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Sexual Selection and Adaptations for Performance of an Elaborate Courtship Display by the Golden-collared manakin (Manacus vitellinus)

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Sexual Selection and Adaptations for Performance of an Elaborate Courtship Display by the Golden-collared manakin (*Manacus vitellinus*)

A dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy in Biology

by

Julia Kristina Barske

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ABSTRACT OF THE DISSERTATION

Sexual Selection and Adaptations for Performance of an Elaborate Courtship Display by the Golden-collared manakin (*Manacus vitellinus*)

by

Julia Kristina Barske
Doctor of Philosophy in Biology
University of California, Los Angeles, 2013
Professor Barnett Schlinger, Chair

Animal courtship displays can be spectacular behaviors employing extraordinary and specialized movements. How and why males perform these displays is still a matter of some debate. The male golden-collared manakin (*Manacus vitellinus*) is a neotropical passerine that spends up to seven months each year on a lek, performing courtship displays to attract females. The display involves the production of mechanical sounds while rapidly jumping around in a cleared court on the forest floor. I hypothesize that females prefer males with optimal neuromuscular control enabling them to excel in the performance of their displays. I propose that courtship displays pose high energetic demands with accompanying behavioral and/or cardiovascular adaptations. As lowland tropical animals are thought to exhibit a slow pace of life associated with a low metabolic rate it is of interest whether male manakins resemble other birds of tropical rainforests in displaying overall low metabolic rate and, if so, how they accomplish this when they also perform what seem like energetically costly courtship displays. Lastly, as testosterone has been shown to activate courtship in male manakins, and the heart is known for being an androgen target in mammals, I propose that testosterone regulates manakin cardiovascular function. I used high-speed videography to film courting male manakins in the forests of Panama. I deployed heart rate transmitters to estimate energy expenditure of courting manakins and used molecular and histological techniques to investigate the male myocardium. Knowing that female manakins are able to distinguish males by tens of milliseconds, choosing faster males for copulations, I show here that females ultimately challenge males by joining them in their display before finalizing their decision to mate. Heart rates rise up to 1300 beats per minute (among the highest recorded for any birds or mammals) during some of the behaviors preferred by females, such as during the production of mechanical sounds. I also found that average daily heart rates are comparatively low, comparable to those of other tropical birds. From a metabolic perspective, male manakin courtship resembles many sprints as opposed to a marathon, as successful males display up to 140 times a day, but each display lasts only ~10 seconds. Compared to a passerine bird of similar size that does not perform elaborate courtship, I found that the muscular walls of the manakin left ventricle are enlarged. The manakin heart expresses androgen receptors at a high level and androgens appear to be involved in regulating calcium handling in the manakin myocardium. A functional approach to animal behavior or sexual selection suggests that physiological costs are associated with mate choice and provides a unique window into anatomical and physiological potential.

The dissertation of Julia Kristina Barske is approved.

Douglas Altschuler

Gregory F Grether

Peter M Narins

Barnett Schlinger, Committee Chair

University of California, Los Angeles

2013

To Jari and Cécilia,

representing generations to come,

may they have the opportunity to experience our nature the way I was able to

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VITA

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Publications

- J. Barske, B. Schlinger and L. Fusani (submitted). Female manakins challenge male motor skills in a paired dance.
- B. A. Schlinger. J. Barske, L. B. Day, L. Fusani, and M. J. Fuxjager (2013) Hormones and the Neuromuscular Control of Courtship in the Golden-collared Manakin (*Manacus vitellinus*). *Frontiers in Neurendocrinology.*
- M. J. Fuxjager, J. Barske, S. Du, L. Day and B. A. Schlinger (2012). Androgens regulate gene expression in avian skeletal muscles. PLoS ONE 7(12): e51482
- M. J. Fuxjager, J. D. Schultz, J. Barske, N. Y. Feng, L. Fusani, A. Mirzatoni, L. B. Day, M. Hau and B. A. Schlinger (2012). Spinal motor and sensory neurons are androgen targets in an acrobatic-like bird. *Endocrinology* 153: 1–12
- K. A Rosvall, C. M. Bergeon Burns, J. Barske, J. L. Goodson, D. Sengelaub, B. A. Schlinger, and E. D. Ketterson (2012). Neural sensitivity to sex steroids predicts individual differences in aggression: implications for behavioral evolution. *Proc. R. Soc. B* 279(1742): 3547-3555
- J. Barske, B. Schlinger, M. Wikelski and L. Fusani (2011). Female choice for male motor skills. *Proc. R. Soc. B.* 278, 1724: 3523-3528
- N. Feng, A. Katz, L. Day, J. Barske and B.A. Schlinger (2010). Limb muscles are androgen targets in an acrobatic tropical bird. *Endocrinology* 151: 1042-1049

Scientific Meeting and Workshop Participations

- Barske J., Umar S., Eghbali M., Kosarussavadi S., Choi E., Schlinger B. Cardiac adaptations to an energetic courtship display in a tropical bird. ISBE 2012. Poster Presentation
- Barske J., Schlinger B., Billo T., Fusani L.Mate choice influences differential introgression in a hybrid zone zone. EOU 2011. Poster Presentation
- Barske J., Fusani L., Wikelski M., Schlinger B. Heart rate as an index of increased metabolic output in a bird with a complex courtship display. SCIB 2011. Oral Presentation
- Barske J., Fusani L., Wikelski M., Schlinger B. Heart rate as an index of increased metabolic output in a bird with a complex courtship display. ECBB 2010. Oral Presentation
- Barske J., Fusani L., Schlinger B. Ist das Balzverhalten des Goldhalspipra (*Manacus vitellinus)* ein Resultat der Sexuellen Selektion? 30. Tagung der Gesellschaft feur Tropenornithologie 2009. Oral Presentation
- Barske J., Fusani L., Schlinger B. Sexual selection and the courtship display of the Golden-collared manakins (*Manacus vitellinus).* Edward Grey Institute Student Ornithological Conference 2009. Oral Presentation
- Barske J., Belloni V., Fusani L. How is sexual selection involved in display evolution in Golden-collared manakin. NAOC 2006. Poster Presentation
- Barske J., Belloni V., Plantamura G., Fusani L. Influenza della selezione sessuale sugli aspetti comportamentali del display di corteggiamento di *Manacus vitellinus*. 22° Convegno Nazionale della Società Italiana di Etologia 2006. Poster Presentation

CHAPTER 1

Introduction

Animal courtship behaviors are true wonders of nature. Calling frogs, head-bobbing lizards with exposed dewlaps, singing birds or roaring deer have always amazed naturalists. They also posed a puzzle to Darwin's theory of evolution and he proposed sexual selection as the driving force for such spectacular behaviors (Darwin 1871). The broad interest in animal courtship has led to many studies, forming a specialized discipline within the science of ethology. The uniqueness of many courtship behaviors has also triggered the interest of scientists intent no integrating the study of animal behavior with studies of animal physiology. In this chapter I will discuss some major findings from this integrated approach on the study of courtship behavior with a focus on my study species, the golden-collared manakin (*Manacus vitellinus* or manakin hereafter). I will discuss areas that to my knowledge have received little attention and how their investigation will contribute not only to the understanding of the evolution of such astonishing behaviors, but might also provide insights to help advance other disciplines, in particular avian endocrinology and cardiovascular biology.

1.1 A Brief Review on Sexual Selection

Darwin defined sexual selection as resulting from the "struggle between individuals of one sex, generally the males, for the possession of the other sex" (Darwin 1871). Sexual selection is an intraspecific process and includes male-male competition, female choice in mate selection as well as sexual conflict. Based on observations that males experience greater variation in number of mates than females, as well as a direct correlation between number of mates and reproductive success, Bateman's principle proposes that one sex (typically males) competes for the other one, which is considered the limiting sex, as it invests the most in producing offspring (Bateman 1948 but see Gowaty et al. 2012).

Secondary sexual characters are sexual features that are not part of the reproductive system; they are sex-specific and appear at sexual maturity. Generally these characters are very conspicuous anatomical or morphological traits such as the peacock's tail, the antlers of deer, the plumage coloration of birds or they are behavioral traits such as the songs of birds and their courtship displays. Darwin (1871) proposed the mechanism of sexual selection where female preference for the exaggerated trait leads to a gain in reproductive success, which outweighs its negative effect on the male being burdensome and consequently not favored by natural selection; but Darwin could not fully explain the evolution of female preference for such traits.

Fisher's (1930) explanation was that female preferences for a trait can evolve due to an arbitrary initial preference. A prerequisite for this model to work is the genetic link of male trait and female preference for choosing a mate based on the trait. Sons of those females who reproduced with males possessing the favorite trait will inherit the trait and, as adults, will themselves be chosen by females, the daughters which in turn express the preference trait. This gives way to a runaway process, increasing female preference for more extreme traits, until natural selection counteracts further evolution of this extreme trait (Fisher 1930). Based on Fisher's hypothesis, Lande (1980, 1981) and Kirkpatrick (1982) used different mathematical models to explore the behavior of genes coding for the male trait and the female preference. They showed that even without an initial selective advantage of the male trait, the frequency of the gene encoding the male trait depends on the frequency of the female preference gene. Kirkpatrick used a haploid two-locus model to find a line of equilibrium for the model along which the force of sexual selection, driven by the female preference, is balanced by the force of viability selection in the male trait (Kirckpatrick 1982). Depending on the strength of viability selection and mating preference, Lande and Kirckpatrick confirm the theoretical possibility of the runaway process.

Another way of explaining the evolution of secondary sexual characters is based on the assumption that selection of female preference occurs only if the preferred mate is favored by natural selection (Zahavi 1975). Zahavi (1975) suggested that sexual selection evolves when the selecting sex can detect the quality of the selected sex through its secondary sexual characters. Zahavi stated “sexual selection is effective only by selecting a character which lowers the survival of the individual” and his handicap principle states that there is a differential cost to the secondary trait, which can be afforded only by high quality individuals (Zahavi 1975). The term “indicator mechanism” is used to describe this process by pointing out the fact that the trait is an index of general viability and condition of the male (Andersson 1994a). Grafen showed in a model using a single haploid locus encoding for both preference and male trait, that the indicator mechanism can cause the exaggeration of male traits and female preference without the self-reinforcing process described by Fisher due to genetic covariation of female preference and male trait (Grafen 1990). The indicator mechanism also includes a functional approach to the evolution of secondary sexual traits like courtship. The physiological specializations that animals exhibit in the performance of their acrobatic displays for example might be directly related to their overall performance in the environment (Lailvaux & Irschick 2006). For example enhanced muscular functioning might enable them not only to perform acrobatic displays but also to be very efficient in predator escape. Thus, the cost of muscular hypertrophy could be outweighed by their fitness advantage not only through sexual but also natural selection. Many different genes are involved in performance, thus the trait might also function as an indicator of genetic quality.

