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THE VOCAL BEHAVIOUR OF LONG-TAILED MANAKINS (*CHIROXIPHIA LINEARIS*): THE ROLE OF VOCALIZATIONS IN MATE ATTRACTION AND MALE-MALE INTERACTIONS.

by

DUGAN FINN MAYNARD

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2012

The vocal behaviour of Long-tailed Manakins (*Chiroxiphia linearis*): The role of vocalizations in mate attraction and male-male interactions

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Declaration of Co-Authorship

I hereby declare that this thesis incorporates material that is a result of joint research, as follows:

For chapter 1, I am the sole author.

For chapter 2, I am the principal author, and I share authorship with Kara-Anne Ward, John Burt, Stéphanie Doucet, and Daniel Mennill. My graduate supervisor Daniel Mennill and my collaborator Stéphanie Doucet made this research possible through funding, assistance with research design, and assistance with field research. Daniel Mennill provided assistance with statistical analyses and writing. Kara-Anne Ward assisted with the execution of the field research and provided feedback on drafts of the manuscript. John Burt and Brian Otis designed the Encounternet radiotelemetry technology and provided support and technical assistance during the execution of the research that forms the basis of Chapter 2. This chapter is formatted as a manuscript for future submission to the journal Ethology.

For chapter 3, I am the principal author, and I share authorship with Kara-Anne Ward, Stéphanie Doucet, and Daniel Mennill. My graduate supervisor Daniel Mennill provided assistance with experimental design, field research, statistical analyses, and writing. Kara-Anne Ward and Stephanie Doucet assisted with the execution of field research. This chapter is formatted as a manuscript for future submission to the journal Animal Behaviour.

Appendix I is a technical manuscript which is included as a supplement that provides support for the new technology (Encounternet) used in Chapter 2. I am a co-author on the manuscript that comprises this appendix, and I share authorship with Daniel Mennill, Stéphanie Doucet, Kara-Anne Ward, Brian Otis, and John Burt. My supervisor Daniel Mennill is the lead author on this manuscript. Together with collaborators Stéphanie Doucet and Kara-Anne Ward, I contributed to the design of this chapter and played a major role in conducting field research, analyzing data, and drafting the manuscript. John Burt and Brian Otis designed the Encounternet radiotelemetry technology and provided support and technical assistance during the execution of the research that forms the basis of Appendix I. This chapter is formatted as a manuscript for future submission to the journal Methods in Ecology and Evolution.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-authors to include the above materials in my thesis.

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Abstract

Tropical birds are little studied relative to temperate birds, despite the fact that tropical species often exhibit unique behaviours, not seen in the temperate-zone. I explore the link between male vocal behaviour and female movement in neotropical lekking Long-tailed Manakins (*Chiroxiphia linearis*) using acoustic recordings to monitor male vocalizations and a novel telemetry system to monitor female visitation behaviour. I report surprisingly few relationships between male vocalizations and female visitation behaviour, in contrast to results from another long-term study population in a different habitat. I examine the timing of male calling behaviour, showing that males avoid overlapping known neighbours, but actively overlap unknown intruders simulated through playback. Overall, my thesis provides insight into acoustic communication in manakins, revealing that male vocalizations play less of a role than expected in female mate choice, and that males modify the timing of their calls in response to the vocalizations of other nearby individuals.

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Chapter 1: General introduction

Bird song

Animals can convey information through an incredible diversity of signals. These can range from the electrical signals of certain species of knifefish, which function in mate attraction (Hopkins 2010); to the scent markings of wolves, which function in territory defence (Sillero-Zubiri and MacDonald 1998); to the dewlap displays of anole lizards, which function in species recognition (Williams and Rand 1977, Bloch and Irschick 2006). Across these diverse signalling modalities, one method that has been extensively studied is that of acoustic signalling (Bradbury and Vehrencamp 2011). Acoustic signals differ from other signalling modalities in many respects. Unlike tactile or electrical signals, acoustic signals transmit over long distances. Whereas chemical signals cannot be modulated rapidly, acoustic signals can be modified during the course of a bout of behaviour. In contrast to visual signals, acoustic signals can be actively interfered with either by conspecifics or heterospecifics (Ficken et al. 1974, Dabelsteen et al. 1997, Catchpole and Slater 2008). Acoustic communication has been particularly well studied in birds (Class: Aves; Catchpole and Slater 2008). Within the class Aves, the order Passeriformes – the songbirds – have the most complex and diverse assortment of vocal behaviours, from the extraordinarily variable songs of the open-ended learning lyrebirds (*Menura spp.*), to the innate, genetically-inherited calls of the suboscines (Suborder: Tyranni; Kroodsma 1984, Kroodsma and Konishi 1991, Zann and Dunstan 2008).

Bird song is generally thought to serve dual roles in territory defence and mate attraction (Catchpole and Slater 2008). In terms of territory defence, playback of male song has been shown to decrease territorial intrusions, trespassing, and occupation by neighbours across a wide array of species including, for example, Red-winged Blackbirds (*Agelaius phoeniceus*), White-throated Sparrows (*Zonotrichia albicollis*), and Anna's Hummingbirds (*Calypte anna*; Yasukawa 1981, Falls 1988, Goldberg and Ewald 1991). Male Red-winged Blackbirds that were surgically modified to be incapable of producing songs experienced more territorial intrusions by prospecting males and higher levels of intrasexual aggression (Peek 1972). When their ability to produce song was restored, they were able to exclude interlopers from their territories again (Peek 1972, Catchpole and Slater 2008). Playback of unfamiliar male song within a bird's territory promotes aggressive behaviour on the part of the territory holder in the form of increased vocal output, approaches to speakers, and attacks of taxidermic mounts, as has been shown in Carolina Wrens (*Thryothorus ludovicianus*), Black-throated Blue Warblers (*Setophaga caerulescens*), and Swamp Sparrows (*Melospiza georgiana*) among others (Hyman 2005, Ballentine et al. 2008, Hof and Hazlett 2010). In these and many other studies, bird song has been shown to function as an aggressive signal important in territory defence.

Bird song also plays an important role in intersexual communication, serving as the primary means of mate attraction in many birds. The evidence for this is diverse and wide-ranging. For example, female *Ficedula* flycatchers will occupy nest boxes where male song is playing but not those where there is no song (Erikson and Wallin 1986). Male Nightingales (*Luscinia megarhynchos*) will continue singing at a high rate until they pair with a social mate, after which point they subsequently decrease their singing rates (Amrhein et al. 2007). Similarly, male Bay Wrens (*Thryothorus nigricapillus*) will increase their song output following the removal of their partner, and decrease their singing rate following re-pairing (Levin 1996). But it is not only the level of output that females attend to; females of many species have been shown to prefer specific types of songs, usually those that are longer, more complex, and more challenging to produce or learn (Catchpole and Slater 2008). Female Canaries (*Serinus canaria*), for example, have been shown to prefer males that produce songs with a higher diversity of syllables (Vallet and Kreutzer 1995). Similarly, female Medium Ground Finches (*Geospiza fortis*) prefer males that produce songs with rapid frequency changes (Podos 2001). Together with the results of many other studies, these findings demonstrate that bird song functions in mate attraction, and that females select males based on the output and the quality of their songs.

It should be noted that the term “song” is typically restricted to oscine songbirds (Passeriforme birds in suborder Passeres); this is because early researchers believed that only oscine songbirds learned their songs, while the vocalizations of all other species were innate or genetically programmed, and were termed “calls” (Kroodsma 1984). New evidence has demonstrated that song learning is not restricted to the oscine songbirds. Parrots (Order: Psittaciformes) and hummingbirds (Order: Apodiformes; Family: Trochilidae) have also been shown to learn their vocalizations (Baptista 1990, Farabaugh and Dooling 1996), and evidence from some species of suboscine birds (Passeriformes in suborder Tyranni) also suggests that vocalizations may be learned (e.g. Trainer et al. 2002, Saranathan et al. 2005). Regardless of what definition of “song” is used, the fact remains that many species possess complex acoustic ornaments important in territory defence and mate attraction. Suboscines and other species without learned songs still use their calls in the context of territory defence and mate attraction (e.g. Bard et al. 2002, Tobias et al. 2011). To follow established convention, in this thesis concerning a suboscine songbird, I will refer to the vocalizations of my study species as “calls” (except when following the convention established by McDonald (2010) of referring to one of the courtship vocalizations of my study species as a “dance song”).

Leks

Leks are aggregations of males that display for females, and are associated with promiscuous mating systems. There are four main factors that must be present for a mating system to be defined as a lek (see Balmford 1991, Höglund and Alatalo 1995 for reviews). (1) Males must provide only gametes; i.e. they do not contribute resources to the female or provision the young. (2) There is some defined arena or display site where males display, and this site is separate from areas that the species use for other activities (e.g. foraging or nesting). (3) The sites where males display are not associated with any resources that the female may benefit from, such as food, water, nest sites, or other physical resources. (4) Females choose freely between the males within the population; i.e. there is no male coercion of females and females are able to inspect males without harassment. If the second factor is not present, and males do not aggregate in a single central area, the lek may be considered to be an "exploded" or "dispersed" lek, as opposed to a "classical" or "concentrated" lek (Prum 1994, Höglund and Alatalo 1995). Lekking in birds is not uncommon. Despite the fact that many mating systems remain undescribed, lek-based mating systems have been documented in over 100 species spanning 14 families (Prum 1994, Höglund and Alatalo 1995).

Lekking animals present an ideal system for studying mate choice. In non-lekking species, females will often select their social mates on the basis of their male's territory quality, or his level of provisioning for nestlings, while seeking extra-pair copulations with males with more elaborate sexually-selected traits (e.g. Westneat et al. 1990, Buchanan and Catchpole 2000). Leks eliminate these confounding variables, since by their very definition males do not provide resources for a female, or provision her offspring (Höglund and Alatalo 1995). Consequently, by studying lekking animals, we can focus directly on the link between male traits and female preference for those traits without the influence of these other potentially confounding factors on females' decisions (Balmford 1991).

Manakins (Family: Pipridae) are widespread neotropical suboscine songbirds. Lekking occurs in at least 32 species of the 52 species of manakin, and can range from the classical leks of the Blue-crowned Manakin (*Lepidothrix coronata*), to the dispersed or exploded leks of the Crimson-hooded Manakin (*Pipra aureola*; Prum 1994). One unusual form of lekking is that of cooperative lekking seen in *Chiroxiphia* manakins (Prum 1994). Males in this genus display in a lek arena where multiple males perform elaborate displays for females in a small display area (McDonald 1989). Within each display area, male social interactions are governed by a linear dominance hierarchy, and typically only the most dominant male has the opportunity to mate with females (McDonald 1989, McDonald and Potts 1994). Several groups of displaying males may be found over a relatively small geographic distance (McDonald 1989). As a result, one can consider the individual display area a lek, based on the concentration of males; but one can also consider the total collection of male groups as a dispersed lek, given that each individual display pair only has one functionally reproductive male (Prum 1994). Throughout this thesis I will refer to the area comprising all of the display perches of a single pair of males as a "display area" and the total collection of display areas in the population as a "lek".

Females visiting leks may benefit by being able to simultaneously assess and compare a large number of males (Balmford 1991). Females may assess males on the basis of their phenotypic or behavioural traits, and use these as a basis for deciding which males to mate with (Balmford 1991). Historically, females were only thought to receive indirect benefits from lek-based mating (i.e. high quality genes from her chosen copulation partner). For example, the vocal and visual displays of males may serve as honest indicators of male quality, which females use when assessing mates (Balmford 1991, Kirkpatrick and Ryan 1991). The quality of male displays and ornaments may be correlated with parasite load, and by selecting males with good traits females may be selecting for increased parasite resistance in their offspring (Balmford 1991, Kirkpatrick and Ryan 1991). Alternatively, the elaborate male traits that females select may represent an innate preference, resulting in runaway selection (Kirkpatrick and Ryan 1991). In this sense, the elaborateness of a trait may not reflect male quality, or any direct benefit, but may still result in a benefit to the female who will produce offspring with the same preference and elaborate trait (Kirkpatrick and Ryan 1991, Kokko et al. 2002).

Recently, there has been increased support for the idea that lek-based mating systems may provide females with direct benefits as well (see Balmford 1991, Kirkpatrick and Ryan 1991 for review). Females can benefit from increased fertility assurance by mating with multiple males, or selecting a male that is most likely to increase her chances of successful fertilization (Balmford 1991). For example, in certain lek-mating frogs, females select males that are approximately 30% lighter than them, as heavier males impede mating success, and lighter males lack sufficient sperm to fertilize her eggs (Robertson 1990, Balmford 1991). It has also been suggested that females may experience reduced predation by mating in a lek where there are many other individuals in attendance (Wiley 1974, Balmford 1991; but see Balmford and Turyhao 1992). By improving our understanding of lekking behaviour within a single species we can gain insight into factors influencing lek formation, social organization, and female mate choice at a larger scale.

Long-tailed Manakins

My thesis research focuses on Long-tailed Manakins, a neotropical suboscine songbird with an interesting lek-based mating system (Fig. 1.1; see McDonald 2010 for review). Males exhibit delayed plumage maturation, going through four pre-definitive (juvenile) plumages before attaining their definitive (adult) plumage in their fifth year (Doucet et al. 2007). Two unrelated males form a long-term association and work together to produce highly coordinated vocal duets to attract prospecting females (McDonald and Potts 1994). When they attract a female, males move to their display perch, where they engage in a series of backwards leapfrog hops, and laboured “butterfly” flight (McDonald 1989). If the female is receptive, she will remain on the perch and mate with the dominant male. The system is governed by female choice and males are never able to successfully copulate with a non-receptive female (McDonald and Potts 1994, McDonald 2010). With rare exceptions only the dominant (alpha) male of a pair mates with the female; the subordinate (beta) male is thought to gain delayed fitness benefits by inheriting the display perch after the alpha male dies (McDonald and Potts 1994). Because females show site fidelity, returning to the same perch between years, a subordinate male is able to improve his chances of future reproductive success by displaying regularly with his dominant partner, thus improving the reputation of the display area (McDonald 1989, McDonald and Potts 1994). Most beta males are at least eight years old, while most alphas are at least 13 (McDonald 2009, McDonald 2010). At each perch area, the alpha and beta males may be joined by up to 13 other affiliate males, these males may range from being first years to definitive adults, and social interactions between these males follow a linear dominance hierarchy (McDonald 1989, McDonald 2009). While a male's position in this dominance hierarchy is largely dependent on age, it is also affected by his connectivity within the network (McDonald 2009). At birth, males have no perch area affiliations; as they age they move through the population interacting with males at different perches, gradually restricting their interactions to a few specific males later in their life (McDonald 2009). Social interactions, and connectivity to dominant males, are important for ensuring a male's future reproductive success in this species (McDonald 2009, McDonald 2010).

Male Long-tailed Manakins have an unusually large repertoire of more than 13 distinct calls, which they use in social interactions and female mate-attraction contexts (Trainer and McDonald 1993). These vocalizations, and their suspected functions are summarized in Table 1. The most important calls in the context of my thesis are the *teeamoo*, *toledo, owng*, *nyanyownh* (or "dance song"), and *buzz-weent* calls.

Table 1.1 The 13 most common calls of male Long-tailed Manakins and their suspected function (information is derived from Trainer and McDonald 1993, and personal observations*).

Call	Intended Receiver	Function
Chitter	Dominant male, or other males*	Given by birds in socially aggressive contexts.
Buzz-Weent	Subordinate partner	Given by the dominant male at the end of a dance display, stimulating the subordinate male to leave before the dominant male can continue with solo display.
Doodoodoo	Unknown	Unknown. Playback of this vocalization stimulates silent approach of males.
Nyanyownh **(or "dance song")**	Female	Given by both male partners during the dance display.
Owng	Female, alpha or beta	Stimulates the female to move to the display perch; may be used to indicate the presence of a female to one or both of the dominant males.
Pee-wit, Pee-wit-oh	Unknown	Unknown, though potentially an unusual derivation of the *teeamoo* call*.
Teeamoo	Partner	Produced by the alpha or beta male to attract his partner; often occurs before bouts of *toledo* duets.
***Toledo* (duet)**	Female	Attracts females to the display area.
Toodleloo	Unknown	Unknown.
Waanh	Males	Close range contact call.
Weet	Threats and other manakins	Alarm call.
Wheeoo	Potential threats, or known males	Can be used as a mobbing call, or as a contact call between males*.
Wit	Partner	Precedes *toledos*, thought to function in synchronizing duet contributions. May also serve as a short-range contact call between males*.

Previous studies by McDonald (1989) on a study population of Long-tailed Manakins in the mountains of central Costa Rica demonstrated that female visitation was positively correlated with many aspects of male display. Female visitation increased with the number of *toledos* males performed, the number of bouts of *toledos* males performed, and the amount of time that two or more males spent in the immediate vicinity of the display perch. Similarly, McDonald's research showed that female visitation was negatively correlated with the number of minutes between successive bouts of *toledos*, the frequency of the *teeamoo* call, and the presence of one or no males (McDonald 1989). Other factors that were weakly negatively associated with female visitation in some years of McDonald's study included the presence of more than two males on the display perch, the length of *toledo* bouts, and the number of a variety of vocalizations associated with male-male interactions (McDonald 1989).

In a later study of the same study population, Trainer and McDonald (1995) showed that the degree of frequency matching between the two males' contributions to a *toledo* duet was highly correlated with female visitation and mating success. They suggest that the level of frequency matching may represent a theoretical ideal that females could be using in their assessment of males (Trainer and McDonald 1995). The level of frequency matching is also correlated with the length of the alpha beta partnership (Trainer et al. 2002). In this sense, females might be using the level of frequency matching to select males that have formed longer associations (Trainer et al. 2002). This might be especially important in Long-tailed Manakins, given that the formation of strong social bonds and longevity are important factors in ensuring reproductive success in this species (Trainer et al. 2002, McDonald 2009, McDonald 2010). Previous researchers have suggested the possibility that the level of temporal synchrony in duets may also be an important measure of quality (Hall 2004). Trainer and McDonald (1995) tested this by measuring the temporal synchrony between the two duet contributions but found no correlation with female visitation or male mating success (Trainer and McDonald 1995).

The majority of the studies conducted on this species to date have taken place in the Monteverde cloud forest, by Dr. Dave McDonald and his research team over an 18-year study (McDonald 2010). While some studies have examined the vocalizations of Long-tailed Manakins in other habitats (Foster 1977, Trainer and Parsons 2001), no one has attempted to document whether the patterns revealed by McDonald and colleagues hold true in other populations and other habitats. This is problematic, given that many studies have shown that females often prefer different traits in different populations (Schluter and Price 1993). For example Dunn et al. (2008) showed that female Common Yellowthroats (*Geothlypis trichas*) prefer males with large black masks in a study population in Wisconsin, but that mask size did not correlate with female preference in New York. The New York females instead appeared to favour males with larger yellow bibs (Dunn et al. 2008). Many studies are only ever conducted on a single population, and the findings from these studies are then applied to the species as a whole (Dugatkin 2001). This is problematic given that different populations will experience different environmental conditions, population dynamics, and selective pressures (Dugatkin 2001). This may be especially true of species that span a large range or exist in many different types of habitats, like Long-tailed Manakins (Dugatkin 2001; Trainer and Parsons 2001).

I studied a population of Long-tailed Manakins living in Santa Rosa National Park, which is a dramatically different habitat from the Monteverde cloud forest where they have been previously studied; this is despite the fact that they are only separated by 115 km (McDonald 2003). Monteverde is a mid-elevation cloud forest, while Santa Rosa is a near-sea-level dry forest (McDonald 2003). These two habitats differ dramatically in the composition and diversity of species and climatic characteristics (Nadkarni and Wheelwright 2000). Santa Rosa has a distinct rainy season and a dry season (Chapman 1988). Rainfall ranges between 900 and 2400mm a year, and occurs almost entirely in the rainy season between May and December (Chapman 1988). Many of the trees are drought deciduous and lose their leaves during the dry season (Chapman 1988). Throughout the year the average monthly daytime temperature within the park ranges between 21 and 34°C (Burham 1997). Comparatively, Monteverde has three seasons: a wet-misty season, a wet season and a dry season and receives an average of 2500mm of rain annually (Bohlman et al. 1995). The average monthly daytime temperature in Monteverde ranges between 16 and 19°C (Johnson et al. 2005).

The Use of New Technology for Monitoring Animals

Technological advances have served to drive forward the field of avian bioacoustics, field ornithology, and behavioural ecology (Catchpole and Slater 2008, Blumstein et al. 2011, Birkhead 2011). For example, the use of aluminum leg bands or rings has allowed us to better understand patterns of migration and identify recaptured individuals (Birkhead 2011). Similarly the invention of miniature radio-transmitters has let us record the movements of secretive species through the densest of forests (Neudorf et al. 1997, Anders et al. 1998). In terms of acoustic data, the advent of programmable digital recorders in the last decade has meant that we are now able to record vocal behaviour of birds over longer time periods than ever before. These new technologies provide us with a means of answering questions that have previously been beyond our ability to address.

New technologies are especially important in tropical research. Historically the scientific literature on birds has been biased towards temperate research, in that the majority of papers published focus on temperate species (Stutchbury and Morton 2001). This is despite the fact that the vast majority of species occur within the tropics, where species exhibit unique and unusual behaviours that are rare or absent from the temperate zone (Stuchbury and Morton 2001). Generally this has been attributed to the fact that the majority of major research institutions are located in the temperate zone, and as a result temperate species are often more easily studied (Stuchbury and Morton 2001). Because tropical study sites often lack the infrastructure or long-term study populations that are more common in the temperate zone, we need to employ new techniques and make use of new technologies to address similar questions.

My thesis research takes advantage of two new technologies to address questions related to Long-tailed Manakin behavioural ecology, lekking, and female choice. I employ both autonomous digital recorders, and an Encounternet radiotelemetry system. In Appendix I, I present the details of a field test of a new Encounternet technology, and demonstrate that this technology provides an effective way for monitoring female visitation behaviour. In chapter 2, I combine Encounternet monitoring of female movement with long-term recordings of male vocal behaviour, in order to understand how male vocalizations influence female visitation choice. In chapter 3, I use autonomous stereo recorders and call playback to experimentally study how males acoustically interact with one another.

