and more complex duets that provide more honest signals of commitment. Canebrake wrens appeared to exert a degree of control over the speed with which they perform their duets, this behaviour raises questions about other mechanisms for pair bond or aggression signalling, that have not yet been the subject of study.

8.7 *Conclusions*

The use in this study of both observations and experiments enabled me to test the different functions for duetting, and to reveal some intriguing behavioural patterns. The study of duet song is a growing field, and this thesis has served to provide new methods for testing and distinguishing between the different functions for duetting, that will hopefully be applied to other species. I have been able to draw attention to the possibility that duets can serve as signals in many of the same ways that have been demonstrated in male solo song. It is perhaps not surprising that I am left with the conclusion that duetting appears to serve many functions in the canebrake wren. Evolved perhaps to aid joint territory defence, and elaborated to allow mate guarding and pair bond maintenance, the duets of the canebrake wren are a diverse and fascinating behaviour that have still to reveal all their secrets.

Chapter 9: References

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