In the case of birds, males not involved in parental care can afford to develop such extreme characters whereas females that must stay on a nest do not develop ornaments that would expose them (and their nests) to predators and thus reduce their fitness. In polygamic systems males mate with multiple females, so being a preferred male is so advantageous as to further promote the evolution of exaggerated ornaments or behaviors that females prefer. Theoretical studies that have tried to distinguish between Fisher's and Zahavi's hypotheses, indicate that the evolution of extreme traits under the indicator mechanism is more likely with the basic Fisherian condition, that is, a genetic correlation between ornament and preference. In fact, if the indicator trait is heritable, females will benefit through both the "good genes" passed to their offspring, and/or through the increased reproductive success of having attractive sons. Therefore it is misleading to see these as distinct hypotheses (Mead & Arnold 2004).

To better understand the underlying mechanism of the evolution of such extreme traits, it is important to identify the characters that are the basis of female choice for certain males and subsequently determine underlying physiological systems and their potential role in male fitness.

1.1.1 Secondary sexual characters as honest indicators

Females often choose males according to the quality and size of their ornamentation (Kirkpatrick & Ryan 1991; Andersson 1994b). A familiar and well-studied example of sexual selection is the peacock's tail. Peahens choose males based on the pattern of the coloration (Petrie et al. 1991). Following the indicator hypothesis, one explanation of the female choice states that the color of the tail feathers is related to parasite load; brighter males have fewer parasites reducing the likelihood of their transmission to the female when copulating or fewer parasites may reveal stronger parasite resistance (Hamilton & Zuk 1982). The ornament might be related to the immunocompetence of the individual in the sense that an individual with a good immune system is better at fighting off parasites and fewer parasites allow him to grow a longer tail (Moller & Petrie 1996). Manning (1985) hypothesized that the ornament indicates male age; choosy females should prefer older males as an indication of genetic quality as these males have survived to advanced age. Thus, once the criteria used for mate choice by the female are identified, it becomes interesting to understand why the female is choosing them and how they reflect the male's genetic quality. A study on the cost of the tail and display of the Jackson's widowbird (*Euplectes jacksoni*) concluded that tail length indicates past investment i.e. energy expenditure during tail growth, whereas display rate and attendance on the lek represented actual metabolic expenditure. Thus both traits are proposed to indicate male quality (Andersson 1994b).

The evolution of courtship traits has been explained by the preference of females for both ornaments and behaviors each as indicators of male quality. However, males of many species perform physically elaborate courtship displays and we do not know why the specific choreographed and acrobatic aspects of elaborate displays evolve. Not uncommonly, animal studies have related displaying intensity and categories of courtship behavior to mating success. Fundamental frequencies of roars of red deer (Charlton et al. 2009), calling rate of crickets (Hunt et al. 2004), leg raising rate in wolf spiders (Hoefler et al. 2008), time engaged in display behavior as well as the number of vocalizations, displays and flights of greater prairie-chickens (Nooker & Sandercock 2008) are all good indicators of mating success. Many male members of the avian family Pipridae, or manakins, perform astonishing courtship displays that include physically complex movements such as acrobatics, jumps and powerful wingsnaps. Little is known, however, about the forces that shape the evolution of these manakin displays.

To help explore some of these outstanding questions, I applied an integrated approach to study evolution of courtship behavior in a species of manakin. The data are presented in the following individual chapters:

Chapter 2) Mate choice in golden-collared manakins

Chapter 3) The role of females in courtship displays

Chapter 4) Energetic demands of courtship

Chapter 5) Specializations of underlying physiological systems with a focus on cardiovascular systems.

To fully appreciate the power of the manakin as a model organism to study, I will provide additional details of the bird's behavioral ecology and physiology that have been already described in previous studies.

1.2 Manakins (Pipridae)

Manakins (Passeriformes; Pipridae) perform some of the most conspicuous and physically complex displays in the animal world that include the performance of acrobatic routines and the production of non-vocal, mechanically produced sounds or sonations (Chapman 1935; Prum 1998; Castro-Astor et al. 2004). Manakins, a family of neotropical suboscine passerines, have evolved the capacity to produce mechanical sounds by movements of the wings or tail first described by Chapman (1935). These sounds vary in intensity and frequency and the different species are characterized by repertoires, which contain up to four distinct sounds (Prum 1998). Manakins are also studied for their visual signaling. Many show substantial sexual dimorphism and males use their colorful plumage to attract females (Stein & Uy 2006). Males carefully choose a certain orientation and level of luminosity to maximize the visibility of their plumage in respect to the background (Heindl & Winkler 2003). Multimodal signaling appears to play a major role in manakin courtship, in fact its importance was underlined by a phylogenetic analysis that suggested that plumage coloration in manakins evolved to enhance their elaborate courtship movements (Prum 1990).

Many members of the Pipridae family are polygamic. Males often aggregate in areas, the so-called leks, where each male exhibits his courtship display on his own arena. Females benefit from the simultaneous presence of males on a lek in order to compare multiple males before accepting a mate and mate choice has been studied in a range of species of this family. DuVal and Kampenaers (2008) studied influences on individual reproductive success of both forces, male competition and female choice, in the lance-tailed manakin, *Chiroxiphia lanceolata*. Males of this species compete for alpha status while performing paired courtship displays, as only alpha males mate; female choice only occurs between alpha males. Investigating differences in within status and between status reproductive success, they found that male-male competition in order to become an alpha male accounts only for about 1/3 and female selection i.e. being chosen between the alpha males, accounts for about 2/3 of the reproductive success obtained by a male. Trainer et al. (2002) examined vocalizations that are part of courtship displays in the long-tailed manakin (*Chiroxiphia linearis*). As in *C. lanceolata*, males also perform in pairs, consisting of an older alpha male and a younger beta male that almost never mates. Females appear to prefer frequency-matched sounds and males gain competence in duet singing with age. The cooperative behavior of beta males is explained by the fact that although young males have only few mating opportunities, they use the time to cooperate with alpha males to attract females, in order to improve their singing quality with the prospect that they will replace the alpha male upon his death. Male mating success on a lek is usually strongly skewed, as has been shown also for the golden-collared manakin (Höglund & Alatalo 1995; Stein & Uy 2006). This provides a good basis for sexual selection to occur as evidenced by the extremely specialized traits that evolve in many lekking species.

1.2.1 The Golden-collared manakin (*Manacus vitellinus*)

The Golden-collared manakin (*Manacus vitellinus*) lives in the secondary forests of Panama. At the beginning of the breeding season (January to August), each adult male of a lek (3-12 males per lek) clears an arena on the forest floor surrounded by small saplings. On this elliptical area (30 x 50 cm), males perform their acrobatic jump-snap displays, bouncing from one sapling to the next while flipping up their wings in midair producing a loud cracking sound, the wingsnap. Males produce another mechanical sound when perched, the so-called rollsnap, a series of 12 or more wingsnaps at a frequency of 50-70 Hz. In addition, they emit vocalizations (e.g. chepoos) using their syrinx, the avian vocal organ. The different vocalizations seem to be directed to both potential mates and to other males (Mcdonald & Potts 1994). Periodically, males will chase one another around a lek, otherwise, males show few aggressive behaviors towards each other (personal observations). Because males provide no benefit to their mates except their sperm, courtship is the only factor that females can use to assess their mate. Male ornaments and the position of the courtship arena within the lek are important factors for female choice (Shorey 2002; Stein & Uy 2006) but they explain only a fraction of the mating success.

Females are attracted to the leks by the noisy activity of male birds. They usually stay in the lower canopy to observe males until they decide to join a male in what has been called a 'duo dance'. Using high speed videography, Fusani et al. filmed the courtship display of several males and using frame-by-frame analysis, found that males significantly differed from each other in various components of the display (Fusani et al. 2007b). In slow motion, the rapid jumping - almost bouncing between the branches- dissolves in a highly acrobatic performance. In fact, the differences between males amount to only tens to hundreds of milliseconds.

Given the higher flicker fusion rate in birds versus humans (birds: 100-120 Hz; humans: 25-30 Hz), I suspected that females are able to distinguish different males by their display and thus the interest in understanding its importance as a signaling trait. Using high-speed videography to investigate the acrobatic courtship display of wild male golden-collared manakins, while also recording their mating success, I was able to quantify several behavioral elements of the display and measure their relevance in female choice. Females prefer males that produce more mechanical sounds, the wingsnaps and rollsnaps, and that differ in certain dance moves, for example reaching the stationary position with their beards exposed by tens of milliseconds faster (more detailed description see Chapter 2). Based on evidence that females prefer males that perform their displays with exceptional neuromuscular capabilities, I hypothesized that males perform with even greater skill in the presence of females during the 'duo dance' when the female makes her final decision. **I tested this hypotheses by analyzing high-speed video recordings to document the behavior of males displaying with and without a female.** These data indicate that indeed components of male courtship are performed faster when a female is present, and, when a duo dance is performed, the female takes the lead, suggesting that she is indeed challenging the male to perform. These data are presented in Chapter 3.