Summary and Goals

In summary, the vocalizations of birds are complex and play an important role in both territory defence and mate attraction (Catchpole and Slater 2008). Females can use both the total output of these vocalizations or their fine structural qualities when assessing males and choosing mates (Podos 2001). Lek-based mating systems represent an ideal system for studying female choice because lekking birds lack many of the factors that confound female mate choice decisions, which may be present in territorial species (Höglund and Alatalo 1995). Long-tailed Manakins have a complex lek-based mating system, which has only been studied in detail in a single study population (McDonald 2010). This is problematic, given that previous studies have shown that female preference for male traits can vary between locations (Schluter and Price 1993). My work employs two recent technological innovations—an Encounternet radiotelemetry system for monitoring female movement, and autonomous digital recorders for long-term monitoring of male vocal behaviour—to examine the mating system and acoustic interactions of Long-tailed Manakins in a wild population.

The main goal of my thesis is to improve our understanding of the inter- and intra-sexual interactions that occur in lekking Long-tailed Manakins. In chapter 2 I examine how male vocal performance affects female mating decisions using automated recorders and a novel radio-tracking technology. In chapter 3 I turn my attention to the interactions between neighbouring males and how they divide up their acoustic environment. Here I employ a new stereo recording technique to discriminate between calling males, and use experimental playback to simulate instances of counter-calling. I include an appendix that details the first field test of the Encounternet system, which provides important support for the methods outlined in chapter 2.

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Figure 1.1. (A) A pair of definitive Long-tailed Manakin males at a display perch. The males sit close to one another while performing their coordinated *toledo* duets. When a female arrives, they will perform dance songs and elaborate leapfrog dances on this perch. (B) A female Long-tailed Manakin wearing an Encounternet radiotag. We monitor the movement of females through the population using autonomous short range radio tags and receivers.

Chapter 2: The relationship between male vocal behaviour and female visitation in lekking Long-tailed Manakins: Analyses with a novel radiotracking technology

This chapter is the outcome of joint research and is co-authored with collaborators K.-A. Ward, J. Burt, S. Doucet, and D. Mennill.

Chapter Summary

The acoustic signals of many animals play an important role in mate attraction, and the vocal behaviour of songbirds provides some of the most well-studied examples. Research on lekking birds presents a unique opportunity for studying mate choice for male acoustic ornaments because female preference for male vocal behaviour is not simultaneously influenced by male territorial behaviour. In this study we explore the vocal behaviour of male Long-tailed Manakins, a neotropical lekking suboscine songbird with a complex repertoire of calls that they use in a variety of contexts. We focused on five vocalizations thought to be especially important in female mate attraction in this species. We recorded male vocal behaviour at 38 display areas over the course of two years, quantified vocal output and the level of synchrony of male vocalizations, and compared these features to female visitation behaviour, which we measured through a novel Encounternet radiotracking system. Three male vocalizations associated with mating displays (*owngs*, dance song bouts, and *buzz-weents*) showed no relationship with female visitation behaviour. The level of synchrony of mate-attraction *toledo* duets, showed no relationship with female visitation behaviour. Output of both *toledo* duets and *teeamoo* calls showed significant relationships with female visitation, but in the opposite direction of our predictions. We discuss the potential explanations for these unexpected findings. We analyzed diel variation in male vocal behaviour and female visitation and found that both female visitation to male display areas and the output of *toledos*, *owngs*, and dance song bouts showed a peak in the morning. We compare and contrast these results to another nearby long-term study population. Our surprising results did not show a strong relationship between male mate attraction vocalizations and female visitation behaviour, but did reveal that male vocal output of some types of vocalizations and female visitation show parallel patterns of diel variation.

Introduction

Many animals use acoustic signals to communicate aggression during competition for resources, and to attract breeding partners (Bradbury and Vehrencamp 2011). In Red Deer (*Cervus elaphus*), for example, females preferentially mate with males with deeper roars, which are associated with large body size (Charlton et al. 2007). In Túngara Frogs (*Engystomops pustulosus*), females preferentially mate with males with the most complex calls (Rand and Ryan 1981). Similarly, in Greater Sac-winged Bats (*Saccopteryx bilineata*), females are attracted to males with more complex vocal repertoires (Davidson and Wilkinson 2004). Birds produce the most well-studied acoustic signals in the animal kingdom, and the widespread use of male vocalizations in mate attraction is well documented across many species (Catchpole and Slater 2008).

Female birds may use male vocal output or the quality of male vocalizations as a means of assessing males and determining which male to mate with (Catchpole and Slater 2008). In some species, male vocalizations provide information about direct benefits available to females. This has been shown to be the case in Sedge Warblers (*Acrocephalus schoenobaenus*), where males with larger repertoires of more complex vocalizations show higher levels of provisioning for their offspring (Buchanan and Catchpole 2000). In other cases, male vocalizations provide information about indirect genetic benefits available to females. For example, Spencer et al. (2005) showed that young Canaries (*Serinus canaria*) exposed to malaria parasites develop fewer, simpler songs later in life. In this way, females who mate with males with larger repertoires of complex songs could be choosing to mate with males that have genes conveying parasite resistance. Whether they provide females with a means of assessing direct or indirect benefits, acoustic ornaments have been shown to be important in mate choice (Gil and Gahr 2002).

Studies of male acoustic ornaments and female mate choice are not always straightforward. For example, under laboratory conditions female Song Sparrows (*Melospiza melodia*) solicit copulations from males with larger, more complex repertoires (Searcy 1984). However, no such relationship exists when tested under field conditions; males with larger high quality repertoires did not attract mates or re-pair after their mate was removed any sooner than males with small repertoires (Searcy 1984). However, it is known that most socially monogamous birds engage in extra-pair copulations with males other than their social mate (Griffith et al. 2002). Consequently, female Song Sparrows may select a social partner using different criteria than the males that ultimately fertilize their eggs (Searcy 1984, 1992). Some support for this idea comes from Great Reed Warblers (*Acrocephalus arundinaceus*), where females seek extra pair copulations from neighbouring males with larger repertoires (Hasselquist et al. 1996).

One way to circumvent the potentially confounding effects of social mates and extra-pair partners when studying female mate choice is to focus on species where males and females do not form social partnerships. Lekking species can serve as an ideal study system for researching female mate choice (Balmford 1991). In lekking species, males provide only gametes and do not provision the young or provide nesting females with territories (Höglund and Alatalo 1995). Males may mate with multiple females and females are free to choose between males (Höglund and Alatalo 1995). Therefore, by studying the relationship between male acoustic traits and female preferences for those traits in a lekking species, we can focus on that relationship in the absence of the potentially confounding variables imposed by social mates (Balmford 1991).

Long-tailed Manakins (*Chiroxiphia linearis*) are neotropical lekking birds with an unusual mating system (Foster 1977). Pairs of unrelated males form long-term partnerships and together produce coordinated vocal duets to attract females to their display area (McDonald and Potts

1994). Upon the arrival of a female males perform a complex leapfrog dance display on a low horizontal “display perch” while producing additional vocalizations, males may also engage in dual “butterfly” flight between the display perch and surrounding vegetation (McDonald 1989). Males may have one to a few display perches that they use regularly within their display area. If the female is receptive, the dominant (alpha) male will mate with her (McDonald 1989). While the subordinate (beta) male gains no direct benefits, it has been shown that the subordinate male receives delayed fitness benefits (McDonald and Potts 1994). By cooperating with the alpha male, subordinate males improve the reputation of the display area, allowing them to attract more females later in life (McDonald and Potts 1994). Females show site fidelity and subordinate males inherit the display area from their dominant partners, as result subordinate males achieve fitness benefits through displaying, but these benefits are offset in time (McDonald 1989, McDonald and Potts 1994).

In this species the number of females seen copulating at a given display area has been shown to be highly correlated with female visitation (McDonald 1989). As a result, we were interested in measuring levels of female visitation to display areas which we thought might serve as a proxy for male mating success, as has been done in previous studies of this species (e.g. Trainer and McDonald 1995).

Long-tailed Manakins have a large repertoire of at least 13 unique vocalizations (see Table 1; or the full description in Trainer and McDonald 1993). Among these there are five vocalizations that function primarily in mate attraction, or are associated with the dance displays that males perform for prospecting females. (1) The *teeamoo* call is a solo vocalization given by an alpha or beta male to attract his male partner, and typically precedes a series of *toledo* duets performed by the pair. (2) The *toledo* duet is the most common vocalization of male Long-tailed Manakins, and is produced by pairs of males to attract females to their display area. (3) The *owng* call is used by the dominant males to indicate the presence of a female near the display perch to their partner, or to stimulate a nearby female to move to the display perch. (4) The *nyanyownh* duet or “dance song” is given while pairs of males dance for visiting females at their horizontal display perch. (5) The *buzz-weent* call is given at the end of dance display by the dominant male to his subordinate partner, stimulating the subordinate to leave; the dominant male then begins a silent solo display that may end in copulation. Each of these vocalizations is associated with female mate attraction, or some aspect of dance displays for prospecting females (Trainer and McDonald 1995).

Previous research on manakins in the montane cloud forests of Costa Rica has revealed some relationships between male vocal behaviour and female visitation and mating behaviour. Based on observations at manakin display areas over a 2-year study, McDonald (1989) showed that the number of *toledo* duets and dances was positively correlated with female visitation and mating success. Conversely, the number of *teeamoos* males gave was negatively correlated with female visitation; this pattern was interpreted as one of the male partners being unresponsive to his partner’s attempt to initiate mate attraction *toledo* duets, resulting in fewer displays (McDonald 1989). In previous studies, females appeared to be influenced by not only the total output of vocalizations, but also the quality of those vocalizations. Trainer and McDonald (1995) showed that the level of frequency matching between the two contributions of a *toledo* duet was positively correlated with female visitation. They hypothesized that duets that were closely matched could reflect a high quality pair of duetting males (Trainer and McDonald 1995, Trainer et al. 2002). Support for this idea came from the observation that males who had been partners for longer periods of time had duets that were more closely matched in terms of frequency (Trainer et al. 2002). Consequently, females might use frequency matching as an indicator of longevity, or the ability to maintain a partnership—factors that are important in the Long-tailed Manakin mating system (Trainer and McDonald 1995, Trainer et al. 2002).

Another unusual feature of the Long-tailed Manakin mating system is that the males' output of these highly coordinated vocal and visual displays appears to remain relatively constant over the course of the day— a pattern that differs markedly from that of several other species of manakin who show pronounced morning and afternoon peaks in output (Snow 1962, Bradbury et al. 1989, Trainer and McDonald 1993, McDonald 2010). Female visitation to display areas also appears to remain relatively constant throughout the day, with visits by females roughly corresponding to male vocal output (McDonald 2010). The fact that male vocal output and female lek visitation appear to show parallel patterns of diel variation provides further support for the idea that these behaviours are linked.

In this field study of Long-tailed Manakins, we address the hypothesis that female visitation behaviour is influenced by male vocal behaviour. We use passive digital recordings to sample male vocal behaviour, and an automated radiotelemetry system to monitor female visitation behaviour. First, we assess whether the output of five types of male vocalizations correspond with female visitation to male lek sites. Second, we evaluate whether the time and frequency coordination of male-male duets corresponds with female visitation to male display areas. Third, we describe patterns of diel variation in male vocal behaviour and female visitation behaviour. For all three of these analyses, we compare the results of our study population in Costa Rica's lowland Pacific dry forests to the published results from a long-term study population of this species in Costa Rica's montane cloud forests, and evaluate whether climatic patterns may shape the similarities and differences that we see between these two study populations.

Methods

We conducted our research in Sector Santa Rosa of the Guanacaste Conservation Area in Guanacaste Province, northwest Costa Rica (10°53’ N, 85°46’ W). The Guanacaste Conservation Area is the world’s largest remaining stand of neotropical dry forest, and is home to a long-term study population of Long-tailed Manakins, which have been captured and colour banded by SMD since 2003. In total, 675 manakins have been outfitted with unique band combinations since 2003.

Our study of the relationship between male vocal behaviour and female visitation began in 2010 and continued through 2011. To address the question of how male vocal behaviour influenced female visitation we collected data following a three-fold approach: (1) we measured female visitation with a new automated radiotelemetry system called “Encounternet” (see Appendix I); (2) we quantified male vocal output using autonomous digital recorders; and (3) we analyzed duet quality using sound analysis software.

Female Visitation

We located the low horizontal branches that males regularly displayed on for females (display perches) by listening for the soft *nyanyownh* vocalization that males produce when dancing (Trainer and McDonald 1993). We confirmed that the perch was being used by adult males to display for females through visual observation. We then captured females using mist nets placed in the area around these display perches and banded the females with an aluminum leg band and a unique combination of coloured leg bands. We outfitted females with an Encounternet radiotag (J. Burt, Seattle, WA). Full details of the Encounternet system are given in Appendix I, including results of a field test that confirms that this new radiotelemetry system provides reliable data on female visitation behaviour. Briefly, over the course of the two-year study period we tagged 82 females, 70 of which we detected more than one day after release. We attached receiver stations to branches above male display perches, to monitor females as they came within range. We analyzed data from reciever stations at 22 different display areas in 2010 and 38 in 2011 where we also recorded male vocalizations (details below). We evaluated all female encounters where the female remained in range of the receiver station for at least 12 seconds (equivalent to three tag pulses). This allowed us to eliminate cases where females may have flown near the receiver station, but not remained in the area to assess males. In 2010 we detected females between April 4th and April 30th, and in 2011 we detected females between April 13th and May 16th. In this study, we focus on the display areas in the core area of our study site, the 'Bosque Humedo' section of Sector Santa Rosa, where we captured and tagged the vast majority of females.

Male Vocal Output

We deployed autonomous digital recorders in display areas near display perches (approximately 3-10m from the display perch). We used a different recording apparatus in 2010 than in 2011. In 2010 we used digital recorders as described by Hill et al. (2006), consisting of Marantz PMD-670 digital recorders connected to Sennheiser ME62/K6 omnidirectional microphones. In 2011 we used Wildlife Acoustics Song Meter SM-2 digital recorders with built-in omnidirectional microphones. Recordings were collected with 22050 kHz sampling frequency, with 16 bit accuracy in WAVE format. We deployed recorders in the early morning (0500-0700H), and allowed them to record until the battery had died or 24 h had elapsed, whichever came first.

We visualized sound using Syrinx-PC sound analysis software (J. Burt, Seattle, WA) and documented the number of *teeamoos, toledos*, *owngs*, bouts of dance songs (i.e. repeated utterances of *nyanyownh* calls with no breaks >1.0 seconds), and *buzz-weents*. We annotated over 330 hours of recording for 25 unique display areas in 2010; and over 831 hours of recording for 38 unique display areas in 2011. In 2010 we analyzed recordings collected between May 30th and June 16th, and in 2011 we analyzed recordings between April 4th and May 9th. In total we annotated more than 85,000 vocalizations in 2010, and more than 123,000 vocalizations in 2011. Our recordings varied in length in both 2010 (range= 1.2-15.8 h), and 2011 (range= 22.5-24.0 h). To control for this we converted the vocal output into rate data (number of vocalizations per hour) to facilitate comparisons.

Duet Quality

Previous research has suggested that the degree of frequency matching between the two males' contributions to the *toledo* duet may be important in female mate attraction (Trainer and McDonald 1995, Trainer et al. 2002). Meanwhile, research on the duets of other species suggests that the degree of temporal synchronization may reflect the quality of the singers (Hall 2004). To quantify the level of both frequency matching and temporal synchrony we followed a modified version of the methods outlined by Trainer and McDonald (1995). We used Syrinx-PC to visualize a single continuous recording and select 10 separate bouts of duetting with a high signal-to-noise ratio. A bout was defined as a series of at least 10 *toledo* duets separated by no more than 10 seconds. Within a single bout we used a random number generator (Microsoft Excel, 2007) to select a single duet. We restricted our sampling to duets that did not start or end a bout (i.e. the first and last duets were never selected) because we were concerned that these would not be representative of the bout as a whole. In some cases males did not perform 10 separate bouts; in these cases we selected another random duet from within one of the previously measured bouts.

Using AviSoft SASLab Pro we computed spectrograms (parameters: FFT length: 512, Frame size 100%, overlap 93.75%) and adjusted the cut-off frequencies to a high pass of 1.00 kHz and a low pass of 2.00 kHz. We used AviSoft's "magic reticule cursor" to trace the frequency of maximum amplitude of each male's duet contribution, setting the cursor's threshold to -50 dB with a snap distance of 82 Hz. We took 10 measurements at specific stereotyped points for each bird's duet contribution, or 20 measurements per duet (see Fig. 2.1 for description of these points). We took the average difference between the 10 time and frequency values and used this as our measures of temporal and frequency synchrony respectively. Larger differences between the two contributions to a duet produce a larger score—therefore, these two measures are best thought of as being measures of temporal and frequency asynchrony, where a higher score indicates less synchronous duets.

Temperature

We used the automated temperature recorders present in our Wildlife Acoustics Song Meters to log the temperature over the course of the day. We measured the temperature at 10 separate locations between May 5th and June 12th 2011. We then calculated the average hourly temperature between 0530 and 1830 at these 10 locations (Fig. 2.4H).

Statistical Analyses

Our method of using Encounternet to study female visitation behaviour produced six measures: the number of unique females that arrived on each perch and visited near each perch (≥5.0m from perch); the total number of visits made on and near each perch; and the average time spent by females on and near each perch. These variables were correlated (r^2: average ± SE: 0.57±0.06; range: 0.22-0.92) so we performed a principal components analysis to condense them into a single multivariate score. This analysis yielded a single principal component with an eigenvalue greater than 1. This principal component explained 67% of the variation in the data, and had positive loadings from all six variables (eigenvectors: average ± SE: 0.40±0.04; range: 0.21 to 0.46; See table 2.2. for loadings). We used this principal component as our measure of female visitation.

We explored the relationship between male vocal behaviour and female visitation using general linear mixed models. In our first model, the female visitation principal component score was our response variable, male vocal behaviours (rate of *teeamoos*, *toledos*, *owngs*, bouts of dance songs, and *buzz-weents*) were our fixed effects, and display area was included as a random effect to account for the fact that some display areas were sampled in both 2010 and 2011. Our output variables were non-normal, however the residuals of our whole model were normal. Our second model was the same, except that the fixed effects were our time asynchrony and frequency asynchrony measures, which were normally distributed, and produced normally distributed residuals.

To examine diel variation in male vocal behaviour we analyzed vocal output at 32 display areas where we had 24 h recordings. To facilitate comparisons with previous research in the Monteverde population (McDonald 2010), we calculated hourly output between the hours of 0530 and 1830H. We present vocal output as a proportion of total daily output (after McDonald 2010). Because some types of vocalizations were never recorded at certain display areas, our sample size varies for each of the different types of vocalizations. To assess female visitation we used two separate measures: on the perch, and near the perch (5.0 m or more from the receiver; see Appendix I). We converted these data to proportions, where we divided the number of visits made to a single perch during one hour by the total number of visits made over the course of the day. This was done to facilitate comparisons to previously published results by McDonald (2010).

Results

Contrary to our predictions based on previous research, we found no strong positive relationships between the mate attraction vocalizations of male Long-tailed Manakins and female visits made to the male display areas. Mixed model analysis of the output of five manakin vocalizations involved in mating behaviour revealed that output of *buzz-weents* ($F_{1, 40}$= 0.1, *n*= 56, *p*=0.7)*, owngs* ($F_{1, 48}$= 0.6, *p*= 0.4)*,* and dance song bouts ($F_{1, 44}$= 0.04, *n*= 56, *p*=0.8) were not correlated with female visitation (Fig. 2.2). The remaining two types of vocalizations, *teeamoos* and *toledos*, were both correlated with female visitation. *Toledo* duets which are understood to attract females to display areas, were negatively correlated with female visitation ($F_{1, 48}$= 4.1, *n*=56, *p*=0.05); and *teeamoos*, vocalizations which are understood to serve in partner attraction, before the initiation of bouts of *toledos*, were positively correlated with female visitation ($F_{1, 48}$= 4.8, *n*=56, *p*=0.03).

In further contrast to our expectation, time and frequency synchronization within male *toledo* duets showed no relationship with female visitation behaviour. Mixed model analysis showed that neither frequency asynchrony ($F_{1, 39}$= 2.8, *n*=49, *p*=0.10), nor temporal asynchrony ($F_{1, 43}$= 0.6, *n*=49, *p*=0.43) were correlated with female visitation (Fig. 2.3).

Analysis of diel variation in vocal output showed pronounced differences with time of day. *Teeamoos* showed a relatively consistent output, with a very subtle increase in the early morning (0630 to 0830 h) reaching its highest levels in the early afternoon (1330 to 1430 h; Fig 2.4A). *Toledos* showed a strong peak in the morning (0630 to 0830 h) and then declined to lower levels, increasing during a second peak in the mid-afternoon (1430 to 1530 h; Fig. 2.4B). *Owngs* showed a strong peak in the morning (0630 and 0830 h) and then declined before later rising to a second, smaller peak in the afternoon (1330 to 1530 h; Fig. 2.4C). Dance song bouts had a dramatic peak from 0630 to 0830, and then occurred at low levels at all other hours of the day (Fig. 2.4D). *Buzz-weents* showed a similar trend to dance song bouts, but with less abrupt changes. *Buzz-weents* peaked between 0730 to 0830, before gradually declining and remaining relatively constant throughout the day (Fig. 2.4E).

Female visitation to display perches showed substantial diel variation, with the highest levels in the morning (0630 to 0930 h) decreasing to lower levels at 0930 h and slowly declining as the day progressed (Fig. 2.4F). Female visitation to the area near the display perch (>5.0m) showed a similar trend that was slightly less demarcated (Fig. 2.4G).

Of the five male vocalizations that we assessed, the diel pattern for male dance songs and *buzz-weents* showed the most similar trend to female visitations; all three showed a single peak in the morning before declining to a lower level throughout the remainder of the day.

Average temperature varied over the course of the day, ranging from 23°C in the early morning to an average high of 28°C in the late afternoon (1330 h), and dropping off to approximately 26°C in the early evening (Fig. 2.4H).

Discussion

Our analyses of digital recordings of male vocalizations and passive telemetry data of female visitation to male display areas shows that the vocalizations of male Long-tailed Manakins have little influence on female visitation behaviour. The *toledo* duets of manakins, which are understood to be important in attracting females to male display areas (McDonald 1989), showed a weak but surprising negative correlation with female visitation. Furthermore, *teeamoo* calls, which are understood to be produced by males to attract their male partner before commencing a bout of *toledo* duets, showed a weak but positive correlation with female visitation. We found no relationship between the output of male *owng* calls (a vocalization used when stimulating nearby females to move to the display perch), male dance song bouts (the vocalizations produced while males perform elaborate dances for females), or male *buzz-weent* calls (a vocalization given at the end of the dance display which signals a late stage in the courtship process), and female visitation behaviour. Furthermore, we found no effect of duet quality on female visitation, either in terms of frequency or temporal asynchrony. We quantified diel variation in male vocal behaviour and female visitation behaviour, and found that almost all measured activities showed high levels in the morning. Female visitation to display areas and the output of male dance song bouts and *buzz-weent* calls followed the most similar patterns of variation. We explore each of these results below, and contrast our findings to those of previous studies.