1.3 Costs of Courtship in the Tropical Environment

As described above in mating systems where males contribute nothing else than sperm to the offspring, females base their mate choice on indicators of quality. Male quality is expressed in his physical condition, which in turn may be reflected by immunocompetence, parasite load, resistance to predators, heterozygosity, or his ability to maintain a high metabolic rate (Hamilton & Zuk 1982; Brown 1997). **As manakins have such highly physical courtship displays, I hypothesize that these behaviors entail high metabolic costs, which might serve as an indicator of quality.**

When exploring physiological performance traits, aerobic capacity, defined as the maximal rate of oxygen consumption or the maximum rate of oxygen inhaled and delivered to the muscles, is a possible indicator of good genes (Chappell et al. 1996). Aerobic capacity can refer to high-energy expenditure during short, very intense exercise or also to less intense exercise performed over a longer period of time. High aerobic capacity is essential for physical endurance during exercises like elaborate courtship displays. High aerobic capacity allows increased performance for such energetically demanding behaviors and therefore might give an advantage to the fitness of the individual. It is a crucial component of an organism's physiological condition and therefore might provide an honest signal of the male's quality. In this way females may use courtship displays to assess aerobic capacity of potential mates (Chappell et al. 1996). Studies on jungle fowl have shown sexual dimorphism in aerobic capacity. This finding proposes that there might be a trade-off to high aerobic capacity. If there were no associated costs, but only a benefit to the individual's survival, male and females should not differ in aerobic capacity. Hence the sexual dimorphism in aerobic capacity indicates that this trait might play an important role in sexual selection (Chappell et al. 1999). The cost associated with high aerobic capacity comes from the fact that high levels of activity, as during these demanding courtship displays, require great amounts of energy. To maintain a high metabolic rate, and so a high aerobic capacity, the organism requires sufficient water, oxygen and calories (food). The ability to maintain a high metabolic rate and its associated costs of living reflects the male's good overall condition.

Metabolic costs of courtship or aggressive behaviors involved in mate attraction have been investigated in several animal taxa by means of various methods. Increased weight loss in successful anurans suggests that calling, which is used to attract females, is energetically costly (Wells 2001). In contrast, no correlation between body condition and courtship effort is found in displaying in *Anolis* lizards (Hurd 2004). Several studies have looked at agonistic behavior in crabs; winners obtain most of the matings and usually have a higher resting metabolic rate (Briffa & Sneddon 2007).

Vehrencamp et al. (1989) studied the energetic costs of display behavior of the sage grouse, *Centrocercus urophasianus*. Several pieces of evidence indicated that the display is costly: displaying males lost weight, display rates decreased during the day and leks were attended less during cold days. All the cues preferred by females required continuous energy. The authors used doubly labeled water to measure daily energy consumption, which was found to be positively correlated to display effort, which in turn was related to mating success. In addition, more active males recovered a larger fraction of their daily display costs by feeding than less active males proposing the high quality of preferred males. A study on the costs of the displays of the great snipe (*Gallinago media*) used the same variables and concluded that actively displaying males were at the maximum metabolic rate they can sustain and lost weight over a period of time, which according to the authors indicated that the display behavior is costly

(Höglund et al. 1992). Another study on the costs of the courtship display of great frigatebirds (*Fregata minor)* found no linear relationship between metabolic rate and proportion of time spent on display. These results were equivocal, however, because low-ability males spent significantly more energy on displays than successful males (Dearborn et al. 2005). Overall the results encountered are inconsistent and further investigations on the energetic costs of courtship behavior are needed in order to understand how they can serve as honest signals.

An alternative method to investigate energy consumption is via heart rate telemetry, which enables the researcher to obtain instantaneous estimates of energy expenditure of freely moving animals. Advanced transmitter designs now allow for measures of heart rate even in small passerines like the manakins that weigh only 18 grams and has the potential to provide detailed insight into the energetics of courtship.

This method relies on Fick's convection equation, which shows that the heart rate is a major component in the response of the cardiovascular system to an increased oxygen demand (Fick 1870). The animal is captured only once to implant the heart rate transmitter and to determine the relationship between heart rate and oxygen uptake. One disadvantage to the use of heart rate to measure energy expenditure is that the linear relationship between heart rate and oxygen uptake can easily be disturbed by other factors such as body temperature. The stress of the surgery to implant the transmitter may be another disadvantage. Two studies (Bevan et al. 1995; Butler et al. 2004) have compared the following methods used to measure energy expenditure: respirometry, doubly labeled water and heart rate. Respirometry has limited use in field studies as the exact amount of oxygen and carbon dioxide exchange needs to be measured. This is normally accomplished by placing the animals in special open-flow respirometer chambers. The doubly labeled water technique, which uses stable isotopes of hydrogen and oxygen to trace over time the flow of water and carbon dioxide through the body, has greater utility in the field, as the animals are able to move freely once the doubly labeled water is injected. The drawback, of course, is that only average metabolic rate over the experimental period can be obtained. A further burden is that the respiratory quotient (CO_2 removed/O_2 taken up) of the animal has to be known in order to estimate the energy expenditure from the carbon dioxide production. Another drawback is that animals have to be captured to obtain blood samples, but several recent improvements to the technique, including the presentation of isotope-injected dead insect prey (Anava et al. 2002) and the elimination of recaptures extracting water from animal droppings (Haggarty et al. 1998) have addressed this. Nevertheless, the doubly labeled water procedure has limited utility for the study of many species and the advancing technique of heart rate transmitters provides a great alternative.

The manakins appear as an interesting study organism to investigate costs of courtship behavior as the production of mechanical sounds and the acrobatic display of the Golden-collared manakin require complex neuromuscular control of the wing and leg muscles. These muscles are sexually dimorphic, being hypertrophied in males, suggesting they incur a cost to male manakins (Schultz et al. 2001, see below). These complex and energetically demanding displays are perfect candidates for being honest signals in terms of high metabolic rate requirements. Most of the studies that investigated energetics of courtship behavior have been performed in the temperate regions (see above). The tropics are of particular interest as the lack of seasonality provides a mild environment: no drastic temperature changes and a rather constant availability of food. Animals living in the tropics are said to have a slow pace of life, which is associated to a low metabolic rate (Wiersma et al. 2007a; Wiersma et al. 2007b). This is valid in particular for animals living in lowland tropical forest as altitude or desert environments might change conditions. As manakins have several traits typical for the slow pace of life including a long life span (~ 14 years; Snow & Lill 1974; Karr et al. 1990; Brawn et al. 1995) and small clutch size (1 – 2 eggs; Ricklefs 1976; Skutch 1985), I was interested in understanding how they manage this apparent trade off between high costs of courtship display and slow pace of life and measured heart rate as a proxy of energy expenditure in reproductively active manakins.

Using transmitters that weigh only ~ 1g, I recorded heart rates of free-living manakins during their courtship season. Heart rates reach peaks of 1300 beats per minute while displaying, but average heart rates are low, comparable to other tropical birds. These data are presented and discussed in Chapters 2 and 4.

1.4 Physiological Systems Underlying Acrobatic Courtship

In order to perform such physically complex behaviors, males likely develop special physiological adaptations such as specialized neural circuits or muscles, but little is known about the evolution of such traits. It is known that sex hormones play a major role in activating and maintaining such characteristics (Wingfield & Farner 1980). In order to optimize courtship success, males develop physiological adaptations for courtship conferring additional costs to the male. Investigating such physiological adaptations related to courtship opens another venue in the study of sexual selection, that is, the measurement of physiological costs (e.g. hypertrophied muscles) in relation to mating success. In considering the development of these adaptations, it is important first to consider the role of sex steroids in the avian annual cycle as well as their influence on different muscles.

1.4.1 Role of sex hormones in the annual cycle of birds

Most birds have an annual reproductive cycle that is divided into phases of breeding and non-breeding. When breeding the gonads enlarge and birds engage in reproductive behaviors like courtship and nesting. They may also acquire other secondary sexual characters like growth of a breeding plumage or anatomical features used during courtship, such as wattles and combs of chickens. During the non-breeding period these characters can be reduced and reproductive behaviors are seldom expressed if at all. It is generally agreed that two factors signal the onset of the breeding period: proximate factors like change in photoperiod, social and environmental cues and/or ultimate factors like food availability. In temperate birds after the winter solstice, the hypothalamic-pituitary-gonadal axis responds to increasing day length by secreting pituitary gonadotropins that induce an increase in gonadal growth and sex-steroid secretion (Ball et al. 2002). These sex-steroids induce the growth of secondary sexual characters and activate reproductive behavior (Wingfield & Farner 1980; Wingfield & Silverin 2009). In the tropics breeding is still periodic, but as seasons are less predictable through photoperiod, breeding duration varies and is less synchronous across species and factors signaling the onset of breeding are mostly of short-term environmental property e.g. end of the rainy season (Immelmann 1971).

The testes are the principal site of testosterone (T) synthesis; in temperate breeding male birds elevated levels of circulating T activate and maintain courtship, copulatory and territorial aggressive behaviors (Balthazart 1983). During the non-breeding season sex-steroids may be unimportant in temperate breeding birds. For example, T levels in wintering stonechats (*Saxicola torquata*) are low during the winter, when T is not required for pair-formation or aggression (Gwinner et al. 1994). In recent studies it has been shown that the neuroendocrine control of tropical breeding birds differs from that of birds breeding in temperate regions. Many tropical birds breed year round and it has been shown that T can circulate at relatively low levels throughout the year in these species (Wikelski et al. 2003). Goymann et al. (2004) showed that the level of T in tropical birds is inversely correlated to the length of the breeding season, allowing higher efficiency in shorter time, and directly correlated to the altitude of the habitat, allowing more T in higher altitudes due to decreased presence of parasites in tropical highlands compared to lowlands. These explanations are based on the immunosuppressive actions of T (Folstad et al. 1992). A study on year-round territorial spotted antbirds has shown that plasma T levels are low and show no correlation with territorial behavior in reproductively inactive males. It has been suggested that the low plasma T levels may be offset by increased androgen metabolism or steroid-receptor-binding in the brain to activate territorial aggression (Canoine et al. 2006).

Within the brain, T activates neural circuits controlling behaviors usually after conversion into its active metabolites i.e. estradiol (E_2) and dihydrotestosterone (5α-DHT). For example, the estrogen synthetic enzyme aromatase is expressed in the brain where it can convert T to E_2 to activate estrogen-dependent pathways (neurons that express estrogen-receptors). Estrogens have been shown to activate masculine behaviors like copulation and birdsong (Schlinger & Brenowitz 2009). Experiments on wintering song sparrows have shown that E_2 is modulate territorial aggression and song (Soma et al. 2000). Another study has shown that the presence of aromatase in the brain regulates certain courtship behaviors in male ring doves (Fusani et al. 2001).

By contrast, T can also be acted upon by 5α-reductase that converts T into 5α-DHT that activates androgen-dependent pathways (neurons that express androgen-receptors; AR). The androgen 5α-DHT is more potent than T because it binds to the androgen receptor (AR) with about 4-times higher affinity than T (Grino et al. 1990). In the avian brain, androgens participate in the growth and activation of neural circuits controlling song (Balthazart 1983) and activate some patterns of courtship behaviors in doves, domestic fowl and quail (Fusani 2008).

Sex steroid hormone receptors, ERs (estrogen receptors) and ARs, can work via two different pathways. There is the slow-action, genomic pathway, where the lipid-soluble steroid crosses the cell membrane and binds to intracellular receptors, which moves to the nucleus where the complex acts as a transcription factor regulated by cofactors. This causes a slow response due to modulation of gene expression. In the more recently discovered fast-action pathway, the steroid binds to a membrane receptor, which through secondary messengers changes the physiological state of the cell for example regulating the cell's ion transport (Heemers & Tindall 2009).

1.4.2 The effect of androgens on muscles

T acts not only on the brain, but ARs are also found in some striated muscles, indicating a direct effect of steroids on muscle. The presence of ARs has been shown in muscle involved in reproduction like the levator ani muscle of mice (Antonio et al. 1999; Johansen et al. 2007) but also in other skeletal muscles (Frogs: Thibert 1986). Castration or treatment with a T antagonist (flutamide) caused atrophy and demasculinization of the perineal muscles of male rats, which could be prevented by co-administration of T (Breedlove & Arnold 1981,1983; Forger & Breedlove 1987). The effects of androgens on skeletal muscles include the increase in muscle mass through an increase in protein synthesis (Scow & Hagan 1957; Bhasin et al. 2001; Ustunel et al. 2003) and an inhibitory effect on adipogenesis (Herbst & Bhasin 2004). In fact, AR in myocytes have been shown to improve body condition by reducing fat mass (Fernando et al.

2010). In murine myoblasts in culture it has been shown that AR associates with the DNA binding protein Serum Response Factor in order to activate genes, which stimulate muscle growth, in particular skeletal α-actin (Vlahopoulos et al. 2005). T also increased the recruitment of satellite cells (stem cells present in between the basement membrane and the sarcolemma, the cell membrane of a muscle cell) to form myoblasts, progenitor cells of myocytes, increasing myofiber size by cell fusion. In addition, T caused an increase in muscle power and velocity by increasing the number of fast twitch fibers (Pansarasa & Antona 2002; Mänttäri et al. 2008) and the aerobic capacity by enhancing myoglobin expression (Mänttäri et al. 2008). Myoglobin is a cytoplasmic hemoprotein expressed in oxidative skeletal muscles and cardiac myocytes and functions as an oxygen store for the muscle. Steroid effects, like those described above, might allow these hormones to influence adaptations in muscles involved in the performance of specialized behaviors like the courtship displays of male manakins.

1.4.3 Role of sex steroids in manakin courtship

Previous findings indicate that T activates male manakin courtship behavior. During the non-breeding season, when T-levels are low in manakins, T-treatment increased courtship behavior in adult males in the wild (Day et al. 2006). Consistent with this suspected role of T, there is a peak of T at the beginning of the breeding season (Fusani et al. 2007a). Other studies found a significant seasonal change in circulating T levels in adult male manakins accompanied by a change in testes volume (Wikelski et al. 2003; Day et al. 2007).

The immunocompetence handicap hypothesis states that there is a trade-off in having high circulating T. On one side T is increasing the sexually selected trait but at the same time it compromises the immune system (Folstad et al. 1992; Edler et al. 2011). After this definition, high T concentrations are affordable only by high quality males, which by means of honest signals get selected through female choice. T in breeding manakin males is circulating at varying concentrations, but I have not found a correlation between T levels and courtship behavior or mating success (Barske et al. unpublished data), providing no evidence for the immunocompetence hypothesis. The lack of correlations of T levels with mating success might be explained by the fact that T plasma levels might vary depending on e.g. social stimuli (Wingfield 1985), and thus it might be difficult to find a consistent difference between males. On the other hand, difference might not lie in circulating T but in differences in the sensitivity to T in various tissues, seen for example in the different number of ARs present. Thus investigating the presence of sex hormone receptors in different tissues establishes which tissues are the targets of these hormones and thus where individual differences in sensitivity might be observed.

In the manakins, first of all there is evidence that, for the activation of courtship behavior, T acts at least in part through AR and not through its metabolites like E_2. Adult male manakins were implanted with a time-release pellet containing flutamide, an AR blocker, at the beginning of the courtship season. After one week there was a significant reduction in courtship activity. This is convincing evidence that flutamide interferes with the activation of courtship behavior confirming that androgens play a role in activating this behavior. Unexpectedly, there was an increase in courtship activity after three weeks of the implantation (Fusani et al. 2007a). The reason for that is not clear but authors still concluded that ARs play a role in controlling courtship behavior. Our lab found also that co-administrating T and fadrozole, an aromatase inhibitor, had limited effects on courtship behavior in adult breeding males and T-treated non-breeders (unpublished data). This suggests that the activation of courtship behavior in male manakins largely involves androgen-dependent pathways with estrogen playing little or no significant role.

ARs are found in various regions of the manakin brain. One particular region is the caudal archistriatum in the forebrain which in vocal learning song birds contains an androgen-sensitive vocal promotor nucleus. As manakins do not have complex songs, this unique AR expression for a suboscine may be related to the control and learning of their elaborated courtship display that also involves auditory signals (Fusani et al. 2003). Aromatase expression was found in several telencephalic loci and in the diencephalon as well as in the cerebellar cortex. This suggests that the manakin forebrain may be capable of synthesizing estrogen, a feature shown only by oscine passerines (Saldanha et al. 2000). Finally, ER expression was found in the septum, the preoptic-hypothalamic areas and the hippocampus (Fusani et al. 2003).

Expression of AR is also seen in the spinal cord and wing muscles of manakins. It has been shown that muscles involved in the production of mechanical sounds during the courtship behavior are sexually dimorphic with the males showing hypertrophy in these muscles (Chapman 1935; Lowe 1942; Schultz et al. 2001). Two muscles (SH: M. scapulohumeralis caudalis; SC: M. supracoracoideus) involved in the lifting of the wings essential for the production of the wingsnaps, are larger with a larger fiber diameter and more fibers characterized as fast twitch (Schultz et al. 2001). Fast twitch fibers compared to slow twitch fibers can hydrolyze ATP more quickly and have a faster electrochemical transmission, so can contract faster. Males express more ARs in the cervical and lumbosacral enlargements of the spinal cord. In particular, AR mRNA has been encountered in motoneurons controlling wing and leg muscles, but also in afferent fibers transmitting somatosensory feedback from periphery to CNS (Schultz & Schlinger 1999; Fuxjager et al. 2012b). This suggests that neuronal circuits underlying the courtship behavior can be activated by T. It has been shown that male wing muscles contain higher AR mRNA expression than two non-displaying passerine species, the zebra finch (*Taeniopygia guttata)* and ochre-bellied flycatcher (*Mionectes oleagineus)* (Feng et al. 2010). AR expression in muscles is more robust than in the brain or spinal cord and its importance has been emphasized through additional experiments. T has been shown to change the transcriptional program of several muscles, increasing IGF1 and parvalbumin, two genes important for muscular growth and contraction (Fuxjager et al. 2012a). This AR-dependent regulation of gene expression might be how manakins optimize muscle performance required for a successful courtship.

The idea of sexually dimorphic organization of neuronal circuits is disputed by the findings that in females, which normally do not perform displays, and juvenile males, T causes the performance of some courtship behaviors (Day et al. 2007). This is the first evidence that females can produce mechanical sounds similar to the ones used by the males during courtship. In fact, this is confirmed by the lack of sex differences of AR mRNA expression in wing muscles (Feng et al. 2010) as well as spinal cords (Fuxjager et al. 2012b). The difference in behavior between the sexes likely stems from differences in circulating T between males and females during the breeding season (Day et al. 2007).

These findings give insight into how trade-offs in the transcriptional program of a cell or tissue may ultimately facilitate and coordinate shifts in life history tactics, such as the transition from non-breeding to breeding states and how the steroid hormones might play an important regulatory role.

The preceding material has focused on tissues and systems that have conserved functions in courtship. As I am investigating cardiovascular functioning during courtship, to complete the picture of physiological systems underlying the acrobatic courtship display of manakins, I am interested in the manakin myocardium and its interplay with T.

1.4.4 The myocardium

The myocardium is the cardiac muscle, a type of involuntary striated muscle found in the walls of the heart. The cells that comprise cardiac muscle are called cardiomyocytes. Cardiac myocytes represent 75% of cardiac mass, but only 30% of cardiac cells. Other non-myocytes in the heart include smooth muscle cells, endothelial cells and fibroblasts, the latter making up 90% of the non-myocyte cells. This muscle has very strong endurance and so relies highly on aerobic respiration. It consequently contains a large number of mitochondria, numerous myoglobins and a robust blood supply through the coronary arteries. Contrary to smooth and skeletal muscles, the myocardium requires extracellular Ca^{2+}, which enters the cells through L-type Ca^{2+} channels and sustains depolarization after Na^{+} entry. This influx of Ca^{2+} causes further release of internal Ca^{2+} from the sarcolemma, which in turn causes the excitation and contraction of the muscle. For many muscles, their relaxation is evoked by the contraction of their counterpart in the example given above, the SC lifts the wings and the pectoralis lowers them. Instead in the heart, the relaxation of the heart, the diastole, with the refilling of the chambers is a passive process and thus fast removal of Ca^{2+} is crucial for fast contraction. SERCA and Na^{+}/Ca^{2+} exchanger are two crucial players in cardiac relaxation (see Iaizzo 2009).

Male golden-collared manakins spend half of the year on their leks performing their highly acrobatic and energetically demanding courtship behaviors when heart rates rise to extremes of 1300 b/min (Chapter 2) only recorded in smaller hummingbirds during their highly energetically demanding hovering flight. **I hypothesize that the manakin cardiac muscle should display specializations in order to sustain such high heart rates during most of the**

days in the 7-month long breeding period. I found, that the manakin myocardium has an increased thickness and in addition an increased expression of Ca^{2+} handling proteins in the manakin heart. These data are described in Chapter 5.