Vocal output

We had anticipated that the vocalizations associated with dance displays (*owngs,* dance song bouts, and *buzz-weents*) would be positively correlated with female visitation given that dances are typically performed in the presence of a female (McDonald 1989). However, we found no such correlations. We found that the output of *toledo* duets was negatively correlated with female visitation in Long-tailed Manakins living in the lowland dry forests of Costa Rica. This result is counter-intuitive given that multiple previous studies suggest that *toledo* duets are used in mate attraction (McDonald 1989, Trainer and McDonald 1993, McDonald 2010). We also found that the output of *teeamoo* calls was positively correlated with female visitation. *Teeamoo* calls are used by the alpha or beta male to attract their partner and usually preceded a bout of *toledo* duets, and previous findings have suggested that a greater output of *teeamoo* calls may reflect a poorly coordinated pair of males (McDonald 1989). As such, it is surprising that *teaamoo* output should be positively correlated with female visitation. Overall our analysis of male vocal output has produced results that contradict our expectations based on previous findings.

There are multiple possible explanations for our unexpected findings. First, it is possible that females carrying Encounternet tags behaved differently from non-tagged females and this gave rise to our unusual findings. This seems unlikely. Our radiotags weighed less than 5% of female body mass (see Appendix I), as has been suggested as an appropriate weight limit for radiotags (Naef-Daenzer et al. 2001). There is little evidence in the literature that appropriately weighted radiotags (<5% of the bird's body mass) affect avian behaviour. For example, radiotagged female Hooded Warblers (*Wilsonia citrina*) did not differ from untagged females in their provisioning rate, or time spent brooding (Neudorf and Pitcher 1997). Similarly, radiotagged Dickcissels (*Spiza americana*) exhibited a brief stress response to being tagged, but their stress level returned to baseline within the first 48 hours after being tagged (Wells et al. 2003). Moreover, radiotagged tits (*Parus* spp.) showed patterns of survivorship that did not significantly differ from untagged birds (Naef-Daenzer et al. 2001). While some studies have found reduced levels of survivorship in radiotagged individuals (e.g. Petty et al. 2004), or reduced locomotory abilities in radiotagged individuals (Logue 2007), the suggestion that tagging would so dramatically alter a female's behaviour, to the point where her behaviour was opposite to that of an untagged female, seems unlikely. Indeed, our analyses of tagged birds showed that females visited many perches after being tagged, often great distances apart (Ward et al. *manuscript in prep*.), and although our sample size is small, we found evidence of tagged females being present in the study population a year after being tagged (Appendix I).

A second possibility for the lack of correspondence between male vocalizations and female visitation is that females in our study population are selecting males on the basis of non-acoustic traits. One trait of importance may be plumage ornaments. Long-tailed Manakins have elaborate plumage ornaments including long tail feathers, carotenoid-coloured crown patches, structurally-coloured blue mantle patches, bright orange legs, and melanin-coloured jet-black body plumage (Slud 1957, Doucet et al. 2007), and plumage has been shown to be of central importance to mating success in other species of manakin (Stein and Uy 2005). Another possible trait is the visual performance of male dance displays. The elaborate leap-frog dances and butterfly displays that precede copulation are performed directly in front of prospecting females (Slud 1957, Foster 1977, McDonald 1989) and may be more important than the acoustic traits we measured here. Yet another trait of importance may be display area centrality. In other species (e.g. Hovi et al. 1994, Stein and Uy 2005) males with display positions located centrally had higher reproductive success. Ongoing research of the plumage, dance displays, and lek placement of manakins in our study population will help to establish whether these traits may show a stronger correspondence with female visitation behaviour.

A third possibility for the lack of a relationship between male vocalizations and female visitation is that female behaviour may drive changes in male vocal output in unexpected ways. Under this explanation, males may adjust their calling behaviour in response to the number of females that visit their display area, and female visitation could either be random or controlled by some other unmeasured cue. Males that perform more *teeamoo* calls might not cause more females to visit by virtue of the vocalization itself; instead, a larger number of females visiting a display perch may incite higher output of *teeamoo* calls from a resident male as he attempts to attract his male partner to display for the visiting females. Similarly, higher levels of male *toledo* duets could be the result of lower levels of female visitation, where males that receive few female visits spend more time producing this mate-attracting call. In many species of songbird, male song rate is highest before males attract a breeding partner (Catchcpole and Slater 2008). For example, in territorial Rufous-and-white Wrens (*Thryothorus rufalbus*) males produce more songs when unpaired compared to when those same birds pair with a partner (Hennin et al. 2009). The idea that female mating behaviour can influence male vocal behaviour is well supported. Female Brown-headed Cowbirds (*Molothrus ater*) have been shown to alter the vocal behaviour of males through their use of non-vocal cues which indicate preferences for specific song features (King et al. 2005). Similar patterns have been noted in lekking birds. Male Satin Bowerbirds (*Ptilonorhynchus violaceus*) adjust their display intensity in response to female receptivity, and actually decrease the intensity of their displays in response to certain female behaviours (Patricelli et al. 2006). In this way, the presence of females and their behaviour could drive male vocal behaviour and alter male display rates consistent with the patterns we found for *teeamoo* and *toledo* vocalizations.

A fourth possibility for our surprising results is that our sample size prevented us from detecting an effect (and, for two vocalizations, resulted in a spurious effect in the opposite direction to our prediction). Based on a comparison with the sample size of prior studies, we consider this possibility unlikely. Our sample size was larger than any previous study, in terms of the number of display areas studied and the number of hours over which we detected females, and our recording of vocal behaviour was comparable to previous studies. Therefore, it seems more likely that any spurious conclusions would be the product of small sample sizes from previous studies. A previous study reporting a relationship between male vocalizations and female visitation (McDonald 1989) relied on data from six display areas, whereas we monitored 38 display areas. This previous study (McDonald 1989) monitored female visits to display areas over 1,396 hours, whereas we monitored visitation of tagged females for over 40,000 hours of receivers being present at display areas. This previous study (McDonald 1989) recorded male vocalizations during 1,396 total hours at six different display areas (an average of 232 hours per display areas), whereas we recorded male vocalizations during 1,161 display areas hours at 38 different display areas (an average of 30.5 hours per display areas). Only for this last comparison is our dataset smaller than prior studies, suggesting that future research should collect more hours of recordings per display areas to confirm the patterns we have documented here with similar rigor to prior studies.

Duet quality

We detected no significant relationship between the level of duet synchrony – measured in terms of both frequency matching and temporal synchrony – and the level of female visitation. Previous researchers have suggested that the level of frequency matching or temporal synchrony within a vocal duet could indicate the duetting pair's quality, and serve an important function in mate assessment (Trainer and McDonald 1995, Trainer et al. 2002, Hall 2004). Indeed, Trainer and McDonald (1995) showed that Long-tailed Manakin duets that were highly coordinated in terms of frequency-matching were associated with higher levels of female visitation. The possibility that our null result was the product of insufficient sampling seems unlikely, given that the previous study of Long-tailed Manakins that found a significant result relied on a sample size of seven display areas, while our study focused on 38 display areas (Trainer and McDonald 1995). We also analyzed female visitation for a longer period of time. While Trainer and McDonald (1995) monitored display areas for 18 to 38 hours, using Encounternet we were able to monitor visits by tagged females constantly throughout an extended breeding period (average total detection time per display perch 990 ± 49 hours). It is worth noting that although we found no statistically significant relationship, our result was in the same direction as Trainer and McDonald's (1995), where females were more likely to visit males that were more closely frequency matched. Trainer and McDonald's (1995) bioacoustic methods involved measuring printouts of spectrograms, whereas our methods involved more rigorous automated measurement using the most up-to-date bioacoustic techniques. As such, it is conceivable that the results of Trainer and McDonald (1995) were driven by a simpler acoustic measurement approach.

It has been suggested that the frequency matching within a Long-tailed Manakin duet may serve as a proxy by which females can assess the duration of a partnership, or the longevity of the males (Trainer and McDonald 1995, Trainer et al. 2002). It is also thought that frequency matching may serve as an indicator of a pair's quality since there is a theoretical maximum (i.e. perfectly synchronized male-male duet contributions), in a manner analogous to that of fluctuating asymmetry (i.e. perfect symmetry; Trainer and McDonald 1995). It is possible that although females have been shown to prefer males with highly frequency-matched duets in another population (Trainer and McDonald 1995), females do not attend to frequency matching in our study population. Such population differences in female preference have been shown in other systems, for example where the effect of fluctuating asymmetry, and female preference for male traits, differs between populations (Møller 1995). This could result from differences in environmental and developmental stressors between populations (Møller 1995).

Population Differences

Why should two studies of Long-tailed Manakins exhibit such different patterns? In our lowland population female visitation did not appear to be influenced by vocal output or vocal quality in the same way as the highland population. Females also exhibited different patterns of diel variation in visitation over the course of the day in the two populations. As discussed above, differences in experimental design and statistical analyses are an unlikely explanation given that our sample sizes are large. Instead, differences between these two populations could be driven by climatic differences between the two populations, or may result from differences in female preferences in the two populations.

The climate of our lowland dry forest study site in Santa Rosa is dramatically different from that in the highland cloud forests in Monteverde (Nadkarni and Wheelwright 2000). Santa Rosa is much hotter (average monthly daytime temperature: 21-34 ˚C) than Monteverde (16-19 ˚C), and has different seasonal patterns of rainfall (Chapman 1988, Bohlman et al. 1995, Burham 1997, Johnson et al. 2005). These differences in seasonality could impact male display and female nesting behaviour. Tropical birds have longer, more variable breeding seasons when compared to their temperate counterparts (Stuchbury and Morton 2001). This is thought to be due in part to a lack of external cues signalling the beginning or end of the breeding season, since day length is relatively constant and climatic changes are less dramatic in the tropics (Stuchbury and Morton 2001). In this sense the more dramatic change between the rainy and dry season observed in Santa Rosa may serve as a more immediate cue compared to the more subtle change between the dry, wet-misty, and wet seasons of Monteverde (Bohlman et al. 1995, Topp and Mennill 2008). This may in turn alter female search strategies and preferences, or male display behaviour, in ways that have yet to be understood.

It is well known that temperature can strongly influence metabolic demand, and it seems likely that males and females should adjust their energetically demanding activities (e.g. performance of the dance display or mate searching) to avoid times of the day when thermoregulation might be more challenging (Kendeigh 1969). This could explain the different patterns of diel variation in female visitation behaviour and male vocal behaviour between Santa

Rosa and Monteverde. The cooler temperatures of Monteverde may be more conducive to the production of energetically demanding dance displays by males, and mate searching by females, throughout the day. This may allow males and females to maintain relatively constant levels of vocal output and mate searching throughout the course of the day (McDonald 2010). Conversely, the high afternoon temperatures seen in Santa Rosa may severely limit animal activity, both in terms of male display behaviour and female mate searching behaviour. Evidence supporting this idea comes from the fact that both female visitation to display areas and the number of dance displays males perform appears to be lowest during the hottest point in the day (Fig. 2.4).

An alternative explanation, that differences between populations could be the result of differences in innate female preferences, must also be considered. Many studies of female preferences for male traits focus on only a single population (Dugatkin 2001). This can result in spurious conclusions when those results are applied to a species as a whole, or even to other populations. This is because different populations can experience dramatically different environmental conditions and evolutionary forces, which can interact with one another resulting in unique female preferences between populations (Møller 1995, Dugatkin 2001, Dunn et al. 2008). It is not uncommon for patterns observed in one population to be different in other populations. For example, Dunn et al. 2008 showed that Common Yellowthroat (*Geothlypis trichas*) females in their Wisconsin study site preferred males with larger black masks, while females in their New York population did not assess males on the basis of their yellow masks and instead preferred males with larger yellow bibs. This is also predicted on the basis of theoretical models, where populations that experience separation are likely to experience divergence in selection for sexually selected traits and female preferences for those traits (Schluter and Price 1993). That is, provided that the benefits females receive from selecting different traits are equivalent (Schluter and Price 1993). The differences between our lowland population and the previously-studied montane population may provide an additional example of this pattern.

Significance and Future Work

This work represents the first field test of a novel radiotracking system: Encounternet. This system is capable of monitoring many animals over long time periods in a challenging environment where previous researchers have had to invest many years to achieve similar results. The behaviour of tropical birds is grossly understudied (Stuchbury and Morton 2001), and our study confirms that Encounternet can provide data on the movement behaviour of tropical birds. Our findings also suggest that more research is required to understand the targets of female choice in lekking tropical animals. Whereas previous studies have found an effect of male vocalizations on female visitation behaviour, our work suggests that other factors must also influence female visitation behaviour, whether they may be plumage ornaments, visual displays, or display area centrality. Finally our research highlights the importance of studying multiple populations, as dramatically different patterns of female preference and male display can often exist between them (Schluter and Price 1993, Dugatkin 2001, Dunn et al. 2008). Future work on our lowland dry forest study population should focus on analyzing additional male ornaments and patterns of female mate choice. Information on mating success, as measured genetically, may provide more important insights than visitation alone (though these measures have been previously shown to be highly correlated; McDonald 1989, Trainer and McDonald 1995, McDonald 2010).

In conclusion, our research on male vocal behaviour and female visitation in Long-tailed Manakins suggests that male vocalizations play less of a role in shaping patterns of female visitation than expected. Our findings differ from those of previous studies, and this may be due in large part to the dramatic climatic differences between these populations, or result from differences in preferences of prospecting females in different populations. Overall our work sheds light on the unusual mating system of a little studied tropical bird, provides a strong test of a novel radiotracking technology, and highlights important differences between populations.

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Table 2.1 The loadings of the variables used to assess female visitation on the principal component. This principal component explained 67% of the variation in the data, and was used as the measure of female visitation in the analyses that follow.

Variable	Loading on PC1
Number of visits on perch	0.46
Number of visits near perch	0.43
Average visit length on perch	0.38
Average visit length near perch	0.21
Number of unique females on perch	0.46
Number of unique females near perch	0.44

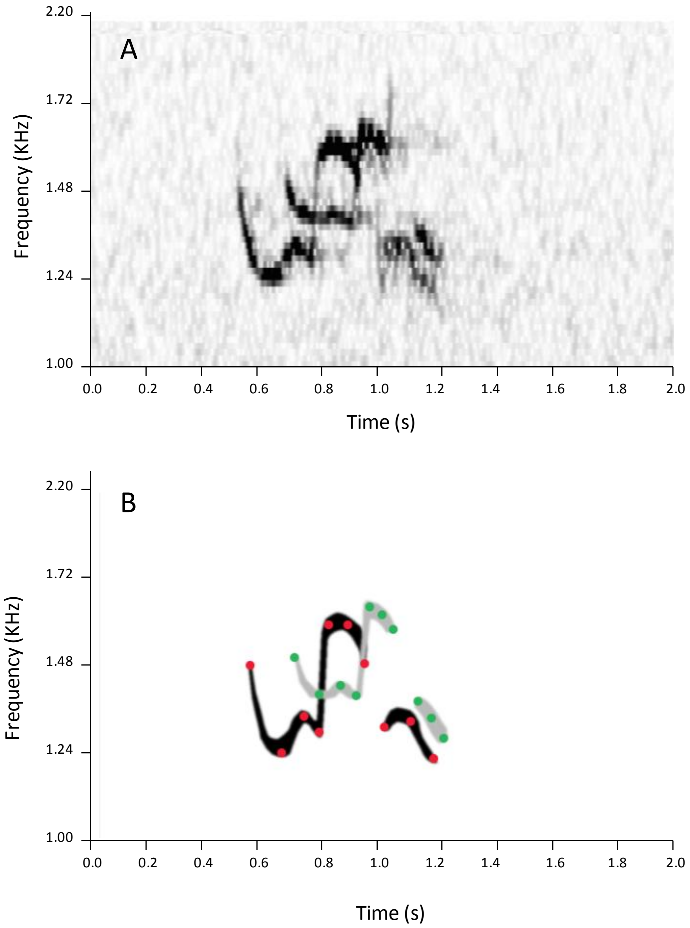

Figure 2.1. A typical *toledo* duet produced by two male Long-tailed Manakins, showing the points that were measured in our analysis of duet structure. The individual male's contributions are highlighted in different colours (grey and black). For each individual's contribution to the duet we measured the exact time and frequency at 10 stereotyped points. These points (from left to right and highlighted in red for one male and green for the other male) were: (1) the start of an individual's *toledo*, (2) the nadir of the first dip following the start of the duet, (3) the apex of the first rise, (4) the nadir of the second dip following the first, (5) the point at the apex of the major rise, (6) halfway between the point at the apex of the rise and the end of the first element of the *toledo*, (7) the end of the first element of the *toledo*, (8) the beginning of the second element of the *toledo*, (9) halfway between the beginning and end of the second element of the *toledo*, (10) the end of the second element of the *toledo*. Phonetically, within the *toledo* call these measures correspond to: *to* (1-4), *le* (5-7), and *do* (8-10).

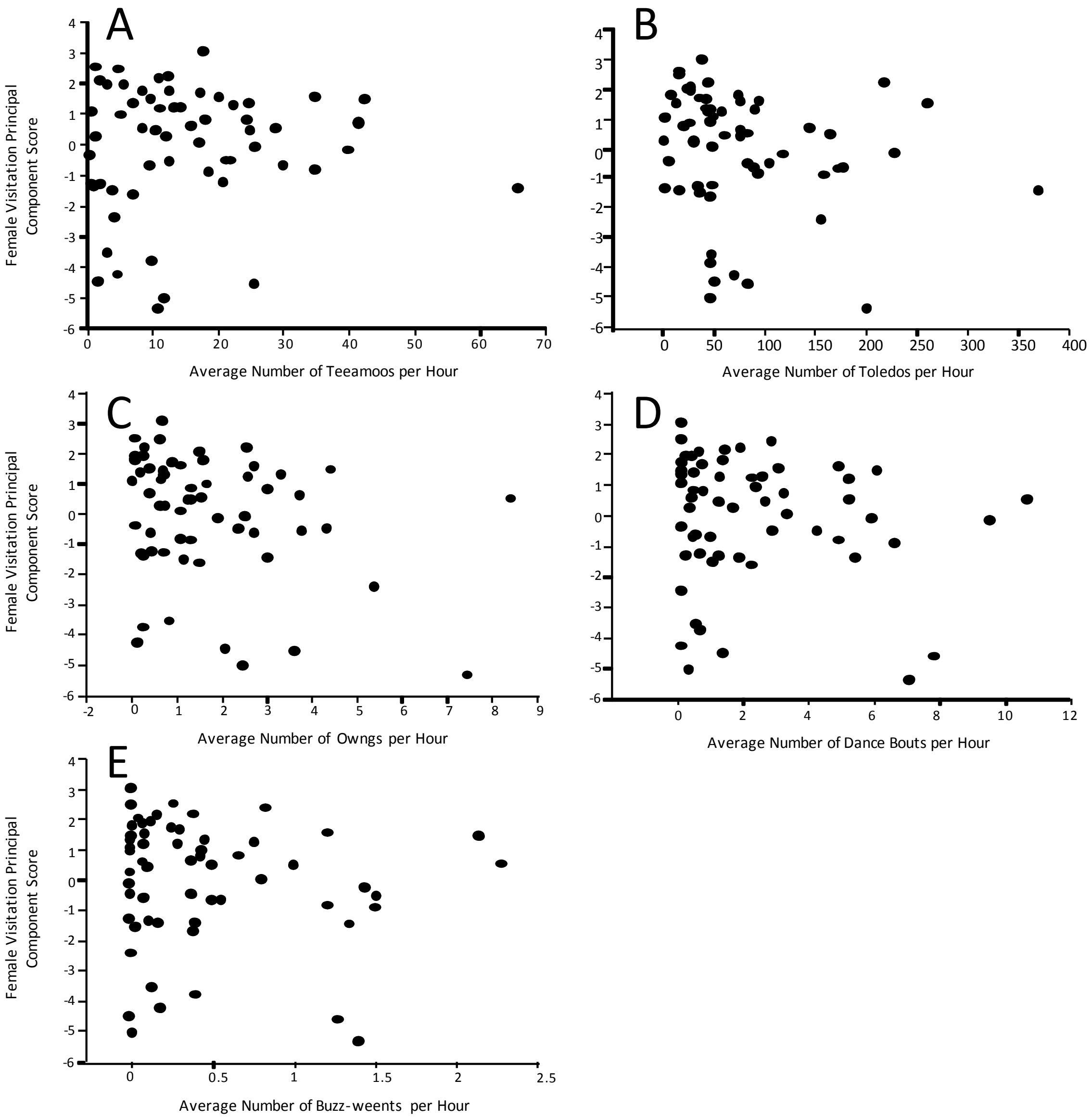

Figure 2.2. The relationship between male vocal output and female visitation at display areas of Long-tailed Manakins in Costa Rica. (A) The production of *teeamoo* calls was positively correlated with female visitation; this call is thought to be used by a male to recruit his partner to begin a bout of *toledo* duets. (B) Conversely, the production of *toledo* duets was negatively correlated with female visitation; this call is thought to be used to attract females to male display areas. (C) The production of *owng* calls was not correlated with female visitation; this call is thought to be used to signal the presence of a female or stimulate a female to move to the display perch. (D) The number of bouts of dancing that males engaged in was not correlated with female visitation. (E) The production of the *buzz-weent* calls were not correlated with female visitation; this call is used by the alpha male to signal dominance and the beginning of a period of solo display. The figures are standard bivariate plots of output versus female visitation, while the statistics discussed in the body of the paper relate to the output of a mixed model.