Given the apparent importance of T in manakins due to the high androgen sensitivity in several tissues and the demonstrated role in regulating courtship behavior, I hypothesize that T plays a role in regulating cardiovascular functioning during courtship. As I will show, I found increased high androgen sensitivity in the manakin heart, which is discussed in Chapter 5.

McGill et al. found evidence of androgen binding proteins in the myocardial cells of two non-human primate species (McGill et al. 1980). Golden (2003) confirmed the presence of AR in cardiac myocytes of male rats. They also identified a positive inotropic (increased force of muscular contraction) effect of T on myocytes. Gonadectomy reduced contractile velocities of isolated male rat ventricular myocytes, which is restored by T (Golden et al. 2003). Gonadectomy of prepubertal male and female rats causes depressed ventricular function and contractile performance, decreases in ventricular myosin ATPase activity and shifts in myosin heavy chain isoenzymes (Schaible et al. 1984). Gonadectomy in postpubertal rats decreased ventricular functioning by altering myosin isoenzyme and ATPase patterns. T treatment in males and T together with E_2 treatment in females prevented such effects. T caused myocardial protein synthesis which gives rise to hypertrophy acting directly or via stimulation of growth hormone secretion (Scheuer et al. 1987). In hypogonadic human males T increased repolarization of the ventricular muscle, i.e. the time for the ventricle to become excitable again is reduced allowing an increased heart rate. In the same study, an inverse relation between the biologically active part of T (the ratio of T over sex hormone-binding globulin) and duration of repolarization has been shown. At the same time, supraphysiologic levels of T in athletes have been shown to cause degeneration of cardiac muscle as well as unhealthy hypertrophies of the left ventricle, smaller ventricular dimensions with thicker walls (Hassan et al. 2009). In summary, there is a diversity of studies pointing to various ways in which sex steroids might influence cardiac function. Using the manakins as a natural model for increased cardiac functioning, which might be induced through steroids, could bring major insights into the field of cardiovascular dysfunction and treatment as cardiovascular disease is the number one cause of mortality in industrialized countries, with high gender disparities up to the age of menopause, suggesting an important role of sex steroids.

1.5 Summary

Combining ultimate and proximate questions, in this work I am attempting to understand the drivers of the evolution of the extremely acrobatic courtship behavior of golden-collared manakins. I have created and tested several hypotheses regarding underlying physiological specializations that enable manakins to perform such acrobatic behaviors. After identifying the basis of female choice, I investigated the energetics of male manakins to understand the potential energetic costs that such courtship displays procure. Subsequently, I investigated the underlying cardiovascular system to obtain insight in how manakins might have armed themselves to withstand such costs.

1.6 References

Anava, A., Kam, M., Shkolnik, A., & Degen, A. A. 2002. Seasonal daily, daytime and night-time field metabolic rates in Arabian Babblers (Turdoides squamiceps). *The Journal of experimental biology*, 205, 3571–3575.

Andersson, M. 1994a. *Sexual Selection.* Princeton, NJ: Princeton University Press Princeton.

Andersson, S. 1994b. Costs of sexual advertising in the lekking jackson's widowbird. *The Condor*, 96, 1–10.

Antonio, J., Wilson, J. D., George, F. W., & Fredrick, W. 1999. Effects of castration and androgen treatment on androgen-receptor levels in rat skeletal muscles. *Journal of Applied Physiology*, 87, 2016–2019.

Ball, G. F., Riters, L. V., & Balthazart, J. 2002. Neuroendocrinology of song behavior and avian brain plasticity: multiple sites of action of sex steroid hormones. *Frontiers in neuroendocrinology*, 23, 137–78.

Balthazart, J. 1983. Hormonal correlates of behavior. In: *Avian Biology*, Vol.VII edn. (Ed. by D. S. Farner, J. R. King, & K. C. Parkes), pp. 221–365. San Diego: Academic Press.

Bateman, A. J. 1948. Intra-sexual selection in drosophila. *Heredity*, 2, 349–368.

Bevan, R. M., Speakman, J. R., & Butler, P. J. 1995. Daily energy expenditure of Tufted Ducks: a comparison between indirect calorimetry, doubly labelled water and heart rate. *Functional Ecology*, 9, 40–47.

Bhasin, S., Woodhouse, L., & Storer, T. W. 2001. Proof of the effect of testosterone on skeletal muscle. *Journal of Endocrinology*, 170, 27–38.

Brawn, J. D., Karr, J. R., Nichols, J. D., Ecology, S., & Jan, N. 1995. Demography of Birds in a Neotropical Forest : Effects of Allometry, Taxonomy, and Ecology. *Ecology*, 76, 41–51.

Breedlove, S. M., & Arnold, A. P. 1981. Sexually dimorphic motor nucleus in the rat lumbar spinal-cord - response to adult hormone manipulation, absence in androgen-insensitive rats. *Brain Research*, 225, 297–307.

Breedlove, S. M., & Arnold, A. P. 1983. Hormonal-control of a developing neuromuscular system .1. complete demasculinization of the male-rat spinal nucleus of the bulbocavernosus using the anti-androgen flutamide. *Journal of Neuroscience*, 3, 417–423.

Briffa, M., & Sneddon, L. U. 2007. Physiological constraints on contest behavior. *Functional Ecology*, 21, 627–637.

Brown, J. L. 1997. A theory of mate choice based on heterozygosity. *Behavioral Ecology*, 8, 60–65.

Butler, P. J., Green, J. A., Boyd, I. L., & Speakman, J. R. 2004. Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods. *Functional Ecology*, 18, 168 – 183.

Canoine, V., Fusani, L., Schlinger, B., & Hau, M. 2006. Low Sex Steroids , High Steroid Receptors : Increasing the Sensitivity of the Nonreproductive Brain. *J. of Neurobiology*, 67, 57-67.

Castro-Astor, I. N., Alves, M. A. S., & Cavalcanti, R. B. 2004. Display behavior and spatial distribution of the Red-headed Manakin in the Atlantic forest of Brazil. *Condor*, 106, 320–335.

Chapman, F. 1935. The courtship of Gould's manakin (Manacus vitellinus vitellinus) on Barro Colorado Island, Canal Zone. *Bulletin of American Museum of Natural History*, 68, 471–525.

Chappell, M. . A. ., Zuk, M. ., & Johnsen, T. . S. . 1996. Repeatability of Aerobic Performance in Red Junglefowl : Effects of Ontogeny and Nematode Infection. *Functional Ecology*, 10, 578–585.

Chappell, M. . A. ., Zuk, M. ., & Johnson, T. . S. 1999. Aerobic Performance Does not Affect Social Rank in Female Red Jungle Fowl. *Functional Ecology*, 13, 163–168.

Charlton, B. D., Reby, D., & Mccomb, K. 2009. Female red deer prefer the roars of larger males Female red deer prefer the roars of larger males. *Society*, 382–385.

Darwin, C. 1871. *The descent of man, and selection in relation to sex.* London, UK: John Murray.

Day, L. B., Fusani, L., Hernandez, E., Billo, T. J., Sheldon, K. S., Wise, P. M., & Schlinger, B. 2007. Testosterone and its effects on courtship in Golden-collared Manakins (Manacus vitellinus): seasonal, sex, and age differences. *Hormones and behavior*, 51, 69–76.

Day, L. B., McBroom, J. T., & Schlinger, B. a. 2006. Testosterone increases display behaviors but does not stimulate growth of adult plumage in male golden-collared manakins (Manacus vitellinus). *Hormones and behavior*, 49, 223–32.

Dearborn, D. C., Anders, A. D., Williams, J. B., Dearborn, C., & Anders, D. 2005. Courtship display by Great Frigatebirds, Fregata minor: an energetically costly handicap signal? *Behavioral Ecology and Sociobiology*, 58, 397–406.

Duval, E. H., Kempenaers, B., & B, P. R. S. 2008. Sexual selection in a lekking bird : the relative opportunity for selection by female choice and male competition. *Proceedings of the Royal Society B Biological Sciences*, 275, 1995–2003.

Edler, R., Goymann, W., Schwabl, I., & Friedl, T. W. 2011. Experimentally elevated testosterone levels enhance courtship behavior and territoriality but depress acquired immune response in Red Bishops Euplectes orix. *Ibis,* 153, 46–58.

Feng, N. Y., Katz, A., Day, L. B., Barske, J., & Schlinger, B. A. 2010. Limb muscles are androgen targets in an acrobatic tropical bird. *Endocrinology*, 151, 1–8.

Fernando, S. M., Rao, P., Niel, L., Chatterjee, D., Stagljar, M., & Monks, D. A. 2010. Myocyte androgen receptors increase metabolic rate and improve body composition by reducing fat mass. *Endocrinology*, 151, 3125–32.

Fick, A. 1870. Uber die Messung des Blutquantums in der Herzventrikeln. *Sitz. Physik. Med. Ges.*, 2, 16.

Fisher, R. A. 1930. *The genetical theory of natural selection.* Oxford: Oxford Press.

Folstad, I., Karter, A. J., Naturalist, T. A., & Mar, N. 1992. Parasites , Bright Males , and the Immunocompetence Handicap. *The American naturalist*, 139, 603–622.

Forger, N. G., & Breedlove, S. M. 1987. Seasonal Variation in Mammalian Striated Muscle Mass and Motoneuron Morphology. *Journal of Neurobiology*, 18, 155–165.

Fusani, L. 2008. Testosterone control of male courtship in birds. *Hormones and Behavior*, 54, 227–233.

Fusani, L., Day, L. B., Canoine, V., Reinemann, D., Hernandez, E., & Schlinger, B. A. 2007a. Androgen and the elaborate courtship behavior of a tropical lekking bird. *Hormones and Behavior*, 51, 62 – 68.

Fusani, L., Donaldson, Z., & Schlinger, B. A. 2003. Distinctive expression of androgen receptor in the brain of a sub - oscine bird with elaborate courtship displays. *Society for Neuroscience Abstract Viewer and Itinerary Planner*, 9411.

Fusani, L., Gahr, M., & Hutchison, J. B. 2001. Aromatase Inhibition Reduces Specifically One Display of the Ring Dove Courtship Behavior. *General and Comparative Endocrinology*, 30, 23–30.