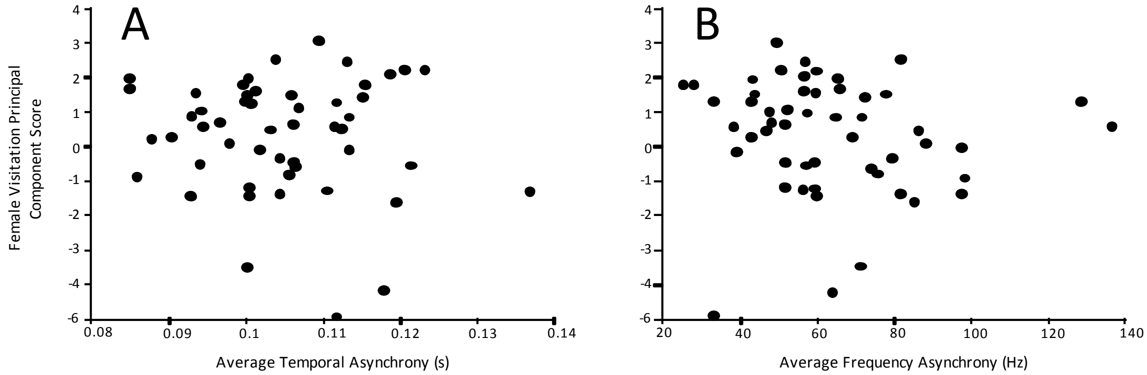

Figure 2.3. The relationship between male duet structure and female visitation at the display areas of Long-tailed Manakins in Costa Rica. The degree to which the two contributions to a toledo duet were mismatched (i.e. the level of asynchrony) did not correlate with female visitation. This was true when synchrony was measured in terms of frequency matching (A; the average absolute frequency difference between 10 stereotyped points within each duet contribution), and temporal synchrony (B; the average absolute time difference between the same 10 stereotyped points within each duet contribution). The figures are standard bivariate plots of asynchrony versus female visitation, while the statistics discussed in the body of the paper relate to the output of a mixed model.

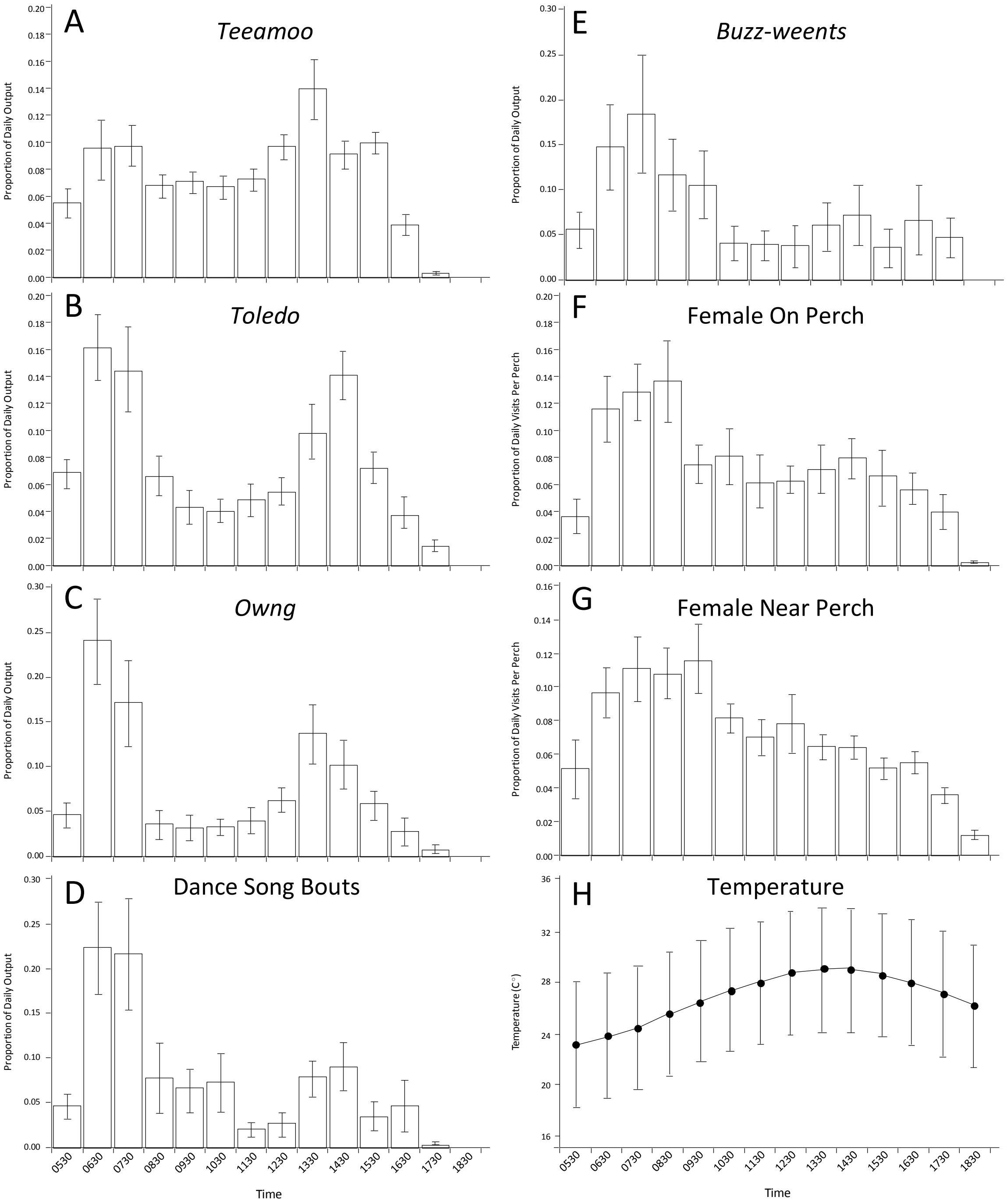

Figure 2.4. Diel variation in male vocal behaviour, and female visitation behaviour of Long-tailed Manakins, and ambient temperature. Patterns of diel variation in 5 vocalizations: (A) *teeamoo,* (B) *toledos,* (C) *owngs*, (D) dance songs, and (E) *buzz-weents*. The pattern of diel variation in female visits to (F) male display areas and (G) the area near male display areas (≥5.0 m from male display perches). (H) Temperature also varied over the course of the day.

Chapter 3: Counter-calling behaviour in lekking Long-tailed Manakins: Duetting males avoid overlapping the duets of neighbours, but not simulated intruders

This chapter is the outcome of joint research and is co-authored with collaborators K.-A. Ward, S. Doucet, and D. Mennill.

Chapter Summary

Animals that live in communication range of multiple conspecific receivers have the potential to interfere with neighbouring individuals' signals, or avoid interference by signalling at different times. We used both an observational and experimental approach to study signal timing in lekking tropical songbirds. We recorded duetting male-male pairs of Long-tailed Manakins (*Chiroxiphia linearis*) during periods when two neighbouring pairs were calling under natural conditions, and during interactions between birds and a playback-simulated pair of intruding males. We use three complimentary analytical techniques to evaluate whether birds varried the timing of their duet calls relative to nearby animals: circular statistics, resampling analysis, and duty cycle models. Our analyses reveal that Long-tailed Manakins produce duets with non-random timing during counter-calling interactions. During natural interactions, all three analytical techniques reveal that manakins time their duets to avoid overlapping nearby males' duets. In response to playback, males showed more variable strategies. Males overlapped playback duets more than expected by chance, but often vocalized in the silent interval as well. During playback males increased their inter-call interval above pre-playback levels, and returned to intermediate levels following the end of playback. Our study shows that males alter the timing of their calls in response to the vocalizations of others around them, and uncovers similarities in the complex acoustic signalling behaviour of lekking birds relative to the better-studied signalling behaviour of territorial birds.

Introduction

When animals live within communication range of multiple receivers, social factors can have a strong influence on signalling behaviour. Most animals produce long-range communication signals, especially in mating and territory defence contexts (Bradbury and Vehrencamp 2011). These signals can transmit long distances, beyond the average spacing between individuals, thus creating many opportunities for interference (McGregor 2005). Animals can vary the timing of signal production either to overlap or avoid overlapping other signallers in order to increase or decrease interference, respectively (Greenfield 1994a; Greenfield 1994b; Naguib and Mennill 2010). The strategies animals use to minimize interference varies with species (Greenfield 1994a), signal modality (Carlson and Copeland 1985; Johnston 1997), and context (Greenfield 1994b), and can have important fitness consequences for the individuals involved (Greenfield 1994a; Greenfield 1994b; Mennill et al. 2002; Mizyzaki and Waas 2002; Amy et al. 2008; Garcia Fernandez et al. 2010).

There are at least six main reasons why animals might overlap each others' signals (reviewed in Greenfield 1994b; Todt and Naguib 2000; Naguib and Mennill 2010). (1) Overlapping may be an agonistic signal that is an important part of counter-signalling exchanges (e.g. in counter-singing exchanges in songbirds, overlap can be associated with threat and contest escalation; Naguib and Mennill 2010; but see Searcy and Beecher 2009, 2011 for an alternate perspective). (2) Overlapping may occur when animals are tightly spaced, and must either produce overlapping signals or else decrease their signal output (e.g. in a chorus of toads or a cluster of fireflies; Greenfield 1994b). (3) Overlapping signals may make it more difficult for predators to detect a single individual, so that producing an overlapping signal is less risky than an alternating signal (e.g. hylid frogs are more susceptible to predation by bats when they produce alternating calls; Tuttle and Ryan 1982). (4) Females may respond to males' mating signals with signals of their own, and males may maximize their opportunity to detect female responses by producing synchronized calls, thereby preserving a silent period in which female signals can be detected. This has been demonstrated in the overlapping signals of fireflies, where males and females exchange bioluminescent flashes with one another; Buck and Case 1986. (5) Females might assess males on their ability to synchronize their signals with those of other males, selecting for the evolution of overlapping calls (e.g. females select for synchronized, overlapping duet calls in some neotropical manakins; Trainer and McDonald 1995). (6) Alternatively, overlap may occur by chance if individuals produce signals with no reference to the timing of signals of nearby animals (Searcy and Beecher 2009; Searcy and Beecher 2011).

There are at least four main reasons why animals might avoid overlap by producing signals in the intervals between other animals' signals. (1) Alternating may communicate decreased threat or a de-escalation of aggressive contests (Naguib and Mennill 2010). (2) Alternating may allow individuals to broadcast their signal with minimal interference by animals around them (Greenfield 1994b). (3) For some signals, such as acoustic signals, animals may not be able to produce a signal and detect the signals of others simultaneously, and mutual listening may therefore favour signal alternation (Hultsch and Todt 1982). (4) Females may select for a higher duty cycle of signals between different areas of grouped males as an evolutionary by-product of sexual selection for high duty cycle from a single individual. Since groups that alternate at a fixed rate will have higher duty cycle than more synchronous groups, selection would favour individuals that alternate (Greenfield 1994b).

The acoustic signals of birds have been well studied from the perspective of signal timing. More than thirty observational and experimental studies have documented overlapping behaviour in many species of birds (reviewed in Naguib and Mennill 2010). While the signal function of overlapping is contentious, and overlapping may occur by chance alone (Searcy and Beecher 2009), the fact that overlapping is associated with specific behaviours suggests that participants adjust their response based on the outcome of overlapping exchanges and use these exchanges to inform their subsequent behaviour (Naguib and Mennill 2010). Previously documented effects of overlapping include: (1) overlapped birds sometimes change their signalling behaviour, for example, by producing truncated signals or changing their signal rhythm (e.g. Dabelsteen et al. 1996; Naguib 1999; though see Searcy and Beecher 2011 who argue that this could be a by-product of jamming avoidance); (2) overlapped birds sometimes exhibit aggressive responses (e.g. Naguib and Todt 1997; Mennill and Ratcliffe 2004); and (3) the breeding partner of an overlapped bird sometimes changes their reproductive behaviour (e.g. Otter et al. 1999; Mennill et al. 2002). Overlapping appears to have wide-ranging effects on the behaviour of wild birds, and understanding whether it is a directed signal is an important area for study.

This study on acoustic overlapping focuses on Long-tailed Manakins (*Chiroxiphia linearis*), neotropical suboscine songbirds with a lek-based mating system (Foster 1977). Males exhibit delayed plumage maturation, going through a graded series of plumages before attaining definitive plumage in their fifth year (Doucet et al. 2007). Definitive adult males form long-term obligate male-male partnerships and attract females to their display area and entice them to mate using a combination of coordinated male-male vocal duets and visual displays (McDonald 1989a, Trainer and McDonald 1995). In other animals, vocal duets occur between the male and female of a mated pair, and are understood to function in acoustic contact, territory defence, and mate guarding among other functions (reviewed in Hall 2004; Douglas and Mennill 2010). Long-tailed Manakins are unusual in that their highly coordinated duets are performed by pairs of unrelated males (McDonald and Potts 1994), a trait they share in common with several congeners (Prum 1994; DuVal 2007). Long-tailed Manakin duets consist of the two males producing nearly identical phrases, onomatopoeically similar to the word "*toledo*", where each phrase is approximately 0.6 seconds in length and the two males' contributions are synchronized within 0.1 seconds of one another (Fig. 3.1a). These are repeated every 2.5 to 4.0 seconds for extended periods of time, and males may produce as many as 1,000 duets per hour (Trainer and McDonald 1995). Male-male pairs call from fixed display areas that remain consistent over many consecutive years (Foster 1977; McDonald 2010). Pairs of males from adjacent display areas are visually isolated from one another by distances of approximately 75 m, but can be as close as 25 m (Trainer and McDonald 1993; pers. obs.). Their vocal signals are long-ranging, transmitting upwards of 100 m (pers. obs.), and as a result, neighbouring pairs are routinely in acoustic contact. Consequently, pairs of Long-tailed Manakins attract females in an acoustically competitive environment and this sets the stage for acoustic interference to influence the signalling behaviour of neighbouring pairs of males.

In this study we address the question: do pairs of duetting male manakins call non-randomly? Do they actively avoid signalling at the same time as other males, or do they interfere with each other's vocalizations by overlapping them? We used both an observational approach and a playback experiment to answer this question during a two-year study of a population of Long-tailed Manakins in Costa Rica. We used stereo digital recorders to monitor the timing of duets during naturally-occurring counter-calling bouts between neighbouring male-male pairs of manakins. We then conducted a playback study where we simulated a pair of males by playing duets near established pairs' display areas. For both the natural counter-calling exchanges and the playback-induced counter-calling exchanges, we compared the level of overlapping calls to levels expected by chance. No single compelling null model for predicting chance levels of overlap has been established (Searcy and Beecher 2009; Naguib and Mennill 2010; Searcy and Beecher 2011). As such, we analyzed the natural and experimental recordings using three complimentary null models to determine whether overlapping differed from levels expected based on chance.

Methods

Study Site

We studied Long-tailed Manakins in Sector Santa Rosa within the Guanacaste Conservation Area in Guanacaste Province of northwestern Costa Rica (10°53’ N, 85°46’ W). This conservation area houses the world’s largest remaining stand of neotropical dry forest and has been designated as a World Heritage Site by the United Nations Educational, Scientific and Cultural Organization (UNESCO). Sector Santa Rosa is home to a large population of Long-tailed Manakins that have been studied in detail since 2003 (Doucet et al. 2007).

We located manakin display perches by listening for pairs of males performing vocal duets and then locating the low horizontal branches, where males perform elaborate dances for prospecting females (McDonald 1989a). A single pair of males may display on one to a few display perches in a small area (McDonald 1989a; Prum 1994). We captured birds using mist nets placed around the display areas and outfitted captured birds with an aluminum leg band and a unique combination of coloured leg bands to aid in identification. Not all birds in the study population were banded, yet observations of the colour-banded males demonstrate that the same birds routinely call from the same display areas. Based on this observation, as well as the background information on Long-tailed Manakin behaviour collected over two decades of field study by D. McDonald (reviewed in McDonald 2010), we are confident that the male-male pairs observed at each display area were unique.

Natural Counter-Calling Exchanges

We recorded natural instances of counter-calling between neighbouring pairs of males between 14 April and 24 May 2010. We used a stereo recording apparatus positioned between two adjacent display areas. We deployed recorders between 0500 and 0700 in the morning and allowed them to run continuously, usually finishing between 1200 and 1400. Given that sound propagates slowly through air, we could exploit time-of-arrival differences between the two microphones to assign calls to the pairs of males on either side of the recording apparatus. Our recording system consisted of two omnidirectional microphones (Sennheiser ME62/K6) connected to a solid state digital recorder (Marantz PMD670; 22050 KHz, 16 bits, WAV format, stereo recording). We placed one of the microphones on the periphery of a pair's display area, approximately 10m from the primary display perch, and the other microphone 15m away, in the direction of the nearest neighbouring display area. We attached both microphones to trees and suspended them approximately 2m above the ground. This recording system is an extension of the system described in Hill et al. (2006), except that our recordings were collected in stereo, rather than monaural. At each recording location, we remained in the area immediately following setup of the recording apparatus. For approximately one hour we made notes on the locations of calling males, allowing us to ground-truth that the stereo recording apparatus could provide reliable information on which pair of birds was calling. We found 100% agreement between our observations in the field (i.e. which pair of males produced which vocalization) and the direction that we determined based on differences in arrival times in the stereo recordings.

Consequently, we are confident that this apparatus allowed us to reliably assign duets to the correct pair of males during a counter-calling bout.

We used Syrinx-PC Sound Analysis Software (J. Burt, Seattle, WA) to annotate the stereo recordings of natural bouts of counter-calling between adjacent pairs of males. We restricted our analyses to instances where the two pairs of males were calling in the absence of any other more distant males. Greenfield (1994a) has suggested that insects and frogs space their calls relative to their nearest/loudest neighbours, but by restricting our analyses to instances where only two pairs of males were calling concurrently we avoid the potentially confounding effect of other nearby animals. To ensure that our measurements were taken from bouts of counter-calling, and not one-off calls from either pair of males, we further restricted analyses to instances where both pairs contributed at least 10 duets, where the duets were at most 10 seconds apart. We recorded at 27 different locations, but had to discard 14 because there was no suitable period where the neighbouring pairs were calling in the absence of other callers. Bouts of counter-calling are common in this species.

Our detailed analyses are based on 13 instances of natural counter-calling between neighbouring pairs of males. Each counter-calling dyad was unique, but six of the 26 pairs were involved in more than one comparison. We consider each dyad to be the relevant unit of sampling because counter-calling is a dynamic process where the response of one pair of individuals is not independent from the response of the other pair of individuals; in this sense the relationship of A to B can be considered different from that of A to C. We also analyzed a subset of our data where each particular male was only measured once, and the same patterns that we report in the Results held true.

Playback Experiment

We conducted playback trials to simulate a pair of duetting males, allowing us to experimentally test how birds space their calls out relative to others. We created nine playback stimuli from recordings collected between 28 April and 3 May of 2008 through 2010 from males that were unlikely to have previously encountered members of our population (playback birds were recorded more than 4km away). We collected recordings from pairs of definitive males using a directional microphone (Sennheiser MKH 70) and digital recorder (Marantz PMD660; 22050 Kbps, 16 bit, WAVE format, mono recording). We conducted these recordings between 0500 and 0700 h. From recordings of nine different display areas, we selected a single duet with high signal to noise ratio (assessed visually) where the recordist identified that they were <10m from the calling males. Using Audition (Adobe Systems Inc.) we removed background noise by selecting the duet with the lasso tool and decreasing the amplitude of the background noise to 1/20th of its original level. We then normalized the sound file to -1 dB. We used these normalized stimuli to create a playback track where the sound was repeated with 2.4 s or 3.5 s of silence between duets (see below), and stored the stimuli as an uncompressed sound file (22050 kHz, 16 bits, WAV format). We repeated this process to create nine unique duet stimuli that shared identical amplitude characteristics. A pilot investigation in early 2010 suggested that males may alter their calling rate when calling concurrently with other males by lengthening their inter-call interval. To assess whether this affected a pair's tendency to overlap or alternate, we created two versions of each stimulus: a "slow rate" consisting of a duet followed by 3.5 seconds of silence, and a "fast rate" stimulus which consisted of a duet followed by 2.4 seconds of silence. Under natural circumstances, when calling in the absence of other males, Long-tailed Manakins tended to space their calls with inter-call intervals of 2.0s (SE ± 0.1). Comparatively, when calling concurrently with other males Long-tailed Manakins tended to space their calls with inter-call intervals of 2.4s (SE ± 0.1). As a result our fast rate playback was the most similar to the rate we observed under natural conditions with concurrently calling pairs.

The playback device (Apple iPod classic) was operated by an observer who sat 10m from the loudspeaker (Anchor Audio Mini-Vox PB-25, Torrance, California; frequency response: 100 - 12,000 Hz). The loudspeaker was mounted on a 1.5m pole and placed within the subjects' display area, approximately 5-10m from their primary display perch. The amplitude of the playback stimuli was held constant across all trials at 80 dB (measured at a horizontal distance of 1m from the upwards-oriented speaker using a RadioShack Sound Level Meter, fast response). Based on our assessment, this matched the source amplitude of live duetting males in this forest (Fig. 3.5).

Playback trials were conducted between 0530 and 1030 h, a time when natural bouts of duetting were common (see figure 2.4B). After setting up the playback apparatus, but before commencing a trial, we recorded the focal males until they had produced a bout of at least 10 duets (i.e. 10 duets produced in repetition with inter-call intervals of less than 10 seconds) or 30 minutes had elapsed, whichever came first. We then waited until 10 seconds of silence had elapsed, before commencing playback. Long-tailed Manakins call at a fairly fixed rate with an inter-call interval of approximately two seconds (Fig. 3.5); by waiting 10 seconds after their final duet we were able to consider any vocalizations they produced a new bout. We repeated the playback stimuli until the focal males produced 10 or more duets in the absence of other neighbouring males' duets (average ± SE playback length: 26 ± 3 min; n=39 trials). Playback continued until the focal males stopped duetting and became silent for 10 seconds. Once playback stopped, we recorded the males' vocalizations for an additional 15 minutes.

We conducted 52 playback trials at 32 display areas. At each site we assigned one of the nine playback stimuli (random selection without replacement until all nine stimuli had been used). We randomly assigned whether each subject received a "slow" or "fast" playback rate. For 20 of the 32 display areas, we returned to the site on a different day (4.5 days later ± 0.8 mean ± SE) and broadcast the same stimuli at the alternate rate; we ensured that these trials were conducted at the same time of day, within 1 hour, to minimize any influence of time-of-day on calling behaviour. We avoided conducting playback at the same site or adjacent sites within the same 48h period. In 13 of the 52 trials the males did not produce 10 duets in a row, reducing our final sample size to 39 trials at 24 separate display areas.

We recorded all playback sessions using autonomous digital recorders (Wildlife Acoustics' Song Meters 22050 kHz, WAV format, 16 bit, stereo recording). These recorders were placed 3-10 m away from the playback speaker. Within a single trial the fine structural qualities of the stimulus, its fixed spacing, and consistent amplitude allowed us to discriminate between it and any response from the males. We assigned duets to either the focal males or the playback speaker.