Fusani, L., Giordano, M., Day, L. B., & Schlinger, B. A. 2007b. High-Speed Video Analysis Reveals Individual Variability in the Courtship Displays of Male Golden-Collared Manakins. *Ethology*, 113, 964–972.

Fuxjager, M. J., Barske, J., Du, S., Day, L. B., & Schlinger, B. a. 2012a. Androgens regulate gene expression in avian skeletal muscles. *PloS one*, 7, e51482.

Fuxjager, M. J., Schultz, J. D., Barske, J., Feng, N. Y., Fusani, L., Mirzatoni, A., Day, L. B., Hau, M., & Schlinger, B. A. 2012b. Spinal Motor and Sensory Neurons Are Androgen Targets in an Acrobatic Bird. *Endocrinology*, 153, 1–12.

Golden, K. L., Marsh, J. D., Jiang, Y., Brown, T., & Moulden, J. 2003. Gonadectomy of adult male rats reduces contractility of isolated cardiac myocytes. *American journal of physiology. Endocrinology and metabolism*, 285, E449–53.

Gowaty, P. A., Kim, Y.-K., & Anderson, W. W. 2012. No evidence of sexual selection in a repetition of Bateman's classic study of Drosophila melanogaster. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 11740–5.

Goymann, W., Moore, I. T., Scheuerlein, A., Hirschenhauser, K., Grafen, A., & Wingfield, J. C. 2004. Testosterone in tropical birds: effects of environmental and social factors. *The American Naturalist*, 164, 327–34.

Grafen, A. 1990. Sexual selection unhandicapped by the fisher process. *Journal of Theoretical Biology*, 144, 473–516.

Grino, P. B., Griffin, J. E., & Wilson, J. D. 1990 February. Testosterone at high concentrations interacts with the human androgen receptor similarly to dihydrotestosterone. *Endocrinology*, 126, 1165–72.

Gwinner, E., Rodl, T., & Schwabl, H. 1994. Pair territoriality of wintering stonechats - behavior, function and hormones. *Behavioral Ecology and Sociobiology*, 23, 321–327.

Haggarty, P., Robinson, J. J., Ashton, J., Milne, E., Adam, C. L., Kyle, C. E., Christie, S. L., & Midwood, a J. 1998. Estimation of energy expenditure in free-living red deer (Cervus elaphus) with the doubly-labelled water method. *The British journal of nutrition*, 80, 263–72.

Hamilton, W. D., & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science (New York, N.Y.)*, 218, 384–7.

Hassan, N. a, Salem, M. F., & Sayed, M. a E. L. 2009. Doping and effects of anabolic androgenic steroids on the heart: histological, ultrastructural, and echocardiographic assessment in strength athletes. *Human & experimental toxicology*, 28, 273–83.

Heemers, H. V., & Tindall, D. J. 2009. Androgen Receptor (AR) Coregulators : A Diversity of Transcriptional Complex. *Endocrine Reviews*, 28, 778 – 808.

Heindl, M., & Winkler, H. 2003. Vertical lek placement of forest-dwelling manakin species (Aves , Pipridae) is associated with vertical gradients of ambient light. *Biological Journal of the Linnean Society,* 80, 647–658.

Herbst, K. L., & Bhasin, S. 2004. Testosterone action on skeletal muscle. *Curr Opin Clin Nutr Metab Care*, 7, 271–277.

Hoefler, C. D., Persons, M. H., & Rypstra, a. L. 2008. Evolutionarily costly courtship displays in a wolf spider: a test of viability indicator theory. *Behavioral Ecology*, 19, 974–979.

Hunt, J., Brooks, R., Jennions, M. D., Smith, M. J., Bentsen, C. L., & Bussiere, L. F. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature*, 432, 1024–1027.

Hurd, P. L. 2004. Conventional displays: Evidence for socially mediated costs of threat displays in a lizard. *Aggressive Behavior*, 30, 326–341.

Höglund, J., & Alatalo, R. V. 1995. *Leks.* Princeton, NJ: Princeton University Press.

Höglund, J., Kålås, J. A., & Fiske, P. 1992. The costs of secondary sexual characters in the lekking Great Snipe (Gallinago media). *Behavioral Ecology*, 30, 309–315.

Iaizzo, P. A. 2009. *Handbook of cardiac anatomy, physiology, and devices.* second edn. Humana Press.

Immelmann, K. 1971. Ecological aspects of periodic reproduction. *Avian Biology*, 1, 341–389.

Johansen, J. a., Breedlove, S. M., & Jordan, C. L. 2007. Androgen Receptor Expression in the Levator Ani Muscle of Male Mice. *Journal of Neuroendocrinology*, 19, 823–826.

Karr, J. R., Nichols, J. D., Klimkiewicz, M. K., & Brawn, J. D. 1990. Survival Rates of Birds of Tropical and Temperate Forests: Will the Dogma Survive? *The American naturalist*, 136, 277–291.

Kirckpatrick, M. 1982. Sexual Selection and the Evolution of Female Choice. *Evolution*, 36, 1–12.

Kirkpatrick, M., & Ryan, M. J. 1991. The Evolution of Mating Preferences and the Paradox of the Lek. *Nature*, 350, 33–38.

Lailvaux, S. P., & Irschick, D. J. 2006. A functional perspective on sexual selection: insights and future prospects. *Animal Behavior*, 72, 263–273.

Lande, R. 1980. Sexual Dimorphism , Sexual Selection , and Adaptation in Polygenic Characters. *Evolution*, 34, 292–305.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences of the United States of America*, 78, 3721–5.

Lowe, R. P. 1942. The anatomy of Gould's manakin (Manacus vitellinus) in relation to its display. *Ibis*, 84, 50–83.

Manning, J. T. 1985. Choosy Females and Correlates of Male Age. *Education*, 349–354.

McGill, H. C., Anselmo, V. C., Buchanan, J. M., & Sheridan, P. J. 1980. The Heart Is a Target Organ for Androgen. *Science*, 2, 1–4.

Mcdonald, D. B., & Potts, W. K. 1994. Cooperative Display and Relatedness among Males in a Lek-Mating Bird. *Science*, 266, 1030–1032.

Mead, L. S., & Arnold, S. J. 2004. Quantitative genetic models of sexual selection. *Evolution*, 19, 5, 264-271.

Moller, A. P., & Petrie, M. 1996. Condition dependence , multiple sexual signals , and immunocompetence in peacocks. *Behavioral Ecology*, 13, 248–253.

Mänttäri, S., Anttila, K., & Järvilehto, M. 2008. Testosterone stimulates myoglobin expression in different muscles of the mouse. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 178, 899–907.

Nooker, J. K., & Sandercock, B. K. 2008. Phenotypic correlates and survival consequences of male mating success in lek-mating greater prairie-chickens (Tympanuchus cupido). *Behavioral Ecology and Sociobiology*, 62, 1377–1388.

Pansarasa, O., & Antona, Æ. G. D. 2002. "' Oxidative stress '": effects of mild endurance training and testosterone treatment on rat gastrocnemius muscle. *Enzyme*, 550–555.

Petrie, M., Halliday, T., & Sanders, C. 1991. Peahens Prefer Peacocks with Elaborate Trains. *Animal Behavior*, 41, 323–331.

Prum, R. O. 1990. Phylogenetic analysis of the evolution of display behavior in the neotropical Manakins (Aves:Pipridae). *Ethology*, 84, 202–231.

Prum, R. O. 1998. Sexual selection and the evolution of mechanical sound production in manakins (Aves : Pipridae). *Animal Behavior*, 55, 977–994.

Ricklefs, R. E. 1976. Growth rates of birds in the humid New World tropics. *Ibis*, 118, 179–207.

Saldanha, C. J., Tuerk, M. J., Kim, Y., Fernandes, A. O., Arnold, A. P., & Schlinger, B. A. 2000. Distribution and regulation of telencephalic aromatase expression in the zebra finch revealed with a specific antibody. *Journal of Comparative Neurology*, 423, 619–630.

Schaible, T. F., Malhotra, a., Ciambrone, G., & Scheuer, J. 1984. The effects of gonadectomy on left ventricular function and cardiac contractile proteins in male and female rats. *Circulation Research*, 54, 38–49.

Scheuer, J., Malhotra, A., Schaible, T. F., & Capasso, J. 1987. Effects of gonadectomy and hormonal replacement on rat hearts. *Circulation Research*, 61, 12–19.

Schlinger, B. A., & Brenowitz, E. A. 2009. Neural and Hormonal Control of Birdsong. In: *Hormones, Brain and Behavior*, (Ed. by D. Pfaff), pp. 799–839.

Schultz, J. D., Hertel, F., Bauch, M., & Schlinger, B. A. 2001. Adaptations for rapid and forceful contraction in wing muscles of the male golden-collared manakin: sex and species comparisons. *Journal Of Comparative Physiology A*, 187, 677–684.

Schultz, J. D., & Schlinger, B. A. 1999. Widespread accumulation of [3 H] testosterone in the spinal cord of a wild bird with an elaborate courtship display. *Neurobiology*, 96, 10428–10432.

Scow, R. O., & Hagan, S. H. 1957. Effect of Testosterone Propionate on Myosin, Collagen And Other Protein Fractions in Striated Muscle of Gonadectomized Rats. *Endocrinology*, 60, 273–376.

Shorey, L. 2002. Mating success on white-bearded manakin (Manacus manacus) leks: male characteristics and relatedness. *Behavioral Ecology and Sociobiology*, 52, 451–457.

Skutch, F. 1985. Clutch size, nesting success, and predation on nests of neotropical birds, reviewed. *Ornithological Monographs*, 36, 575–594.

Snow, D. W., & Lill, A. 1974. Longevity records for some neotropical land birds. *The Condor*, 76, 262–267.

Soma, K. K., Tramontin, A. D., & Wingfield, J. C. 2000. Oestrogen regulates male aggression in the non-breeding season. *Proceedings of the Royal Society B: Biological Sciences*, 267, 1089–1096.

Stein, A. C., & Uy, J. A. 2006. Plumage brightness predicts male mating success in the lekking golden-collared manakin , Manacus vitellinus. *Behavioral Ecology*, 17, 41–47.

Thibert, P. 1986. Androgen sensitivity of skeletal muscle: Nondependence on the motor nerve in the frog forearm. *Experimental Neurology*, 91, 559–570.