Data Analysis

Which null model is most appropriate for estimating chance levels of overlap is a controversial topic (see Searcy and Beecher 2009; Naguib and Mennill 2010, Searcy and Beecher 2011). Research on invertebrates and anurans favours the use of circular statistics and phase response curves (e.g. Greenfield 1994a; Greenfield 1994b). Some cetacean and avian researchers use a resampling approach (e.g. Miller et al. 2004; Fitzsimmons et al. 2008; Schulz et al. 2008). Other avian behavioural biologists use duty cycle models to estimate chance levels of overlap (Ficken et al. 1974; Searcy and Beecher 2009). Given the diversity of null models for estimating chance levels of overlap, and the fact that each model can provide different interpretations, we opted to use three complementary null models to assess whether male Long-tailed Manakins space their duets non-randomly relative to rival males to increase or decrease levels of overlap: (1) circular statistics, (2) resampling analyses, and (3) duty cycle calculations. The circular statistics approach assesses whether males called non-randomly relative to one another and accounts for the cyclic nature of counter-calling interactions. The resampling approach, in contrast, assesses whether overlapping is more or less frequent than expected if males were calling without reference to one another while maintaining the same call rate. Finally, the duty cycle approach assesses whether the total number of overlapping calls is different than we would expect based on chance, and whether birds are more likely to overlap versus avoid being overlapped.

In our analysis of playback trials, we treated the playback sounds as the reference against which the natural males' response was measured. In naturally-occurring interactions, there is no obvious reference pair of males, because the two pairs of males call back-and–forth in succession. We arbitrarily chose a reference pair in natural counter-calling bouts, assigning the pair of males that produced the final duet within a bout of counter-calling as the reference pair. The reference pair can be considered analogous to the playback males in the playback trials, in that all measurements are made relative to this pair. We then re-analyzed each two-way interaction in our naturally-occurring interactions, treating the other pair of birds as the reference pair. By performing these analyses both ways, and representing each as a separate analysis, we avoid pseudoreplication in that we do not have two points from the same trial represented in one analysis.

Null Model 1: Circular Statistics

To calculate the timing of each duet relative to the reference males we performed calculations following a modified version of the methods outlined in Zar (1999) (Equation 1.).

Equation 1.

$$A = \frac{360\,(X - R_1)}{R_2 - R_1}$$

Where *A* is degrees between the onset of the responding males' duet and the last duet from the reference males; *X* is the time of the onset of the response of interest (in this case the duet of the responding males); R_1 is the time of the onset of the reference males' duet preceding the responding males' duet; and R_2 *i*s the time of onset of the next duet from the reference males. A value for *A* of 180 degrees would represent a duet from the responding males that began exactly halfway between two successive duets from the reference pair; a value of 10 degrees would represent a duet from the responding males that began shortly after the onset of a duet from the reference pair; and a value of 350 degrees would represent a duet from the responding males shortly before that of the reference males' next duet.

To calculate the average response for each trial we converted *A* to X, Y coordinates by taking the sine (X coordinate) and cosine (y coordinate) of the angle *A*. By taking the average of the X and Y points for each recording we were able to calculate an average response within each group (Zar 1999). When plotted on a canonical plot this generated a single point, where the angle from the origin represented the average timing of where the responding males spaced their duets relative to the reference pair's duets (analogous to *A* described above), and where the distance from the origin (*r*) represented the consistency of call spacing (*r* varies from 0 to 1; a value of 1 would mean males were invariant in where they spaced their duets relative to the reference males' duets, while a value of 0 would indicate that males were calling completely randomly). We then calculated the "mean of the means" by taking the average of all of the groups' responses, giving a mean angle and associated effect size for the population (Zar 1999). We then performed a parametric one-sample second-order analysis of angles to test the null hypothesis of no directionality that one would predict if males were calling randomly (Zar 1999). When the combined trials showed an effect that was significantly different from the null value of 0, we calculated 95 percent confidence limits for the second-order mean angle where possible (Zar 1999; for circular statistics, confidence limit calculations are not possible when the 95 percent confidence interval exceeds 180 degrees).

To analyze playback responses using circular statistics, we repeated the method described above of calculating an average response for each trial, and for all of the trials pooled together, using the playback as the reference against which we judged the responding males. In six of the 39 trails where males responded, there was a playback period (>10 playback duets) where a lone male produced solo calls (Fig. 3.1b), in contrast to the typical response where both males responded with coordinated duets. Our preliminary observations suggested that these "solo males" timed their calls differently, often overlapping the playback; we therefore chose to analyze solo responses separately from the duet responses. We analyzed solo responses using only the circular statistics method of analysis; solo responses were not easily analyzed with resampling or duty cycle techniques (below) because they were often non-continuous meaning they could be interspersed with a period of duets. Resampling and duty cycle models both require that analyses are conducted over an interval where both individuals are calling (See

Planck et al. 1975 for a formal description of this problem), and as such we could not analyze these non-continuous data using these two methods.

Null Model 2: Resampling Statistics

Our resampling approach involved comparing the observed amount of overlapping in calls during counter-calling exchanges (either naturally occurring, or in response to playback) to the amount of overlapping during counter-calling exchanges where the relative timing of calls had been altered by the addition of a fixed random interval multiple times. This approach removed the association of timing between the two counter-calling pairs' duets, permitting a calculation of the chance level of overlap if the pairs of males were calling with the same spacing, but with no reference to one another. We assigned the males producing the final duet as being the reference pair against which we measured the responding pair. Keeping the timing of the calls of the reference pair fixed, we added a single random value to the start time of each of the responding males' duets between zero and the average rate of the reference males' duets (range: 0 to 4.2 sec). We then measured the total amount of time (in seconds) that overlapping of duets occurred in this manufactured bout of counter-calling. Using a custom-written macro (Microsoft Excel, 2007) we repeated this process 5,000 times for each recording, varying the random number each time, to calculate a null distribution of the total amount of overlap. We calculated the median of the null distribution for each pair of males and then compared this to the observed value using a Wilcoxon signed-rank test. As described above, we then flipped the identity of the reference and responding males and repeated the analysis.

To analyze playback responses using a resampling approach, we followed the same method, assigning the playback duets to be the reference against which we judged the natural males' response. We calculated the total amount of overlap (i.e. playback overlapping responding males and responding males overlapping playback), and compared these to our null value using two separate Wilcoxon signed-rank tests, one for slow-rate playback and one for fast-rate playback.

Null Model 3: Duty Cycle Analysis

We followed a modified version of the duty cycle methods outlined by Ficken et al. (1974) for determining the expected number of overlapping vocalizations (Equation 2).

Equation 2.

$$P_T = N_A D_B + N_B D_A$$

Where P_T is the total number of calls that would be expected to be overlapping if males were calling randomly with no reference to one another; N_A is the total number of calls produced by the first pair of males; D_B is the duty cycle, or proportion of time spent calling, of the second set of males; N_B is the total number of calls produced by the second pair of males; and D_A is the duty cycle of the first set of males. We compared the predicted number of overlaps based on duty cycle to the observed number using a Wilcoxon signed-rank test. This equation determines the total number of times that A is expected to overlap B, plus the number of times that A is expected to be overlapped by B if A and B are calling with no reference to one another.

To analyze playback responses with a duty cycle approach, we additionally tested whether males overlapped playback or were overlapped by playback more or less than would be expected (Equations 3 and 4 respectively). By analyzing the data using these different approaches we can determine whether birds overlapped more duets than we would expect based on chance, and whether they were selectively calling after the onset of playback (overlapping the playback), or before the onset (being overlapped by the playback). We calculated the expected and observed values for each of these methods and tested for significance using a Wilcoxon signed-rank test. We did this for each playback rate, so each pair of males was only represented once in each test.

Equation 3.

$$P_M=N_AD_B$$

Where P_M is the number of playback calls that males are expected to overlap; N_A is the number of calls produced by the males; and D_B is the duty cycle of the playback.

Equation 4.

$$P_P=N_BD_A$$

Where P_P is the number of playback calls that are expected to overlap the males; N_B is the number of calls produced by the playback; and D_A is the duty cycle of the males.

Behaviour During Playback

To characterize the responses of males to playback, we compared the interval between their calls during pre-playback, playback, and post-playback periods. We were interested in understanding whether males adjusted their calling rate in response to playback, and whether their calling rate varied with the two rates of playback. As explained above, the pre-playback and playback period varied in length depending on the behaviour of the subjects. For this analysis, we focused on the first 10 consecutive (where the time between successive calls did not exceed 10 seconds) duets of the pre-playback, playback and post-playback periods. Our response variable was the average inter-call interval between the first 10 calls within a bout. We normalized this response variable using a log transformation, but present the non-transformed values in our figures. We performed a Student's *t*-test to examine differences between treatments.

Circular statistics were calculated manually using Microsoft Excel 2007. All remaining statistics were conducted in JMP (SAS Institute; version 8). All values are reported as means ± SE.

Results

Natural Counter-Calling Exchanges

During natural counter-calling exchanges between neighbouring pairs of duetting males, Long-tailed Manakins selectively called in the silent interval between their neighbours' duets (e.g. Fig. 3.1c). We used circular statistics to analyze these counter-calling exchanges, using each of the two pairs of males as the reference pair in turn. In both analyses, there was a significant departure from the null model of no directionality (parametric one-sample second-order analysis of angles; Fig. 3.2a: $F_{2, 11}$= 57.1, *N*=13, *P* <0.001; Fig 3.2b: $F_{2, 11}$= 59.5, *N*= 13, *P* <0.001). The mean phase angles were 176.6° (Fig. 3.2a) and 181.3° (Fig. 3.2b) with *r* values of 0.28 and 0.21 respectively, placing the average timing of calls almost perfectly out-of-phase with the reference males' duets. In other words, circular statistical analysis demonstrates that counter-calling pairs of Long-tailed Manakins alternate their calls during bouts of concurrent calling.

We used a second null model to evaluate call overlapping using a resampling approach. This approach showed that Long-tailed Manakins overlapped significantly less than expected by chance (Wilcoxon signed-rank tests: Fig 3.2c: W=45.5, N=13, P< 0.001; Fig 3.2d: W=45.5, N=13, P< 0.001). Males overlapped one another almost 50% less than would be expected if they were calling at the same rate with no reference to one another. In other words, resampling analyses supports the conclusion that Long-tailed Manakins avoid overlapping the duets of neighbouring males.

We used a third null model to evaluate call timing involving duty cycle calculations. This approach showed that the number of duets that neighbouring males overlapped was significantly less than would be expected if males were calling randomly (Equation 2; Wilcoxon signed-rank tests: Fig. 3.2e: W=45.5, n=13, P<0.001; Fig 3.2f: W=45.5, n=13, P<0.001). This analysis revealed males overlap nearly 50% fewer calls than would be expected under a null model of random calling.

Playback Experiment

In experimental counter-calling exchanges where duetting male Long-tailed Manakins responded to playback of a nearby unfamiliar pair of rivals, birds behaved differently, calling with substantial variability with respect to the timing of playback duets (e.g. Fig. 3.1d). Circular statistics revealed that responses to playback of duets at a slow rate revealed significant departure from the null hypothesis of no directionality (Fig. 3.3a; $F_{2,13}$= 6.4, N=15, P=0.01). The average phase angle was 171.2°, representing males calling near the middle of the silent interval between playback duets, on average; yet the strength of this relationship was weak (r=0.03) indicating substantial variation in the timing of calls (Zar 1999). Similarly, responses to playback at the fast rate also showed a significant departure from the null hypothesis of no directionality (Fig. 3.3a; $F_{2,19}$= 11.3, N=21, P<0.001). Here, the average phase angle was 282.3°, representing males calling late in the silent interval between playback duets, on average; however, the strength of this relationship was also weak (*r*=0.06) indicating substantial variation in the timing of calls (Zar 1999; note that the small *r*-values for both slow- and fast-rate playback preclude the calculation of confidence intervals). Taken together, these results suggest that pairs of males do not call randomly, but they do not space their duets relative to playback according to the more consistent pattern as we observed during natural counter-calling exchanges.

Using a null model based on resampling to calculate chance levels of overlap, we found that male-male pairs of Long-tailed Manakins overlap playback more than would be expected by chance. Pairs overlapped playback up to 50% more compared to the null hypothesis of chance overlapping. This higher-than-chance level of overlap was true for playback duets presented at a slow rate (Fig. 3.3c) and fast rate (Fig. 3.3d), although only the fast rate was significant at the two-tailed level (Wilcoxon signed-rank test: slow rate *W*=39.5, *N*=18, *P*=0.09; fast rate: *W*=64.5, *N*=21, *P*=0.02;).

Using a null model based on duty cycle to calculate chance levels of overlap, we found that the total number of overlapping calls (i.e. the sum of males overlapping playback and playback overlapping males; Equation 2), was not significantly different from levels expected by chance. This was true for both slow rate playback (Fig. 3.3e; *W*= 22.5, *N*=21, *P*=0.45) and the fast rate playback (Fig. 3.3f; *W*=30.5, *N*=18, *P*=0.20). Males also did not significantly alter the number of playback duets that they overlapped either at the slow rate (expected: 11.8 ± 2.6; observed: 11.8 ± 2.6; *W*=7.5, *N*=18, *P*=0.8) or the fast rate (expected: 7.0 ± 1.0; observed: 9.6 ± 3.1; *W*=7.5, *N*=18, *P*=0.8). However, the number of times playback duets overlapped the subjects' duets was lower than expected under a duty cycle model for both the slow rate playback (expected: 11.2 ± 2.5; observed: 9.9 ± 2.6; *W*=48.5, *N*=18, *P*=0.03) and the fast rate playback (expected: 7.3 ± 1.0; observed: 6.3 ± 1.1; *W*=50.5, *N*=21, *P*=0.08) although this was statistically significant only for the slow rate analysis. Consequently, the duty cycle model demonstrates that males call in such a way as to avoid being overlapped.

Throughout the majority of all 39 responses, birds responded to playback with vocal duets. However, during six trials there was a period where a single male produced solo *toledo* calls (e.g. Fig. 3.1b) in response to the playback. These solo callers timed their calls to begin just after the playback (Fig 3.1d; Fig. 3.4). The small sample size (N=3 instances in both the slow and fast rate playbacks) precludes the use of circular statistics to test for directionality, yet the strength of the effect appeared high (r=0.49 and 0.90 respectively) and the direction in which males were spacing their calls was consistent (17.2° and 4.1° respectively). Therefore, solo-calling males appear to space their calls to actively overlap with playback, calling immediately after the onset of playback duets.

Behaviour During Playback

Compared to pre-playback call rates, males slowed their rate of calling during playback. A linear mixed-effects model of inter-call interval before, during, and after duets played back at slow and fast rates revealed significant variation, where the inter-call interval was significantly shorter before playback than it was during playback, returning to an intermediate level following the end of playback (Fig. 3.5; playback period fixed effect: $F_{2, 77}$ = 3.1, P = 0.05); call rate did not vary significantly between slow rate and fast rate playback treatments (playback rate fixed effect: $F_{1, 87}$ = 0.0, P = 1.0). In 22 of our 52 playbacks, male Long-tailed Manakins approached the area near the playback speaker, sometimes coming as close as 5m from the loudspeaker, but usually 10m or farther. This occurred at 15 of the 32 display areas where we performed playback. These males would often flick their wings and give *chitter* vocalizations, which are thought to function in agonistic interactions (see table 1; Trainer and McDonald 1993).

Discussion

Stereo recordings of naturally-occurring bouts of counter-calling between Long-tailed Manakins revealed that males timed the production of duets to avoid overlapping the duets of males at neighbouring display areas. Males were significantly more likely to produce duets in the middle of the silent interval between their neighbours' duets, and they overlapped their neighbours' duets less frequently and for less total time than would be expected under two different null models. In contrast to natural counter-calling behaviour, males behaved differently when presented with playback simulating a pair of unfamiliar males calling close to their display perch. Under these circumstances males called with little reference to the timing of playback duets. Different analytical models yielded different conclusions with respect to the incidence of overlapping during playback; circular statistics showed that males called non-randomly but with substantial variation; a resampling approach showed that males overlapped fast rate playback more than expected by chance and slow rate playback showed a trend in the same direction; conversely, duty cycle models showed that overlapping did not exceed levels predicted by chance. Interestingly, males sometimes produced solo calls in response to playback; these solo callers appeared to actively overlap the playback, calling immediately after the onset of playback and effectively turning the playback duets into trios. Overall, our analyses reveal that males avoid overlapping known neighbours at adjacent display perches, but produce duets with more variability in timing when counter-calling with playback simulating unfamiliar rivals in close proximity to their display perch.

Call Timing During Natural Bouts of Counter-calling

More than 30 studies have examined overlapping during vocal interactions in birds (reviewed in Naguib and Mennill 2010). These studies reveal that animals may alter the degree of overlap depending on context (e.g. paired Nightingales overlap playback less than bachelors; Kunc et al. 2007), or they may alter their behaviour after being overlapped (e.g. overlapped European Blackbirds decrease song duration and avoid song posts where they had been overlapped; Todt 1981). Several studies reveal that levels of overlap under natural circumstances are lower than expected based on chance (e.g. Foote et al. 2008; Fitzsimmons et al. 2008), just as we found for natural counter-calling bouts in manakins. At least one previous study has demonstrated that neighbouring males vary their song rate relative to their neighbours, producing a predictable pattern of alternation (Smith and Norman 1979). Compared to these previous investigations, our findings most closely resemble those of Brindley (1991), who found that European Robins overlapped playback of familiar neighbours less, and unknown territorial intruders more. Taken together, these results support the following conclusions: under natural circumstances with known neighbours, birds tend to avoid overlap; during encounters with other rival individuals, either simulated through playback or under natural circumstances during intrusions, birds show more variable call timing and sometimes an increase in their level of overlap.

During natural bouts of counter-calling, Long-tailed Manakin males avoid overlap. They may do so for several reasons. First, if overlapping sounds masks the identity of the callers, or the quality of their vocalizations, males may strive to preserve the fine structural qualities of their duets by avoiding overlap. Second, if overlapping is a threatening or aggressive signal, neighbouring birds may avoid overlap in order to minimize aggressive interactions between frequently-encountered animals. Third, males may assess the quality of adjacent, rival animals by listening to their duets, and avoid overlapping so that they can have the opportunity to assess their neighbours in their own inter-call intervals. Fourth, by alternating the spacing of their calls males can create a higher duty cycle of species-typical vocalizations for the area around their display areas, which might increase female mate attraction opportunities. Our playback results, where males did not avoid overlap to the same degree as in the natural counter-calling interactions, do not provide support for the third or fourth explanation. The third explanation is not supported given that unknown intruders are typically considered a greater threat than known neighbours, and males should be particularly attentive to assessing the quality of the unknown potential competitors (Ydenberg et al. 1988), which we did not observe in our playback data. Similarly, the fourth explanation is not supported, given that enhancing the duty cycle of an area should be just as common if the contributors are familiar or unfamiliar rivals. Given that the duet calls of Long-tailed Manakins are understood to function primarily in mate attraction, and the fine structural details are thought to be associated with mating success (Trainer and McDonald 1995), the first of these four explanations seems most likely; males may maximize their sexual advertisements if they minimize their broadcast of overlapping calls.

All of the previous research examining acoustic overlap in birds has focused on territorial songbirds (with the exception of Miyazaki and Waas, 2002, who found that female Little Blue Penguins preferentially approach an overlapping loudspeaker). By focusing on non-territorial birds, our study provides an expanded perspective for understanding how animals space their signals to avoid interference with one another in an acoustically competitive environment. Like all lekking species, Long-tailed Manakins do not exhibit resource-based territoriality (Trainer and McDonald 1993). Instead, up to 13 males form dominance-structured social groups and display for females (McDonald 1993; Trainer and McDonald 1993). While individuals may defend their position in the dominance hierarchy through aggressive interactions, aggressive territorial interactions in this species are unknown (McDonald 1993). Our analyses reveal that manakins avoid signal overlap, in a pattern that matches what is known from several studies of territorial birds (Naguib and Mennill 2010). As with territorial birds, lekking animals are in regular contact with other conspecific animals that are simultaneously attempting to attract breeding partners in an acoustically competitive environment. Our results reveal that call timing in a lekking manakin shows similar patterns to those seen in territorial songbirds.

Call Timing in Response to Playback

Our playback results show a different pattern, where male Long-tailed Manakins called with greater variation during interactions with unfamiliar rivals, and often overlapped unknown rivals while avoiding being overlapped themselves. These results suggest that lekking birds behave differently when counter-calling with unfamiliar rivals or rivals calling in immediate proximity to their display perch. This may be analogous to the "dear-enemy" phenomenon known in territorial songbirds, where neighbours' songs represent less of a territorial threat because their position and behaviour is known (Fisher 1954; Temeles 1994). Conversely, the overlapping directed at the playback (as revealed through our resampling analysis of fast-rate playbacks) might represent an aggressive form of signal interference, as has been suggested by previous studies of territorial songbirds (Naguib and Todt 1997; Mennill and Ratcliffe 2004; Naguib and Mennill 2010). Another possible explanation for the difference between the response of males to familiar neighbours versus unfamiliar playback-simulated rivals is that the responding males did not perceive our simulation as a pair of manakins. This explanation is unsatisfactory for a variety of reasons, including the fact that our circular statistics showed that males were calling non-randomly with respect to playback sounds; males altered their call rates during playback; total overlap exceeded chance levels in the fast-rate playback trials (and showed a nonsignificant trend in the same direction for slow-rate playback trials); solo males actively overlapped the playback sounds; males avoided being overlapped by the playback; and males would often move to the area immediately around the speaker when responding.

Alternatively, pairs of male Long-tailed Manakins may have responded differently to playback duets compared to neighbours' duets because of the location of the acoustic competitors. We presented playback 5-10m away from the primary display perch for each subject pair, which is closer to their display area than is typical for counter-calling males (typically the closest neighbouring perch in our population is approximately 75m away, although they can be as close as 25m; pers. obs.). Although lekking birds are understood to be non-territorial, hearing such a nearby pair of rivals may incite different forms of behaviour. Female Long-tailed Manakins show site fidelity, returning to previously favoured display areas in successive years (McDonald 1989; McDonald and Potts 1994), and males are thought to build the "reputation" of the display area through their duets and visual displays each year (McDonald and Potts 1994; McDonald 2010). As a result, unknown males performing duets near the focal pairs' display area could negatively influence the reputation of the area, or the unfamiliar males could be seen to be taking advantage of a display area's reputation in an attempt to enhance their own mating opportunities (McDonald 1993). Future work could tease these two theories apart by presenting playback from familiar and unfamiliar males at different distances from the display perch, while examining the responding males' overlapping behaviour. If proximity to the subject's display perch is a key factor, this effect should disappear when sounds are presented from a distance more akin to what we see under natural circumstances (i.e. between 25 and 75m).

Although anecdotal, our results on the solo-calling behaviour of subjects in response to playback provides interesting insight into call timing. Our study animals, like many *Chiroxiphia* manakins, produce joint male-male displays to attract females to their display perch and entice them to mate (McDonald 1989b; DuVal 2007). However, unlike some *Chiroxiphia*, this male-male association is obligatory for Long-tailed Manakins; males displaying alone have never been observed to copulate with a female (Trainer and McDonald 1993; Prum 1994; DuVal 2007). As a result, a solo male's only opportunity to reproduce lies in producing coordinated displays with other males. Turning playback duets into trios may represent an attempt to join the queue at the display perch (McDonald 1993; Trainer et al. 2002). It is interesting to note that the only previous study to quantify the number of solo toledo calls found that "no bout of more than eight consecutive solo *toledos* was noted during more than 3,000 hours of scheduled observation" (Trainer and McDonald 1993). In contrast, we found six instances of males producing bouts of solo *toledo* calls, including five instances where males produced more than eight consecutive solo *toledos* (average = 15.4; in the sixth case, a bout of 10 solos was interrupted by a short bout of duets). This occurred over a comparatively small time scale (the total recording time for the playback experiment was just over 27 hours). Our findings suggest that solo males respond differently to playback than pairs of males.

Null Models for Assessing Overlap

Currently there is no well-accepted null model for calculating chance levels of overlap, for comparison to the measured behaviour of communicating animals (Searcy and Beecher 2009; Naguib and Mennill 2010; Searcy and Beecher 2011). For this reason, we used three different null models in our analyses of call timing; circular statistics, resampling analysis, and duty cycle models. We demonstrate that under some circumstances one can draw very different conclusions from these different null models. For example, in our investigation of male responses to playback, our resampling analyses showed that males overlapped playback for longer than would be expected based on chance, whereas our duty cycle analyses revealed that male duets overlapped the playback at levels that would be expected based on chance. While both showed higher levels of overlap than what we observed under natural conditions. Had we only used a null model based on duty cycle and only performed a playback experiment we would conclude that counter-calling males do not deviate from chance levels of overlap, whereas the resampling approach would indicate that males overlapped playback significantly more often than expected based on chance. Consequently, the analytical approach used to calculate chance levels of behaviour must be considered carefully before sound conclusions can be drawn.

The three different analytical approaches used here provide different perspectives, and each approach has strengths and limitations. Circular statistics provide an excellent descriptive tool, as they allow the researcher to easily assess whether there is deviation from randomness. Circular statistics are an effective tool for detecting subtle patterns in cyclical data, and the back-and-forth dynamics of many animal signalling contests can be recorded as cyclical data. The circular statistics approach is limited when call rate or call duration of the reference signal varies greatly, and this technique is unable to detect even strong effects at low sample sizes, as we observed here. Resampling analysis is an effective tool for preserving even highly variable timing of calls, and for establishing chance levels that incorporate the actual spacing of calling birds. Resampling requires a great deal of computing power, however, as the bouts of signals must be shuffled several thousand times to create appropriate estimations. Analyses based on duty cycle models are relatively simple to perform, and as such they have received more widespread use (Searcy and Beecher 2009). Duty cycle models provide a very similar approach to resampling analysis, and may be more robust when analyzing natural counter-calling exchanges with variable call rates or very long inter-call intervals. Duty cycle measurements are less appropriate for short counter-calling exchanges, where changes in timing to just one call can lead to dramatic changes in calculated levels of overlap. Both duty cycle models and resampling analyses must be restricted to a single continuous bout where both individuals are calling (Ficken et al. 1974; Planck et al. 1975). We recommend the use of circular statistics to determine if the spacing of calls between counter-calling individuals is random. As we showed here, circular statistics provide a compelling tool for visualizing that counter-calling males place their calls midway between the onset of their opponent's previous and next calls. If circular statistics reveal non-random call timing, we recommend resampling as a follow-up analysis to quantify the amount of overlap that occurs, given that this technique appears capable of detecting subtle differences from background variation in call timing.

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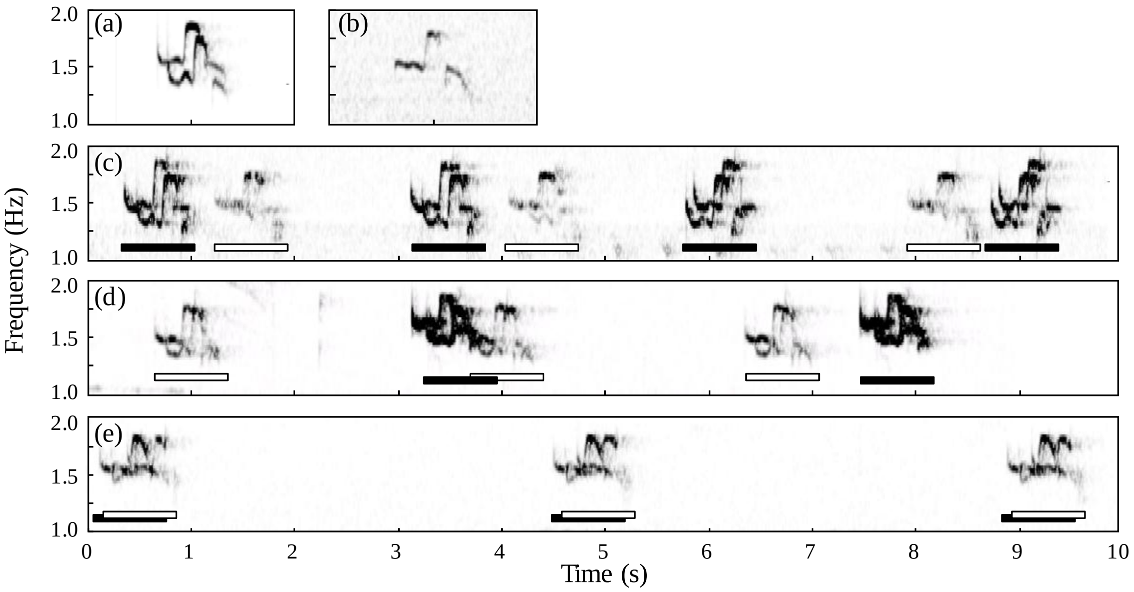

Figure 3.1. Sound spectrograms depicting the *toledo* calls of Long-tailed Manakin males (*Chiroxiphia linearis*). (a) A typical duet, comprising the tonal calls of a pair of males produced in near perfect synchrony. (b) A solo call, produced by a lone male; such vocalizations are uncommon, but occurred in 15% of playback trials. (c) Spectrogram of two counter-calling pairs of manakins at adjacent display perches. The vocalizations from one pair of males are underscored in black bars and the other pair in white bars. In this bout of naturally-occurring counter-calling, the two males produced duets in the silent intervals between each others' calls in alternation. (d) Spectrogram showing playback stimuli (underscored in black bars) and the counter-calling responses of a pair of manakins (underscored in white bars). (e) Spectrogram showing playback stimuli (underscored in black bars) and the solo calls of a lone manakin, who overlapped the playback stimuli (underscored in white bars) to create trios. These recordings were collected with omni-directional microphones in the field, resulting in low signal-to-noise ratios.

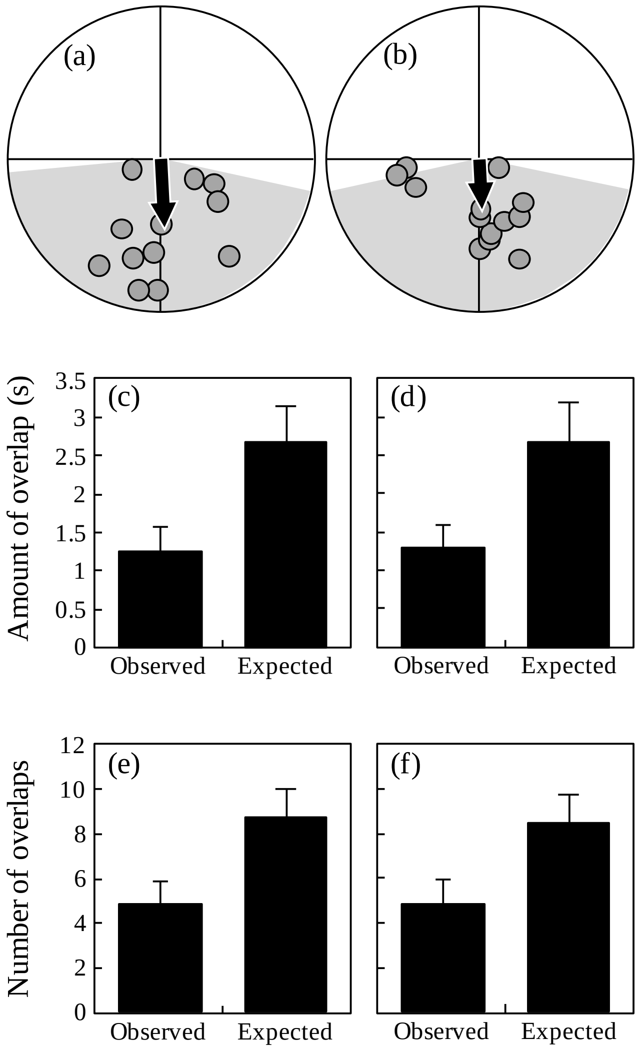

Figure 3.2. Neighbouring pairs of Long-tailed Manakins time their duets to avoid overlap during natural bouts of counter-calling. Circular statistical analyses (a, b; see text) reveal that duetting males call in the silent interval between their neighbours' calls (0 degrees represents the onset of the duet from the reference males, so that perfectly alternating calls are represented by a phase angle of 180 degrees; grey points show the mean for each trial; black arrows show means for the population where the length of the arrow corresponds to the effect size, *r*; the shaded areas correspond to the 95% confidence interval around the population mean; note that the perimeter of the circle corresponds to a value of *r*=0.6). Analysis involving a resampling approach (c, d; see text) reveals that adjacent pairs of males produce fewer overlapping duets (measured in seconds of overlapped sounds) than expected by chance. Analysis involving duty cycle models (e, f; see text) similarly reveal that adjacent pairs of males produced fewer overlapping duets than expected by chance. Values in (c) through (f) are means ± SE. In each analysis, the same pattern held true whether the males producing the final duet in the bout of counter-calling were used as the reference pair (a, c, e) or if the males producing the penultimate duet were used as the reference pair (b, d, f).

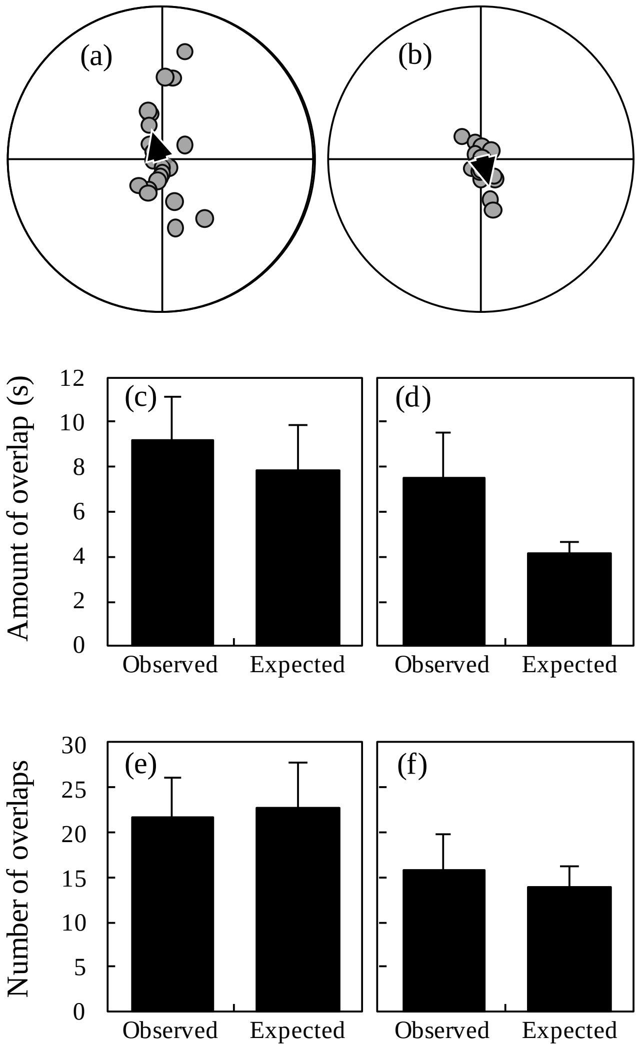

Figure 3.3. In response to playback simulating a rival, intruding pair, male Long-tailed Manakins called with substantial variation, both for playback broadcast at a slow-rate with an inter-call interval of 3.5 seconds (a, c, e) or a fast-rate where calls had an inter-call interval of 2.4 seconds (b, d, f). Circular statistical analyses (see text) reveal no strong directional pattern in the timing of subjects relative to playback duets either at a slow rate (a) or a fast rate (b), as indicated by the short vectors corresponding to the mean phase angles (0 degrees represents the onset of the playback duets; grey points show the mean for each trial; black arrows show means for the population where the length of the arrow corresponds to the effect size, *r*; in this case the edge of the circle corresponds to an *r* value of 1). Analysis involving a resampling approach (c, d; see text) reveals that subjects overlapped playback duets significantly more often than expected by chance for duets broadcast at a slow rate (c), and showed a tendency in the same direction for duets broadcast at a fast rate (d). In contrast, analysis involving duty cycle models revealed no difference between the observed and expected numbers of overlapping calls for duets broadcast at a slow rate (e) or a fast rate (f). Values in (c) through (f) are means ± SE.

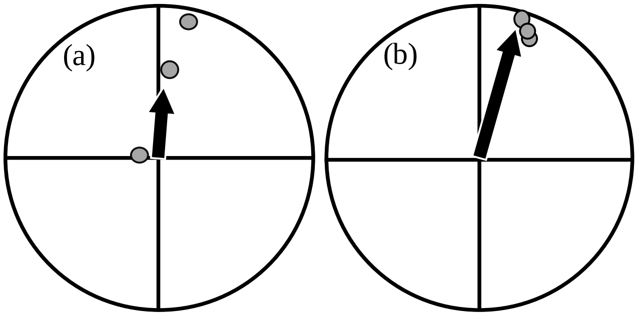

Figure 3.4. In a minority of playback trials to Long-tailed Manakins, males produced solo *toledo* calls in response to playback, instead of the more typical duets. Circular statistical analysis of the timing of these solo calls reveals that the solo callers showed a strong tendency to produce their solos immediately after the start of playback (0 degrees represents the onset of the playback duets; grey points show the mean for each trial; black arrows show means for the population where the length of the arrow corresponds to the effect size, *r;* in this case the edge of the circle corresponds to an *r* value of 1). This pattern was evident for both duets broadcast at a slow rate (a) and duets broadcast at a fast rate (b).

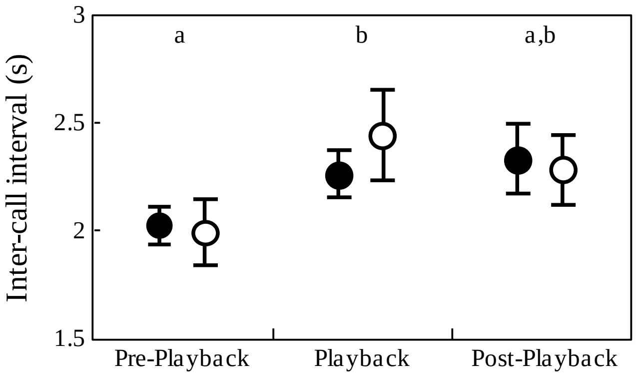

Figure 3.5. Pairs of male Long-tailed Manakins varied their calling rate, represented here as inter-call interval, in response to playback. Prior to playback, birds called with the shortest inter-call intervals; during playback, males called with significantly longer inter-call intervals; after playback had ceased, males called at statistically intermediate levels. Dots represent means and whiskers show standard error. Black dots represent slow-rate playback responses, and white dots represent fast-rate playback responses. Letters show a post-hoc test of honestly significant differences, where categories not connected by the same letter are statistically different.

Chapter 4: Thesis summary

Chapter summary

Many animals produce acoustic signals which can function in agonistic intrasexual contexts as well as intersexual communication (Bradbury and Vehrencamp 2011). The elaborate songs of birds are a well studied example of signals that function in both territory defence and mate attraction (Catchpole and Slater 2008). Understanding the role vocalizations play in these contexts can often prove challenging, because the inter- and intra-sexual aspects of communication are often tightly connected. For example, measuring female preference for male acoustic ornaments is often confounded as a result of different factors being favoured in social mates versus extra-pair partners (Searcy 1984). By focusing on a lekking species where males are non-territorial we can eliminate this confounding effect. My thesis focused on the female attraction function (as measured through visitation) of male vocal behaviour in the absence of the potentially confounding influence of territory defence.

In Chapter 2 I examined the effect of male vocal behaviour on female visitation in a population of Long-tailed Manakins in Costa Rica over the course of a two year field study. To quantify female visitation I analyzed the visitation behaviour of Long-tailed Manakin females at 38 unique display areas using a novel radiotracking system called Encounternet (system details provided in Appendix I). To quantify vocal behaviour I collected and annotated over 1,100 hours of vocal recordings and quantified the level of temporal synchrony and frequency matching in over 510 duets. This is arguably the largest study of the link between female visitation and male vocal behaviour that has been performed to date in this species (McDonald 1989, Trainer and McDonald 1995) and one of the larger such studies across all avian species. Surprisingly, my analyses revealed that the production of male-male *toledo* duets, which are typically thought to function in female mate attraction, were negatively correlated with female visitation. The total output of *teeamoo* calls, a vocalization understood to serve in attracting a male partner to perform duets, was positively correlated with female visitation. Other vocalizations that were predicted to be positively correlated with female visitation (the output of *owng* calls, dance song bouts, and *buzz-weents*) showed no relationship with female visitation. The quality of the *toledo* duets, both in terms of frequency matching and temporal synchrony, were also not correlated with female visitation behaviour. These results are counter-intuitive, and stand in contrast to previous investigations of a highland cloud forest population of this species that have been previously published (McDonald 1989, Trainer and McDonald 1995). I suggested that males that fail to attract females invest more time and energy in producing *toledo* duets in an attempt to increase their reproductive success, explaining the negative association between *toledos* and female visitation. Similar patterns of female visitation and behaviour shaping male vocal output have been seen in other tropical lekking birds as well as territorial species (Patricelli et al. 2006, Hennin et al. 2009). My results stand in contrast to some previous studies by McDonald and colleagues (McDonald 1989, Trainer and McDonald 1995, McDonald 2010), which have found positive correlations between female visitation and mating success and the output of *toledos*, dance songs and *buzz-weents*, as well as the degree of frequency synchrony between the two duet contributions. Similarly, those same studies found a negative correlation between the production of *teeamoo* calls and female mate attraction. This could be the result of climatic differences or differences in preferences between females within my study population versus McDonald's study population. My research highlights the importance of studying multiple populations, where different factors may influence male vocal behaviour and female visitation behaviour.

In Chapter 3 I employed an observational as well as experimental approach to assess how male Long-tailed Manakins coordinate the timing of their calls, by increasing or decreasing levels of temporal overlap. First, I employed a novel stereo recording system to collect long natural bouts of concurrent calling between pairs of neighbouring males. I collected recordings from 13 unique dyads of neighbouring male pairs where each pair contributed at least 10 duets. I used three widely supported methods for studying call spacing and quantifying chance levels of overlap: circular statistics, resampling, and duty cycle analysis. Under natural conditions, with known neighbours, all three methods showed that neighbouring pairs space their calls out non-randomly to avoid overlapping one another. I also performed a playback experiment where I presented pairs of males with recordings of *toledo* duets to simulate an unknown pair of rival males calling close to their display perch. I did this at 52 locations, and of these 39 responded with at least 10 duets. I performed the same three statistical analyses and found a pattern that differed from the natural bouts of counter-calling. Under playback conditions males called with little uniformity (as measured through circular statistics), but appeared to overlap the playback for longer than would be expected based on chance (as measured through resampling). This was in spite of the fact that they did not appear to selectively overlap more duets than would be expected based on chance (as measured through duty cycle analysis). However, the number of duets they overlapped was greater than what was seen under natural conditions. In a few cases where birds responded to playback with solos instead of duets, they actively overlapped the playback turning the duets into trios. A small sample size of solo toledos precluded statistical analyses. Overall these results show that males alter their calling behaviour based on those around them, in order to minimize overlap with known neighbours, but not to minimize overlap with playback simulating an unfamiliar rival pair calling close to their perch.

More studies of male Long-tailed Manakin vocal behaviour with large sample sizes will be required to clarify the relationship between vocal behaviours and their influence on female mating behaviour. My recordings of male vocal behaviour lasted up to 24 hours. By assessing the relationship between male vocal behaviour and female visitation across longer timeframes, we may better understand female preferences for male vocal performance, and how it changes over the course of a breeding season. The use of experimental approaches, either in the lab or in the field, provides potential for future research in this species in documenting female preference for male traits and assessing the relationship between female behaviour and male performance in this species. For example, to assess the role of female behaviour on male display Patricelli et al. (2006) used robotic models of Satin Bowerbirds that they could manipulate to determine if males altered their behaviour in the response to female signals. Similar methods could be used in Long-tailed Manakins to assess the effect of female behaviour on male courtship behaviour. To assess female preferences for male vocal quality independent of other effects would require an experimental design. Females could be placed in a choice arena either in a laboratory or outdoor aviary and presented with artificial male duets varying in their level of synchrony. Similar analyses have been performed to assess differences in female preferences for visual ornaments between populations of Common Yellowthroats (*Geothlypis trichas*; Dunn et al. 2008). Future analyses should also consider other traits or factors that females might be using in their mate selection process (e.g. plumage colouration, male condition, centrality, male visual behaviour, etc.).