Trainer, J. M., Mcdonald, D. B., & L, W. A. 2002. The development of coordinated singing in cooperatively displaying long-tailed manakins. *Behavioral Ecology*, 13, 65–69.

Ustunel, I., Akkoyunlu, G., & Demir, R. 2003. The Effect of Testosterone on Gastrocnemius Muscle Fibres in Growing and Adult Male and Female Rats : A Histochemical , Morphometric and Ultrastructural Study. *Journal of Veterinary Medicine Series C*, 32, 70–79.

Vehrencamp, S. L., Bradbury, J. W., & Gibson, R. M. 1989. The energetic cost of display in male Sage Grouse. *Animal Behavior*, 38, 885–896.

Vlahopoulos, S., Zimmer, W. E., Jenster, G., Belaguli, N. S., Balk, S. P., Brinkmann, A. O., Lanz, R. B., Zoumpourlis, V. C., & Schwartz, R. J. 2005. Recruitment of the androgen receptor via serum response factor facilitates expression of a myogenic gene. *Biochemistry*, 280, 7786 –7792.

Wells, K. D. 2001. *The energetics of calling frogs.* Press, Smithsonian Institution.

Wiersma, P., Chappell, M. A., & Williams, J. B. 2007a. Cold- and exercise-induced peak metabolic rates in tropical birds. *Proceedings of the National Academy of Sciences*, 104, 10866–10871.

Wiersma, P., Muñoz-Garcia, A., Walker, A., & Williams, J. B. 2007b. Tropical birds have a slow pace of life. *Proceedings of the National Academy of Sciences*, 104, 9340–9345.

Wikelski, M., Hau, M., Douglas Robinson, W., & Wingfield, J. C. 2003. Reproductive seasonality of seven neotropical passerine species. *The Condor*, 105, 683.

Wingfield, J. C. 1985. Short-Term Changes in Plasma Levels of Hormones during Establishment and Defense of a Breeding Territory in Male Song Sparrows , Melospiza melodia. *Hormones and*, 19, 174–187.

Wingfield, J., & Farner, D. S. 1980. Control of seasonal reproduction in temperate zone birds. *Progress in Reproductive Biology*, 5, 62-101.

Wingfield, J. C., & Silverin, B. 2009. Ecophysiological Studies of Hormone–Behavior Relations in Birds. In: *Hormones, Brain and Behavior*, (Ed. by D. W. Pfaff, A. P. Arnold, S. E. Fahrbach, A. M. Etgen, & R. T. Rubin), pp. 817–855. San Diego: Academic Press.

Zahavi, A. 1975. Mate Selection-A Selection for a Handicap. *Journal of Theoretical Biology*, 53, 205–214.

CHAPTER 2

Female Choice For Male Motor Skills[1]

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2.1 Abstract

Sexual selection was proposed by Darwin to explain the evolution of male sexual traits such as ornaments and elaborate courtship displays. Empirical and theoretical studies have traditionally focused on ornaments and quantity of displays, but the reasons for the evolution of elaborate acrobatics during courtship remain unclear. I addressed the hypothesis that females choose males on the bases of subtle differences in display performance indicating motor skills that facilitate survival. Male golden-collared manakins (*Manacus vitellinus*) perform elaborate, acrobatic courtship displays. I used high-speed cameras to record the displays of wild males and analyzed them in relation to male reproductive success. Females preferred males that performed specific display moves at greater speed, with differences of 10s of milliseconds strongly impacting female preference. In additional males, I recorded telemetrically the heart rate during courtship using miniature transmitters and found that courtship is associated with profoundly elevated heart rates, revealing a large metabolic investment. This study provides evidence that females choose their mates on the basis of subtle differences in motor performance during courtship. I propose that elaborate, acrobatic courtship dances evolve because they reflect motor skills and cardiovascular function of males.

2.2 Introduction

Darwin proposed that elaborate ornaments and courtship displays evolve because they increase reproductive success of males showing traits that are most attractive to females (Darwin 1871). According to sexual selection theory, the large tail of the peacock and the courtship dances of male birds of paradise are the result of a preference by females to mate with males that have the largest tail or perform the best dance. In the last decades, theoretical and empirical work has focused on mechanisms that link female preference and male traits. The exaggerated ornaments of males in many vertebrate and invertebrate species were the objects of most studies in this respect, because it is relatively simple to obtain quantitative measures of such traits. Thus, a number of characteristics such as the size (Andersson 1992), coloration (Hamilton & Zuk 1982), symmetry (Nooker & Sandercock 2008), and other physical attributes of ornaments have been shown to be objects of female choice. However, female choice appears to operate in many species in which males have no ornamentation. In these species, the attention of researchers focused on what Darwin called 'vigor', i.e. the performance intensity of activities that require considerable energetic investments (Darwin 1871). There is a long list of courtship displays that reflect vigor, from the display flights of many bird species to loud vocalization of frogs to leg-waving in spiders (reviewed by Byers et al. 2010). Nevertheless, a large proportion of variance in reproductive success among males cannot be explained by individual differences in ornament features or display activity even when females rely solely on these traits for selecting mates (Höglund et al. 1995). Furthermore, the relationship between details of elaborate courtship dances and performance vigor is not well understood. At present, there is scarce if any evidence for sexual selection being the driving force for the evolution of these elaborate courtship behaviors (Byers et al. 2010).

One obvious result of courtship displays is that of making ornaments more conspicuous, i.e. signal amplification (reviewed by Candolin 2003). Multi-component signals can be detected, discriminated, and memorized better than single-component signals, particularly when multiple sensory modalities are involved (reviewed by Rowe 1999). Nevertheless, in many courtship displays movements are very rapid and acrobatic and it seems unlikely that their only function is that of catching the attention of the observers or amplifying the signal value. Fusani et al. proposed that the display performance is an indicator of sensorimotor co-ordination and power and thus of condition and overall quality (2007). This hypothesis was advanced also in a recent review on female choice based on male motor performance (Byers et al. 2010). Females might use the skill with which individual males perform challenging actions as indicators of overall performance (Byers et al. 2010). Challenging actions are those which require high precision in motor coordination close to the limits allowed by anatomical and physiological characteristics (e.g. Clark 2009). In an elaborate courtship display, males employ their neuromuscular and sensory systems, and need more than just good condition. Thus, females could evaluate from motor skills not only the good general health of males, but also their developmental history. To support this hypothesis, it is necessary to demonstrate that males differ significantly in the performance of courtship displays with respect to skills, and that female choice is associated to subtle differences in the performance of male displays (see also Byers et al. 2010).

Manakins (Passeriformes; Pipridae) perform some of the most conspicuous and physically elaborate courtship dances in the animal world that include acrobatic routines and mechanically produced sounds (Chapman 1935; Fusani et al. 2007; Prum 1998). The courtship dance of the male Golden collared manakin (*Manacus vitellinus;* GC manakin) is called the jump-snap display (Figure 2.1). Males jump between small saplings that delimit an arena, which they clear on the forest floor. Midair in the jump males powerfully flip their wings upwards to produce a loud wingsnap and after touchdown they rapidly rotate their body to resume a statuary position in which they expose their erected bright yellow throat feathers -'beard'- towards the center of the arena (beard up). After observing a male displaying, a female can engage with the male (courtship success) and then copulate (mating success). GC manakins have a lek mating system where females choose among several males courting concurrently. Mating success is highly skewed in *Manacus* spp. and a minority of males obtain most copulations (Lill 1974; Shorey 2002; Stein & Uy 2006). Because males provide no direct benefits to their mates, courtship is the only cue that females can use to choose their partner. Male plumage and the position and background of the court are important factors for female choice but they explain only a fraction of mating success (Shorey 2002; Stein & Uy 2006; Uy & Endler 2004). The elaboration and high levels of specialization of the motor patterns strongly suggest that these components of the display are subject to sexual selection as well (Prum 1998). Phylogenetic analyses indicate that the complex motor patterns of manakin courtship evolved before the morphological traits (Prum 1990), which may have arisen secondarily to increase the value of the visual stimulus and/or to highlight the motor skills (Byers et al. 2010).

In a previous study using high-speed videography, Fusani et al. showed that male display performance differs significantly between male GC manakins (2007). In particular, some males were faster then others in resuming the 'beard up' posture at the end of each jump and made shorter intervals between jumps (Fusani et al. 2007). I hypothesized that female GC manakins choose their mates based on their motor skills as shown in the courtship dances as they might reflect overall physiological qualities of males. In this work, I studied if male differences in display performance are related to female preference. I found that females preferred males that performed specific dance moves at greater speed, with differences of 10s of milliseconds strongly impacting female preference. A parallel study on additional males showed that heart rate recorded telemetrically with miniature transmitters increased during courtship compared to flight and other non-courtship activities, reaching extreme levels previously recorded only in very small hummingbirds. Thus, this study shows that female GC manakins prefer males that perform with greater skills their very challenging courtship displays.

2.3 Methods

2.3.1 Behavioral observations and morphological measurements

The study was conducted between February and May, the most active period of the 7-months long breeding season (January to July), in 2006 and 2009 in the secondary forests around Gamboa, a town along the Panama Canal in the Republic of Panama (09°07′ N, 79°42′ W). All males were marked with colored leg bands. I measured tarsus, beard, and wing length, and the body weight. I used the focal sample method to record behaviors of male manakins at leks. During each 30-min observation sessions, I documented the following behavioral variables: number of jump-snap displays; number of wingsnaps produced during each display; number of cheepoo vocalizations; number of rollsnaps. Rollsnaps are a rapid series of wingsnaps (up to 12 wingsnaps at 50 Hz). Cheepoos and rollsnaps are produced by males perched above or close to the arena and are involved in both female attraction and male-male interactions (Lill 1974). In addition, I recorded the courtship and mating success of each male. Courtship success is the number of times a female joins a male in his display arena, a measure that in *Manacus* is highly correlated to mating success, the number of times a male copulates (Stein & Uy 2006). Together with collaborators, I recorded a total of 895 30-min observation units from 31 males of 4 different leks.