Future experimental work on how male pairs time their vocalizations in the presence of known neighbours could build on the research presented in Chapter 3. Work from this thesis has demonstrated that males exhibit different calling behaviour when counter-calling with familiar neighbours versus unfamiliar males. Future research could assess the effect of distance and familiarity independently of one another by presenting playback of duets from known and unknown individuals at varying distances from focal display areas. Further studies investigating whether the act of overlapping vocalizations has fitness consequences could be performed (as has been documented in other species; Mennill et al. 2002). The use of taxademeric mounts has been employed in this species in the past (so-called “manakin mannequins”; McDonald 1993); employing taxidermic mounts in combination with call playback could provide researchers with a means of assessing aggressive responses (i.e. are overlapped males more likely to attack taxidermic mounts than non-overlapped males, as tested through interactive playback). This would be especially interesting given that this species is not territorial (McDonald 1989, McDonald 1993).

The tropics are home to an incredible diversity of animals that exhibit unique and unusual behaviours seen nowhere else (Stuchbury and Morton 2001). Understanding how male vocal behaviour influences female mate choice and male-male interactions has been studied extensively in the temperate region, but is only beginning to receive attention in tropical species (Stuchbury and Morton 2001). By improving our understanding of these systems we gain insight into sexual selection, factors governing female mate choice, and the relationships between neighbouring males. My research adds to our understanding of the function of male vocalizations in a tropical lekking bird and highlights the important insights that can be gained from tropical research.

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Appendix I: A novel digital telemetry system for tracking wild animals: a field test for studying female movements in a lekking tropical bird

This appendix is the outcome of joint research and is co-authored with collaborators D. Mennill, S. Doucet, K.-A. Ward, B. Otis, and J. Burt.

Appendix Summary

1. Radiotelemetry provides a tool for monitoring animals that are difficult to observe directly. Recent technical advances have given rise to new telemetry systems that present expanded opportunities for field research. We report the results of a field test of Encounternet, a new digital radiotelemetry system comprising portable receiver stations and digital tags designed for long-term studies of the social behaviour and ecology of free-living animals.

2. We present results from a series of field tests designed to evaluate the utility of Encounternet for monitoring animals in a neotropical forest, with an emphasis on evaluating the system's capacity for studying mate sampling behaviour in female Long-tailed Manakins. In this tropical species, females visit male display areas where males perform elaborate dances on horizontal perches. Females are highly cryptic in both plumage and activities, and therefore Encounternet might provide unique insights into female behaviour and ecology.

3. Our first two tests revealed that pulse strength and probability of detection decrease with the distance between tag and receiver, and that tags placed on a fixed perch near a receiver showed significantly different patterns of reception than more distant tags. Our third test revealed that antenna angle had only a small influence on pulse strength.

4. Blind analysis of simulated bird movements confirmed that the system provides reliable information on animal activity. Data from multiple receivers permitted accurate reconstruction of simulated bird movements. Tag detections showed low levels of false negatives and false positives.

5. Female manakins responded well to carrying Encounternet tags attached by an elastic leg harness. Birds flew well upon release and were detected, on average, for 7.5±0.8 days after

release. Recaptures and re-sightings of females were rare in our large study population, yet there were two occasions where we confirmed that the tag fell off within one year.

6. We conclude that Encounternet technology provides a very effective tool for monitoring animal ecology and behaviour. We discuss the opportunities presented by Encounternet for studying the ecology and behaviour of free-living animals.

Introduction

Tracking technologies have revolutionized ecological research by allowing long-term monitoring of animal movement and behaviour. The ecological and behavioural insights provided by radiotelemetry (e.g. Hinch and Rand 1998; Westcott and Graham 2000), satellite transmitters (e.g. Weimerskirch et al 2000; Hooker and Baird 2001), geolocators (e.g. Stutchbury et al. 2009; Montevecchi et al. 2011), microphone arrays (e.g. Blumstein et al. 2011), and related technologies have expanded our understanding of vertebrate biology beyond what was possible through direct observation or mark-recapture methods. Radiotelemetry, the most longstanding of these technologies, has facilitated monitoring silent or covert animal movements and behaviours, and has become a widely used approach for monitoring animals (reviewed in Cooke et al. 2004; Ropert-Coudert and Wilson 2005). Movements of animals that cannot be studied through direct observation can be quantified effectively with telemetry.

Recent technological developments have produced new innovations in radiotelemetry, particularly due to the exponential enhancement in chip performance delivered by the semiconductor industry. Traditional radio-receivers were handheld devices, and animals were monitored primarily while researchers followed the tagged animal with an antenna (Amlaner and MacDonald 1980). More recently, autonomous receivers have allowed animals to be monitored while researchers are absent from the area (Cooke et al. 2004). Consequently, animals can be monitored around the clock, without the influence of the presence of the researcher. Analogue transmitters broadcasting signals at different frequencies can be manually monitored by receivers that scan multiple channels (e.g. Crofoot et al. 2010). Recent developments in digital transmitters allow for multiple tags to transmit at the same frequency with unique codes. These technical advances dramatically increase the opportunities for radiotelemetry technology to be used in the study of the ecology and behaviour of wild, free-living animals. Until very recently, these advancements in digital radiotelemetry have only been available for large-bodied animals (e.g. Hazen et al. 2010).

Encounternet is a new radiotelemetry technology that brings the advantages of digital radio and automated monitoring to small animals for the first time. In an Encounternet system, tags as light as 0.8g are worn by small animals such as passerine birds (Fig. A.1a, b). The tags are similar in form to an analogue radiotag, but rather than a fixed-function analogue radio circuit, these tags contain a programmable microprocessor and a digital transceiver, allowing much greater flexibility in tag functionality. Encounternet tags are equipped with digital interface ports and analogue data converter inputs, allowing tags to include sensors to log temperature, sound, acceleration, GPS location, etc. The tags can be configured via radio commands, and tags can operate in many modes, such as a conventional transmitter tag, a tag-to-tag proximity logger, a radio transponder, or a radio repeater. The research described here – representing the first field test of this system – focuses exclusively on using Encounternet as a digital, automated telemetry logging system, where each tag periodically broadcasts a brief digital radio pulse encoded with a unique ID number.

The second component of Encounternet is the wireless receiver station (Fig. A.1c, d). Receiver stations contain a microprocessor, transceiver radio, and a high capacity flash memory card. They function as automated monitoring devices, logging the ID number, time, and signal strength of every tag pulse that they receive. Receiver stations are environmentally hardened and powered by an external battery pack; with two rechargeable D-cell batteries, they can log ID pulses for weeks without maintenance. Receiver stations can be placed in strategic locations to monitor the presence of tagged animals, or be placed throughout the habitat to record the overall movement patterns of animals.

The third component of Encounternet is a manually operated “master node”, which serves as the user interface to the Encounternet tags and receiver stations. The master node consists of a modified receiver station mounted on a high-gain directional antenna, and attached to a laptop computer running custom interface software. The master node is used primarily to wirelessly configure tags and receiver stations, and to download tag ID pulse logs from receiver stations in the field. The master node can also operate as a radio tracking system to locate and track tagged animals, as in conventional radiotracking.

We tested the capabilities of Encounternet in a field test designed to evaluate the utility of this technology for studying the movements of small birds in a neotropical forest habitat. In particular, we are interested in using Encounternet to study female movement and visitation behaviour in Long-tailed Manakins (*Chiroxiphia linearis*), a neotropical suboscine passerine bird. In this lek-mating species, males congregate in small groups (3-13 males) where social relationships follow linear, age-graded dominance hierarchies (Foster 1977; McDonald 1989a, b). The top two males in the hierarchy, the alpha and beta, attract females to their display areas through vocal duets and then perform complex, cooperative dances for prospecting females on “display perches”, low horizontal branches in the forest understory (Foster 1977; McDonald 1989a, b). The collection of Long-tailed Manakin display areas within a population can be characterized as a single exploded lek, where different display areas are in visual but not acoustic isolation (Gilliard 1963; Foster 1977). Unlike most lek-mating species, however, females choose among alpha males at different display areas rather than within males at a single display area (McDonald 1989a). Mating success is highly skewed, with a small proportion of alpha males securing most of the copulations (McDonald 1989b; McDonald and Potts 1994). Previous studies have shown that synchronization of duets, duetting rates, and components of male behavioural displays influence female mate choice decisions (McDonald 1989b; Trainer and McDonald 1995).

Yet, we know little about how females go about selecting their mates. Females rarely vocalize, they are very cryptic in both behaviour and appearance, and re-sighting data indicate that they have much larger home ranges than males (McDonald 1989b). If Encounternet provides reliable data, devices attached to female Long-tailed Manakins would facilitate tracking of the display areas visitation behaviour of females and could provide novel insight into female mate choice behaviour and male mate attraction behaviour.

In this study we conduct the first field test of Encounternet telemetry technology. Our field test is designed to evaluate the efficacy of this system for research on the visitation behaviour of female Long-tailed Manakins living in a dense neotropical dry forest, although this versatile system is expected to be useful for tracking many species of animals in a wide variety of habitats. We present a series of tests involving active Encounternet tags and receiver stations, each an investigation of the system's capabilities for studying the ecology and behaviour of females (Encounternet tags) as they visit male display perches (Encounternet receiver stations).

(1) We placed Encounternet tags at varying predetermined distances from receiver stations, and evaluated the signal strength values and the probability of detection of the tags across a range of distances; our goal was to quantify how detection varied when tagged animals changed their distance from a receiver station.

(2) We placed Encounternet tags on the display perches at Long-tailed Manakin display areas, and quantified variation in signal strength values and the probability of detection over time; our goal was to understand how detection varied when a tagged animal sat in a fixed position near a receiver station for a period of time.

(3) We changed the angle of the Encounternet tag antennas relative to the receiver station antennas, and evaluated whether their relative angles influenced signal strength value or the

probability of detection; our goal was to understand how detection varied as tagged animals changed their orientation, but not distance, relative to receiver stations.

(4) We conducted simulated “flight tests” by moving active Encounternet tags around the forest to mimic the behaviour of female Long-tailed Manakins, and assessed how accurately the technology allowed us to reconstruct the behaviour of the simulated females; our goal was to evaluate whether we could correctly record the movements of simulated female manakins in a blind test.

(5) Finally, we evaluated how tagged female Long-tailed Manakins responded to being outfitted with an Encounternet tag, and tested whether the receiver stations detected the tagged females after release.

We discuss the utility and versatility of this system for conducting future studies of female Long-tailed Manakins, for studying mate choice in lekking animals, and for studying the ecology and behaviour of wild animals generally. This is the first test of an Encounternet automated telemetry system in the field.

Methods

General field methods. Our field test of Encounternet took place in Sector Santa Rosa of the Guanacaste Conservation Area in northwestern Costa Rica from April to May of 2010 and 2011. This site is designated a World Heritage site by the United Nations Educational, Scientific, and Cultural Organization (UNESCO) and is home to one of the largest remaining stands of dry forest habitat in the neotropics. The study took place in the mature humid forest section of this study site, where the thick vegetation makes direct observation of animals difficult (Mennill and

Vehrencamp 2008). Moreover, wet tropical habitats are expected to limit the accuracy of telemetry (Millspaugh and Marzluff 2001) as well as the function of electronic equipment. Consequently, this habitat provides a challenging environment for testing the utility of a radiotelemetry system.

***Equipment**.* The tags used in this field test were designed and built by the University of Washington Encounternet project (http://encounternet.net) and consisted of a 7x15mm circuit board (Encounternet tag version 3a) containing a Texas Instruments MSP430 microprocessor and Texas Instruments CC1101 digital radio transceiver operating at 433MHz, with a 16.5cm steel-wire antenna attached. Two size 5 zinc-air hearing aid batteries powered the tags. All components were embedded in an epoxy resin matrix for weatherproofing. Prior to placing tags on females, we used a green marker to colour the dried epoxy so that the tag would blend in with the female's green plumage. Tags were programmed to transmit a unique ID pulse every 4 seconds in 2010, and every 5 seconds in 2011 (we decreased the pulse rate to once every 5 seconds in 2011 to enhance tag battery life).

Receiver stations consisted of a circuit board (Encounternet receiver station version 1.1) with a Texas Instruments MSP430 microprocessor, Texas Instruments CC1101 digital radio transceiver operating at 433MHz, and a 2 gigabyte Micro-SD flash card for data storage. Receiver stations were housed in a 9x9x6 cm waterproof ABS plastic enclosure and were powered by two rechargeable Imedion NiMH D-cell batteries in an external battery holder attached via a cable. A 12 cm omnidirectional 433MHz antenna was mounted on the case. Receiver stations were programmed to log all tag ID pulses to flash memory. Each log entry contained the ID of the receiver station and the tag, the time and date the ID pulse was received, and the received signal strength indication (RSSI) of the pulse.

We used an Encounternet master node for collecting data from the receiver stations in the field. The master node consisted of a modified receiver station attached to a directional Yagi antenna, with a serial cable connection to a laptop computer. To download logs from the receiver stations, we walked within approximately 20m of receiver stations and issued a command on the computer to initiate transfer of tag ID pulse logs from the receiver station to the memory card on the master node. Data downloaded from the receiver stations in the field were transferred to the computer and saved in a tab-delimited text file format.

Encounternet tags were attached to females with a figure eight leg harness, modified from Rappole and Tipton (1991). Elastic thread was fed through two Teflon tubes embedded in the epoxy that coated the tag, and tied off to create two loops that fit around birds' legs so that the tag rode just above their preen gland, with the antenna running down and beyond the tail (Fig. A.1). The elastic thread we used to create the leg harnesses consisted of a rubber inner core and an outer layer of braided cotton thread. So that the harness would deteriorate more quickly and fall off over time, we created a weak point in the outer layer, using a scalpel to cut the cotton threads in a small section of the harness, near where the harness joins to the tag, exposing the rubber inner core for a small section of the harness.

Field tests of Encounternet. To address the five goals of this study, we conducted a series of tests to evaluate the capabilities of Encounternet. The first three tests served to evaluate the system's capabilities in the field, and calibrate the data collected by the receiver stations. The fourth test served to evaluate the accuracy of the system in measuring the behaviour of simulated female Long-tailed Manakins. The fourth test, based on the data collected in the first three tests, was a blind test (female movements were simulated by DJM; data were assessed by KAW, DFM, and SMD). The fifth and final test was an evaluation of how female birds responded to the presence of the Encounternet tags.

For all of the tests below, the Encounternet receiver stations were mounted at fixed locations near male display perches at our field site. We mounted the receiver stations on a vertical branch as close as possible to the male display perch (0.3 to 1.4m from the area where males would dance for females), at a height of approximately 1.5m (Fig. A.1). We pointed the antennas for all receiver stations directly towards the ground.

Test 1: Variable tag-to-receiver distances. Our first test involved recording the pulses from Encounternet tags at variable distances from the receiver stations. We attached tags to the top of a 1m wooden pole and placed this pole at eight different distances from the receiver stations, measured with a measuring tape: 0.0m (directly beneath the receiver station), 5.0m, 10.0m, 15.0m, 20.0m, 25.0m, and 30.0m. At each position, we rested the pole on the ground so that tags were consistently 1m above the ground, and we manually rotated the pole at a rate of ca. 6 rotations per minute, to simulate the subtle movements of a bird making small perch changes while sitting on a branch, and to simultaneously rule out an effect of a particular angle of the tag antennas in this test. Tags were recorded at each position for 60 sec. We conducted this test at n=24 different receiver stations using n=8 different tags.

Test 2: Variation between receiver stations. Our second test involved recording pulses from Encounternet tags that were set atop male display perches. We placed tags on the perch in an orientation that matched the way tags would sit when females visit the perch, with the antenna perpendicular to the axis of the perch, and hanging down slightly below the horizontal. In the wild, female Long-tailed Manakins vary where they sit on the display perch during courtship visits, resulting in on-the-perch distances that may vary by approximately 2m relative to the receiver stations. During this test, we placed the tags near the centre of the perch at a distance of approximately 0.3 to 1.4m from the receiver station (see below), whenever possible in places where we had seen females sit at that particular perch. We held the tag still and recorded pulses for 60 sec. We then rotated the tag by 180 degrees, and recorded pulses for another 60 sec with the tag at the opposite, perpendicular angle. We conducted this test at n=64 different display perches (i.e. n=64 receiver stations) using n=5 different tags.

Test 3: Variation with antenna angle. Our third test was an assessment of whether the recorded pulses varied with the angle of the Encounternet tag antenna relative to the receiver station antenna. We mounted tags on a pole, as in test 1, and positioned the tags exactly 2.00 metres from the tip of the downwards-oriented antenna of the receiver station. We held the orientation of the tags' antenna steady for 60 sec pointing the antennas towards the receiver station and parallel to the ground. We then rotated the tags by 90° so that their antennas pointed at a direction orthogonal to the receiver antenna and parallel to the ground. We conducted this test at n=9 different display perches (i.e. n=9 receiver stations) using n=2 different tags. During each trial, we held the tags in a position with no vegetation in the space between the tags and receiver station.

Test 4: Simulated female behaviour. Our fourth test involved simulating female movement behaviour, to test the accuracy of the system for measuring the behaviour of female Long-tailed Manakins. In the field, we mounted two active Encounternet tags on the end of a 1m wooden pole, and carried the pole around the forest, positioning the tags near the active receiver stations that were mounted at the display perches for all known display areas in the study area. The field tests were conducted by DJM, and the data were analyzed by K-AAW, DFM, and SMD, who were blind to all aspects of the path the tags had traveled in the field. We conducted 11 tests with 22 different tags, including two tags in each test to check that the system produced similar results for both tags. Each tag was carried to 2 to 5 receiver stations (average±SE: 3.1±0.3), and was set on the perch (thereby simulating a female sitting on the display perch), or at a 5m horizontal distance from the perch (thereby simulating a female perching nearby, but not alighting on the display perch), and held at each position for 1.0 to 8.0 minutes (average±SE: 2.68±0.25 min).

After downloading the data from all receiver stations, we established a method for determining whether females visited the male display perch (as would a female inspecting males performing a courtship dance on the perch) or sat at a position ≥ 5m away from the display perch (as would a female listening to the vocalizations of males or watching them from afar, but not directly inspecting a courtship dance). Based on the data from test 1, we calculated a signal strength threshold that would be consistent with a tag emitting pulses from the display perch, or from ≥5.0m away from the display perch. We calculated the threshold in three different ways based on how much data we had collected for each receiver station. (1) For perches where we had data from both test 1 and test 2 (n=24), we calculated the threshold as the difference between the lower 25th percentile of the on-the-perch tag test data and the upper 25th percentile of the 5.0m tag test data. (2) For perches where we had data from test 2, but not test 1 (n=64), we calculated a threshold as the lower 10 percent of the on-the-perch tag test data (we determined this cut-off from the average percentage of on-the-perch tag test points that fell below the threshold of each perch from the previous method). (3) For perches where we had data from neither test 1 or test 2 (n=24), we calculated the population average values of the difference between the lower 25th percentile of the on-the-perch tag test data and the upper 25th percentile of the 5.0m tag test data, and used these values to determine our threshold. Based on the data from test 2 (see Results), it was clear that signal strength values at fixed distances fluctuate over time. We therefore used a criterion of 3 detections within a 30 second period at the appropriate thresholds to conclude that a female was present on or near the display perch. If fewer than three detections were recorded, we concluded that the tagged bird had moved through the area without stopping. The length of the visit was determined as the beginning of such a 30-second period, until the last tag detection that fell within the appropriate threshold.

Statistical analysis

For tests 1 through 4, we evaluate two response variables. (1) *Signal strength* is a numerical estimate of how close the Encounternet tag is to the receiver. Signal strength values are whole numbers that vary on a scale from -50 to 25, where lower, negative numbers imply large distances between tag and receiver, and higher, positive numbers imply small distances between tag and receiver. Each pulse received by an Encounternet receiver station records the date, time, and identity of the signal, as well as the pulse's signal strength value. When tags were left at a particular distance or orientation for a period of time, we calculated an average signal strength value. (2) *Probability of detection* is the proportion of total tag pulses received, where the numerator is the number of pulses recorded by the receiver station and the denominator is the number of pulses emitted by the tag.

We used linear mixed models to analyze signal strength and probability of detection, our response variables. To control for the fact that some tags were sampled repeatedly, and that each receiver station was sampled at multiple distances (test 1), we included tag and receiver station identity as random factors. We used the expected means squares (EMS) approach for our linear mixed models. Our sample sizes varied across the four tests, because there were instances where pulses were not detected (for example, in cases where there was a substantial distance between the tag and the receiver station). All statistics were conducted in JMP 8.0 (SAS Institute, Cary, NC). All tests are two-tailed and all values are presented as means±SE.

Results

Test 1: Variable tag-to-receiver distances

The strength of Encounternet tag signals detected by receiver stations decreased significantly with the distance between tag and receiver (Fig. A.2; linear mixed model; whole model: $F_{34,402}=124.6$, $p<0.0001$; fixed effect of distance: $F_6=599.3$, $p<0.0001$). For example, signal strength varied from 15.1±0.5 for tags directly under the receiver station, to -15.5±1.3 for tags 30m away. A post-hoc test revealed significant differences between distances of 0, 5, 10, and 15m between the tag and the receiver station, with overlapping signal strength values for 20, 25, and 30m (Fig. A.2). The random effects in the model revealed significant individual variation both for tags (random effect: $F_7=4.7$, $p<0.0001$) and receiver stations (random effect: $F_{21}=21.8$, $p<0.0001$).

The proportion of pulses detected by the receivers also decreased with the distance between the receiver and the tag (Fig. A.2; linear mixed model; whole model: $F_{34,693}=70.2$, $P<0.0001$; fixed effect of distance: $F_6=288.2$ $P<0.0001$). For example, the proportion detected varied from 96.2±2.6% for tags positioned directly beneath the receiver station, to 4.4±2.6% for tags positioned 30m from the receiver station. A post-hoc test revealed significant differences in the proportion of pulses detected between all distances except for 25m versus 30m, which were similarly low (Fig. A.2). The random effects in this model also revealed significant individual variation for both tags (random effect: $F_7=4.0$, $P=0.003$) and receiver stations (random effect: $F_{21}=27.8$, $p<0.0001$).

Test 2: Variation between receiver stations

The global average signal strength value for Encounternet tags placed on male Long-tailed Manakin display perches was 5.1±0.7. A test of tags set on display perches for 2 min revealed variation from one receiver station to the next (linear mixed model; whole model: $F_{34,693}$=70.2, *P*<0.0001; fixed effect of receiver station: F_{41}=122.2 *P*<0.0001; random effect of tag: F_4=208.0, p<0.0001). The average signal strength values varied between display perches from -14.4 to 25.0. This variation likely arose due to variation in the distance between the display perch and the receiver station (range: 0.3 to 1.4m), reflecting differences in the nearest vertical branch for mounting the receiver station.

One important goal for future studies of Long-tailed Manakin mating behaviour is distinguishing between females who travel near to a display perch (i.e. prospecting females assessing males from a short distance) and females who visit a display perch (i.e. prospecting females watching males dance at close range). We found that Encounternet tags positioned on the display perch had significantly higher signal strength values than tags positioned 5m from the receiver station (on the perch: 6.75±0.63; 5m from receiver: -4.41±0.59; paired *t*-test for average signal strength value for on-the-perch versus 5m data averaged at each receiver station: *t*=5.7, *P*<0.0001, *n*=24 receiver stations where we collected on-the-perch and 5m-from-perch data using the same tags). Consequently, signal strength facilitates distinguishing females visiting male display perches from females observing display perches from a short distance. Tags positioned on the display perch recorded a statistically higher proportion of pulses, compared to tags positioned 5m from the receiver station (on the perch: 90.5±1.7%; 5m from receiver station: 59.5±3.6%; paired *t*-test: *t*=11.5, *P*<0.0001).

Test 3: Signal strength and tag antenna angle

The signal strength of pulses from Encounternet tags held at a distance of 2.00m with antennas oriented parallel to the receiver antennas (signal strength: 5.8±3.5) was higher than when tag antennas were oriented perpendicular to the receiver antennas (signal strength: 3.1±3.3; linear mixed model; whole model: $F_{10,25}$=1.6, *P*=0.18; fixed effect of orientation: F_1=5.8

P=0.02; neither random factor showed a significant effect: tag: F_1=0.33, *P*=0.33; receiver station: F_8=1.1, *P*=0.39). The difference between the means was subtle (parallel: 5.8; perpendicular: 3.1) and the ranges for parallel antennas (minimum: -4.1; median: 5.8; maximum 10.4) and perpendicular antennas (minimum: -1.7; median: 2.9; maximum 11.2) were overlapping. The probability of detection was 100% for both tag orientations at a close proximity to the receiver station (2.00m with no intervening vegetation).

Test 4: Simulated female behaviour

Our blind analysis of simulated flights and perch visits of female Long-tailed Manakins showed that the Encounternet system can capably reconstruct the behaviour of moving animals. The tags were set on 28 display perches, simulating prospecting females arriving to inspect males during their dance displays, for an average "perch visit length" of 2.62±0.30 minutes. Our blind analysis detected 27 of the 28 simulated perch visits (96.4%; i.e. a "false negative" rate of 3.6% for on-the-perch visits) and measured an average perch visit length of 2.53±0.33 min. The Encounternet-estimated perch visit length showed a strong correlation with the actual perch visit length (Pearson correlation: *r*=0.87, *P*<0.0001, *n*=27). The blind test of perch visits also detected four "false positives" where Encounternet incorrectly indicated that a tag was placed on the display perch (i.e. a "false positive" rate of detection of 12.9% for on-the-perch visits). In all four cases, the system incorrectly identified a tag that was placed on a branch 5m from the display perch as being positioned on the perch. The perch visit length for the false positive visits (6.5±1.4 tag pulse detections per visit, *n*=4) was significantly shorter than the perch visit length for the true visits (29.0±4.2 tag pulse detections, *n*=27; unpaired t-test: t_{29}=2.0, *P*=0.05).

During the "flight tests", the tags were also set on 26 branches that were 5m from display perches, simulating females coming near to male display perches but not visiting the perches directly, for an average visit length of 2.95±0.26 minutes. Our blind analysis detected 25 of the 26 simulated near-the-perch visits (96.1%; i.e. a "false negative" rate of detection of 3.9% for 5m-from-the-perch visits), and measured an average perch visit length of 2.88±0.39 minutes. The Encounternet-estimated perch visit length showed a positive correlation with actual perch visit length for tags placed 5m from male display perches, although this relationship was not as strong as for tags placed on male display perches (Pearson correlation: r=0.48, P=0.02, n=25). Our blind analysis detected an additional 27 instances of Encounternet detecting a female ≥ 5m from the display perch; all 27 were cases where the researcher carrying the tag through the forest walked in the vicinity of the receiver (estimated closest distances of approach: 10 to 30m), but did not pause to alight the tags on a branch. The perch visit length for these 27 visits (5.1±2.9 tag pulse detections per visit, n=27) was significantly shorter than the perch visit length for the true visits (23.7±3.0 tag pulse detections per visit, n=26; unpaired t-test: t_{51}=4.4, P<0.0001).

Test 5: Responses of females to wearing Encounternet tags

Our final field test involved evaluating the responses of female Long-tailed Manakins fitted with an Encounternet tag, and determining whether tagged females were detected in the study site after release. Including the harness, tags weighed 0.88±0.01g (n=12 measured tags); females weighed 18.76±0.43g (mean±SE for the n=12 females carrying these tags). Therefore the tags weighed 4.69% of the female's body mass, less than the 5% body mass guideline that is thought to be appropriate for radiotagging wild birds (Caccamise and Hedin 1985; Naef-Daenzer 1994).

Females responded well to being fitted with tags. During a two-year period, we tagged 82 females. In 79 cases, the female flew well on release, gaining altitude and perching in the mid-story at distances of 20 to 50m and preening before flying out of sight, or flying out of sight immediately upon release. In the remaining three cases, females flew only a very short distance and landed on a low perch or on the ground. In the first case, the female made a second, normal flight soon thereafter, and flew off without any further sign of impediment. In the second case, we attempted to minimize stress on the female by leaving the area while she was perched on a low branch; when we returned later she had left the area. In the third case, when the female made a second strained flight we immediately recaptured the bird and removed the tag; she showed no further sign of impediment on re-release without the tag.

Seventy of the tagged females were detected moving around the area near the receiver stations for days to weeks after being tagged; the remaining females were not detected after the day they were released. Encounternet-tagged females moved around the study site, generating 46,222 detections by the receiver stations (Fig. A.4). There were many detections with a signal strength value of 0 to 2 (Fig. A.4), which we assume are indicative of females sitting on male display perches. We calculated the length of female visits to male display perches, and found that most visits to male display perches were brief (3.8±0.2 min; range: 12 seconds to 86 minutes; Fig. A.4).

The average length of time from tag deployment to final detection was 7.5± 0.8 days (range: 1 to 24; n=70). We do not have sufficient data to determine whether the batteries in the tags died after this period, or whether females exited the area (female home range can be as large as 80ha; McDonald 1989b). In 2010, we located the active nests of two tagged females; in both cases the female was engaging in normal nesting activities and did not show any sign of impediment due to the presence of the Encounternet tag.

Our study population is very large, and recapturing or re-sighting females is a rare occurrence, making it difficult to confirm what proportion of the tags fell off over time. In 2011 we re-sighted three females who had been tagged previously (two were detected visually in the field; one was detected on a video recording of a display perch). Two females who were tagged in 2010 had lost their tags. One female who was tagged earlier in 2011 was still wearing a tag that was no longer transmitting (25 days between tagging and re-sighting).

Discussion

The results of our field test of an Encounternet telemetry system demonstrate that this new technology provides a compelling tool for automated tracking of animal movement in the challenging field environment of a neotropical forest. The system, comprising digital radiotags worn by free-living animals and small receiver stations that autonomously log encounters with tags, met our expectations during a series of five tests. The tests were designed to evaluate the efficacy of the system for research on the visitation behaviour of females (i.e. animals carrying Encounternet tags) relative to the display perches of tropical manakins (i.e. the Encounternet receiver stations). (1) Encounternet tags showed significantly higher signal strength values and probability of detection as the distance between tags and receivers decreased, demonstrating that Encounternet can quantify the distance between a tagged animal and a receiver station. (2) Signal strength values were consistently higher when tags were placed on display perches compared to distances ≥5.0m from display perches, demonstrating that Encounternet can distinguish between tagged animals near a receiver station versus more distant animals. (3) Tag versus receiver antenna orientation influenced signal strength, but not probability of detection, and this variation was small in comparison to variation due to distance, demonstrating that tag orientation will have only a small influence when monitoring freely-moving tagged animals whose antennas vary in orientation over time. (4) Importantly, blind analysis of simulated Long-tailed Manakin movements demonstrated that the system can provide accurate reconstructions of the movement of Encounternet tags, with reasonable rates of false positive and false negative detections. (5) Females responded well to being fitted with Encounternet tags, and anecdotal records confirm that tags fell off over time. The 40 receiver stations produced more than 46,000 detections from 70 tagged animals, and capably quantified the length of visits made by tagged animals to male display perches. Taken together, these results confirm that Encounternet provides an effective tool for measuring and monitoring the movements of tagged birds in a tropical forest. This is the first field test of this new technology.

Long-tailed Manakins exhibit a unique and complex mating and social system (McDonald 2010), and Encounternet offers a special opportunity for understanding the behavioural ecology and evolution of this tropical bird. Males form social groups structured by stable linear dominance hierarchies, and entice prospecting females to mate by performing complex acoustic displays (Trainer and McDonald 1995), showcasing multiple elaborate plumage ornaments (Doucet et al. 2007b), and performing intricately-coordinated visual displays at their display perches (McDonald 1989b). Whereas males are conspicuous and reliably found in the same location, females are highly camouflaged (Doucet et al. 2007a) and their movement patterns are elusive. Consequently, the behavioural patterns of females are difficult to measure. In a study of this species at a montane site in Costa Rica, McDonald (2010) collected an incredible dataset on female visitation through thousands of hours of visual observations (15,000 display perch observation hours over an 18 year period) at a relatively small number of display areas (6 to 8 display areas per year). Such intensive data collection is expensive and time consuming. By contrast, automated detection by Encounternet permits vastly extended sampling opportunities. For example, in our two-year study we collected approximately 40,000 observation hours at dozens of display areas per year. This system also facilitates tracking of individual females between each of the monitored display areas. Therefore Encounternet presents novel opportunities for understanding topics as varied as female mate sampling behaviour to the influence of male ornaments on female visitation.

One important concern for telemetry research is the influence of tag weight and the harness mechanism on the tagged animal's well-being (Naef-Daenzer 1994; Dixon 2011). One common guideline for birds is that the weight of all components attached to a bird should be less than 5% of the bird's mass (Caccamise and Hedin 1985). Tracking studies of a variety of birds confirm that tags weighing <5% of passerine birds' body mass allow animals to continue normal activities, in species as varied as Hooded Warblers (*Wilsonia citrina*; Neudorf and Pitcher 1997), Jackass Penguins (*Spheniscus demersus*; Heath and Randall 1989), and Javan Hawk-Eagles (*Spizaetus bartelsi*; Gjershaug et al. 2004). Our analysis of 82 tagged tropical Long-tailed Manakins suggest that these birds can capably handle the presence of Encounternet tags weighing less than 5% of their body mass. Of 82 tagged female Long-tailed Manakins, 79 flew well upon release, 2 struggled with their first flight but appeared to quickly recover and adapt to the tag's presence, and 1 bird was quickly recaptured so that the tag could be removed. Our field experience suggested that minimal handling time and minimal banding stress produced the optimal results for females upon release. We therefore encourage other researchers to attach tags and release birds as quickly as possible, without additional stressors such as bleeding or intensive morphological measurements, to minimize stress.

Recapture and re-sighting of birds in our large study population is very rare, but multiple lines of evidence confirm that tagged birds continued to move through the study population. (1) Our receiver stations continued to encounter birds moving through the study site for extended periods after release (Fig. A.4); their activities are the subject of forthcoming behavioural studies (Maynard et al., Ward et al., unpublished data). (2) Two tagged females were found at their nest and were engaged in normal parental activities. (3) Three tagged birds were re-sighted after extended periods in the wild; all three were engaged in normal activities, and (4) two birds re-sighted a year after release had lost their tags.

Encounternet has many possible applications in future studies. Encounternet systems will be useful whenever tracking of animals relative to fixed-position receiver stations provides information on the behaviour and ecology of tagged animals. In our study, we focused on female birds visiting display areas; however, this system may be used to monitor a variety of animals and is applicable to a variety of mating systems. For example, Encounternet can monitor visitation at nests, watering holes, feeding areas, and roosting sites. Encounternet can also be used to monitor habitat selection at different scales. Receivers can be set up at specific sites-of-interest, as we did in this study. Alternatively, receivers can be set up in a grid-like pattern throughout a study site to monitor spatial and temporal use of habitat; the size of the area monitored will be limited by the fact that receivers can detect tags at distances of up to 30m. Such an application would provide a compelling approach to study species of conservation concern, to better understand movement and habitat use patterns (Rasmussen and Litzgus 2010). General coverage of a study site with receivers can also be used to identify territory boundaries and to monitor resident excursions into neighbouring territories (e.g. for covert animal movements, such as extra-pair copulations; Double and Cockburn 2000).

As an autonomous detection tool, Encounternet has enhanced capabilities beyond previous technologies for automated monitoring of tagged animals. Radio frequency identification (RFID), usually involving passive integrated transponder devices (PIT tags), is a widely-used technology also involving small devices worn by animals that are detected by fixed-position receivers (Bonter and Bridge 2011). PIT tags require no battery and therefore the devices are lightweight and provide information over extended periods (Gibbons and Andrews 2004). Although Encounternet tags are heavier and have finite battery life, they are capable of transmitting signals that can be detected as far away as 30m (Fig. A.2), whereas PIT tags can typically be detected at ranges of ≤0.1m. An RFID system could not be used in the application we were testing here, for example, because the length of the male display perch (1 to 2m) would require many PIT tag readers. The other established technology most similar to the one we tested here is the use of receiver towers in radiotelemetry studies, where multiple telemetry towers can triangulate the position of tagged animals (e.g. Taylor et al. 2011). This technology has similar limitations to Encounternet in terms of tag weight and tag battery life. Tower-based telemetry, however, is limited by the logistics and cost of setting up the large towers, the transmission of tag signals over long distances to be received by the towers, and by the scale of resolution. Encounternet receiver stations are small and easy to set up (e.g. we easily set up 40 receiver stations in just a few hours), and facilitate positioning of birds on the scale of meters. It is worthwhile to note that tower-based telemetry is compatible with Encounternet, and the two systems could work in concert.

In conclusion, our field test of Encounternet digital telemetry confirms that this system provides a compelling approach for monitoring wild birds. The results of trials designed to evaluate the efficacy of the system for monitoring Long-tailed Manakins can be readily applied to studies of other animals. A new iteration of this technology permits tags to receive pulses from each other, and this development will facilitate unprecedented quantification of animal social networks, extra-pair behaviour, and other social interactions that are difficult to quantify in other ways. Uniquely, Encounternet provides round-the-clock monitoring of tagged animals, and represents a significant advance in the study of animal ecology and behaviour.

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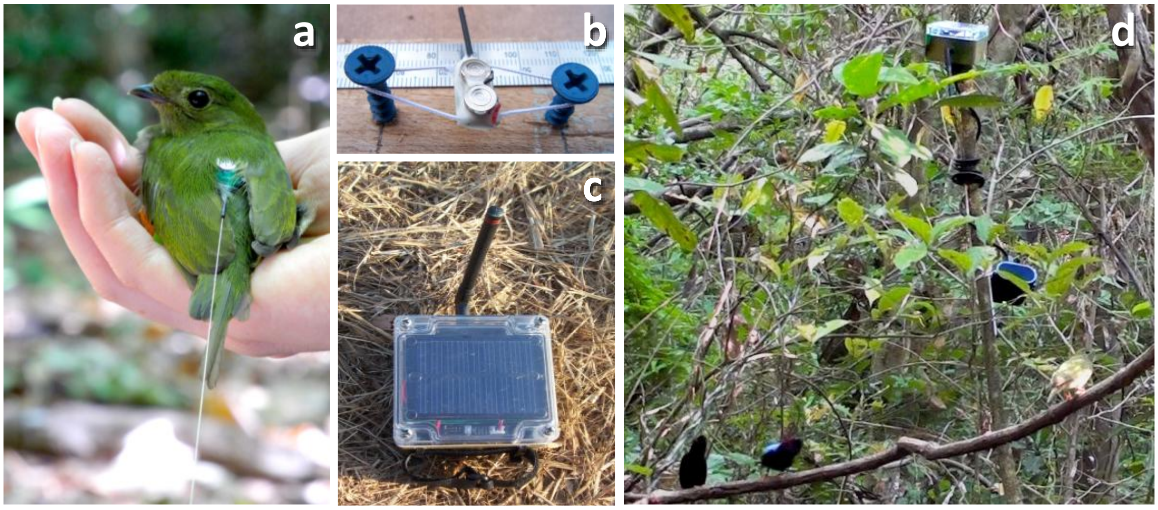

Figure A.1: Photographs of the components of the Encounternet automated telemetry system used in a field test to study Long-tailed Manakins in Costa Rica. (a) A female Long-tailed Manakin fitted with an Encounternet tag; the tag sits above her preen gland and the antenna runs down and beyond her tail. (b) An Encounternet tag, comprising two air-zinc batteries, an antenna, and a programmable chip that controls the timing of the radio pulses. The components are embedded in epoxy for waterproofing, and an elastic thread is fed through Teflon tubes at the two ends of the tag to create a leg harness. Here the tag is suspended between two blue screws. (c) An Encounternet receiver station; the receiver hardware is contained in a waterproof box, a cable that attaches to an external battery back, and a movable antenna is located on one side. (d) A screen capture from a video at a Long-tailed Manakin display perch. Two black males (lower left) dance on a display perch for a green female (lower right). The Encounternet receiver station (top) is mounted above a display perch the external battery pack can be seen below it).

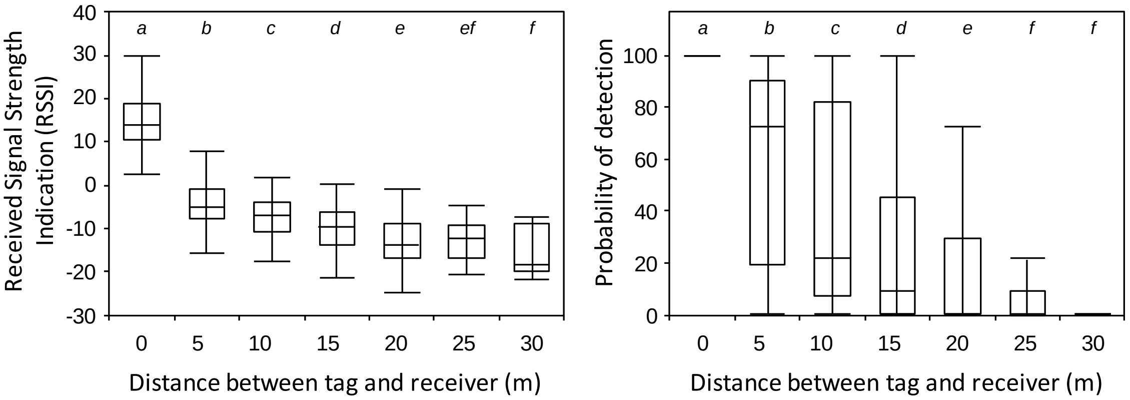

Figure A.2. The signal strength (left) and the probability of detection (right) decreased with the distance between Encounternet digital tags and receiver stations in a neotropical forest in Costa Rica. Box plots show the full range of data for the mean values from each receiver station; the boxes show the 25th, 50th, and 75th percentile, and the whiskers show the maximum and minimum value. Letters above the box plots show the results of a post-hoc test of honestly significant differences; plots that are not connected by the same letter are statistically different.

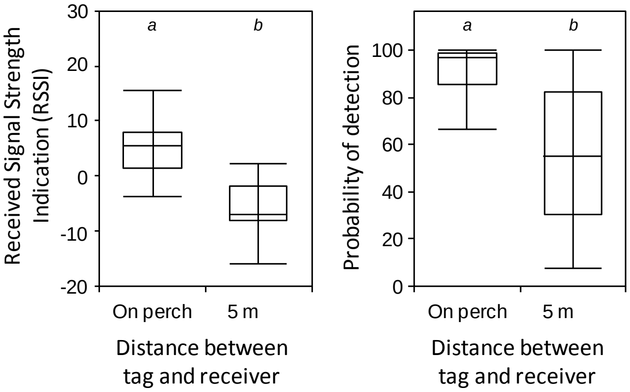

Figure A.3. The signal strength (left) and the probability of detection (right) was significantly higher for tags placed on the display perch at Long-tailed Manakin display perches (approximate distance of 0.3 to 1.4m) than for tags placed 5.0m from the receiver station. Box plots show the full range of data for the mean values from each receiver station; the boxes show the 25th, 50th, and 75th percentile, and the whiskers show the maximum and minimum value.

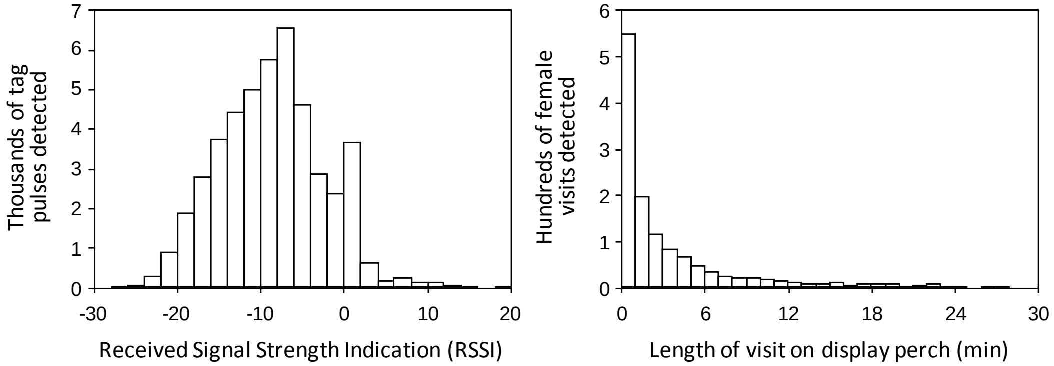

Figure A.4. Summary data from n=70 female Long-tailed Manakins carrying Encounternet tags as detected by autonomous receiver stations at male display sites in a tropical forest in Costa Rica. A histogram of the strength of the received pulses (left) shows a high number of pulses with an RSSI value of 0 to 2 are indicative of females observing male courtship displays while visiting a male display perch. A histogram of the length of female visits to male display perches (right) shows that most female visits to male display perches were brief, although hundreds of visits were longer. Twenty visits longer than 30 min are excluded from the histogram; the longest visit detected in this dataset was 86 minutes.

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