2.3.2 Video recordings

High-speed video recordings (125 frames/s; Supp. Movie 2) were obtained using a MotionMeter camera (RedLake Inc., San Diego, CA, USA). The tripod-held camera was placed about 5 m from the court and controlled remotely. Video sequences were later analyzed using The Observer Video Pro 4.0 (Noldus Information Technology, Wageningen, The Netherlands; Fusani et al. 2007). I analyzed a total of 87 high-speed video sequences from 17 of the 31 males, that is, 5.12 ± 0.32 (SEM) sequences of 9.45 ± 0.51 s per male, and measured the following variables: jump duration; jump speed: distance between the two saplings /duration of the jump; wingsnap rate: number of wingsnaps per second during a jump-snap display; on perch: time spent on a sapling between two jumps; beard up: time required for the bird to resume his statuary posture with the erected beard at the end of the jump, from the moment of landing to the freezing of the posture.

2.3.3 Heart rate recordings

Heart rate was recorded telemetrically using lightweight (~1g) transmitters (Sparrow systems, Fisher, IL, USA) that emit continuous amplitude signals which are frequency modulated by heart muscle potentials and which have been previously validated to record heart rate of small birds as a measure of metabolic rate (Bisson et al. 2009; Cochran & Wikelski 2005; Steiger et al. 2009). Together with collaborators, I was able to record the heart rate of 4 males during displays in parallel to wingsnap activity. Files were analyzed using Cool Edit 2000 (Syntrillium Software, Phoenix, AZ, USA). Band-pass filtering was applied to distinguish heart rates from the continuous transmitter carrying frequency as well as from noise produced by skeletal muscle potentials. Heart rate was calculated only when a minimum of 5 sequential peaks was observed; skeletal muscle potentials demonstrated much less regularity. The relationship between heart rate and metabolic rate (oxygen consumption) for this species was established in a separate study (Chapter 4).

2.3.4 Statistical analyses

SPSS Base 13.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. For behavioral analyses, I used individual mean values of each variable, and I used z-scores to normalize the data. I excluded the morphological variables of six males that had been ringed in previous years to reduce measurement error. Principal Component Analysis of courtship success and mating success was applied to extract a single factor, display success, with an Eigenvalue of 1.889 that explained 94.5% of the variance, and was positively associated with mating (0.972) and courtship success (0.972). Using display success as the dependent variable, step-wise multiple regression analyses were used to investigate which dance variables best predict male reproductive success.

2.4 Results

Female preference was strongly, positively correlated with all measures of display activity (Table 2.1, Figure 2.2). Males performing jump-snap displays, wingsnaps, rollsnaps and producing cheepoos vocalization at higher frequency had a higher display success, an index that explains 94.5% of courtship and mating success (Figure 2.2a-d; Table 2.1). Female preference was associated not only to display activity but also to the performance of specific moves of the jump-snap display. Males differed in these behaviors in the order of 10s or 100s of milliseconds (range of beard up: 0.100-0.146 s; range of on perch: 0.463-1.327 s). In particular, females preferred males who were fractions of second faster in executing dance moves such as 'beard up', 'on perch' - the time the 'beard up' position was held - and produced more wingsnaps per second (Figure 2.2e,f and Table 2.1). The significance of the tests did not change substantially if the very successful male with a display success of 3.98 was removed from the analyses. Similarly, all tests with the exception of wingsnap rate remained significant after applying a serial Bonferroni correction (Rice 1989; Table 2.1). I found no significant correlation between morphological variables and display success (Table 2.1).

Multiple regression analyses were performed using display success as dependent variable to determine which variables best predict male success. Using step-wise regression models for courtship activity ($N = 31$) with display, wingsnap, rollsnap and cheepoo frequency as independent variables, the best model explaining (adjusted R^2) 69.7% of display success was obtained with a single predictor variable, the wingsnap frequency, which accounted for 84.1% of the model's predictive capacity (β; $P < 0.001$). Using courtship choreography variables jump speed, jump duration, 'beard up', 'on perch', and wingsnap rate as independent variables, step-wise multiple regression analyses showed that the best model for predicting female preference included ‘on perch’ alone as predictor variable (adjusted R2, 31.9%; β = 60.1%; N=17; *P* < 0.05). Differences in courtship between males did not depend on females: most displays (>90%) were recorded when no female was in the court.

The velocity and the challenging moves of the courtship dance suggested that besides accurate neuromuscular coordination, it has high metabolic costs. I was able to record telemetrically the heart rate of four males during the courtship displays. An average daytime baseline heart rate of 563 ± 35 beats min^{-1}, (mean ± SEM) was recorded in males perched at the lek but not actively displaying. Heart rate nearly doubled during displaying, increasing significantly to 1017 ± 28 beats min^{-1} (Figure 2.3). The production of wingsnaps in particular appears to be the most energetically demanding action during the jump-snap display, with a maximum recorded of 1374 b min^{-1} (Figure 2.4). An increase in heart rate from 563 to 1017 b/min corresponds to an increase of metabolic rate from 64 to 131 kJ d^{-1} (Chapter 4). Thus, courting in manakins is energetically very expensive, and this is indicated also by a significant, 18% decrease in relative body mass (t = 8.79, df = 6, p= 0.001) over the course of 6-7 weeks of daily courting activity.

2.5 Discussion

Current theories of sexual selection state that, in absence of direct benefits, females choose genetically superior males that will provide higher reproductive success and/or viability to the offspring (Andersson 1994; Fisher 1930; Zahavi 1975). In lekking species such as the GC manakin, males provide no parental investment, therefore females can only rely on the courtship to assess potential mates. Typically, females assess male quality by examining indicators that signal costs, which males withstand such as high levels of testosterone, parasite load and increased risk of predation (Folstad & Karter 1992; Moeller & Petrie 2002). Studies of other species have related display intensity to mating success; good indicators of success include the roar intensity of red deer (Charlton et al. 2007), the calling rate of crickets (Hunt et al. 2004), leg raising rate in wolf spiders (Hoefler et al. 2008), and courtship display rate of greater prairie-chickens (Nooker & Sandercock 2008). This work adds that acrobatic displays and motor skills are important indicators of male quality by providing novel evidence that female choice is associated with subtle difference in display performance in males.

Previous work in *Manacus manacus* did not reveal a correlation between displaying behavior and mating success (Shorey 2002). However, the latter study did not focus on courtship performance and observations were interrupted during females' visits (Shorey 2002). Thus, the present study confidently illustrates very strong correlations between displaying activity and mating success in manakins. This is not a surprising result as the extreme specialization of manakin courtship displays appears clearly to be the result of strong, ongoing sexual selection (Prum 1990; 1998). Thus, the intensity of displaying activity joins other courtship traits that have been shown to be associated with female choice in GC manakins, such as the color of the plumage (Stein & Uy 2006) and the contrast of the bird with the arena's background (Uy & Endler 2004).

Courtship choreography may highlight morphological traits and thus enhance male conspicuousness (Johnsgard 1994). Slow-motion analysis of *M. vitellinus* displays show that males turn their brightly colored collars towards the center of the arena at the end of each jump – the 'beard up' move (Fusani et al. 2007). This action might increase the apparent size of the beard (i.e. the beard is closer to the female) and its visibility. However, Prum (1990) suggested that in manakins morphological specialization might be evolved to increase the visibility of motor patterns and not vice versa. The duration of 'beard up' shows a strong negative correlation with display success: the shorter the time required to restore the position after a jump, the higher the success. The examination of the jump-snap display at slow motion suggests indeed that the action of turning towards the court's center at the end of each jump is one of the most demanding moves of the display. The whole action resembles the landing of a gymnast after a routine, technically defined as the reduction of total body momentum at touchdown (McNitt-Gray et al. 2001). In humans, such task presents a significant challenge to the neuromuscular system (McNitt-Gray et al. 2001). Clearly, similar challenges are encountered by male manakins. The variable that regression analyses indicated as the best predictor of male success among choreography variables, 'on perch', reflects another obvious challenging limit to display performance, because males, after resuming the 'beard up' posture, need to reload the leg muscles to spring into the next jump. Altogether, these data support the hypothesis that the choreographic performance of the jump-snap display indicates to females males' sensorimotor co-ordination and power, and thus condition and overall quality (Fusani et al. 2007). They also provide novel evidence in support of the more general hypothesis that females might use the skill with which individual males perform challenging actions as indicators of overall performance (Byers et al. 2010).

Our results suggest that females visually discriminate 10s to 100s of millisecond differences in the male dance performance. The capacity of females to discriminate slight difference in male choreographed motor patterns (dances) had been shown previously only in humans (Neave et al. 2010). However, several data suggested that animals, and in particular manakins, have similar capacities. First, there is ample evidence that animals can discriminate slight differences in motor performance in contexts other than courtship (e.g. Byers et al. 2010). Secondly, flying species need to integrate information at a higher rate than other species (Kare 1965), and in pigeons and domestic fowls the critical fusion rate (the frequency at which a flickering light source is perceived as continuous) is 2-3 times higher than in humans (reviewed in Maddocks et al. 2001). Manakins are known for the quickness of their movements in the forest, presumably an adaptation for avoiding predation. Thus, the capacity of female manakins of discriminating courtship moves differing of fractions of seconds is not surprising.

The other major finding of the present study was that courtship activity in manakins is accompanied by transient, exceptional increases in metabolic rate which even exceeds 1200 beats min^{-1} recorded in tiny hummingbirds (Lasiewski 1964). There is no consensus that energy expenditure is a cost evaluated by females because high metabolic rate is found to be related to reproductive success in some species (Höglund et al. 1992; Vehrencamp et al. 1989) but not in others (Hurd 2004). However, conventional techniques to measure metabolic rate such as body weight changes and doubly labeled water lack fine temporal resolution (Bevan et al. 1995; Butler et al. 2004). Heart rate, on the contrary, provides a real-time measure of energy demand. This is one of the first studies in which heart rate and thus direct energy expenditure were measured during the performance of an elaborate, acrobatic courtship display, and I showed that energy expenditure doubles during courtship compared to other activities performed immediately before and after the display. Therefore, manakin displays show off the energetic investment and the cardio-muscular capacity of the performer.

In conclusion, this study shows that female choice in Golden-collared manakins is associated to subtle differences in male motor performance during courtship displays, a challenging action for the nervous, neuromuscular, and cardiac system. I propose that elaborate, acrobatic courtship dances evolve because they reflect motor skills and cardiovascular function of males.

2.6 Figures and Tables

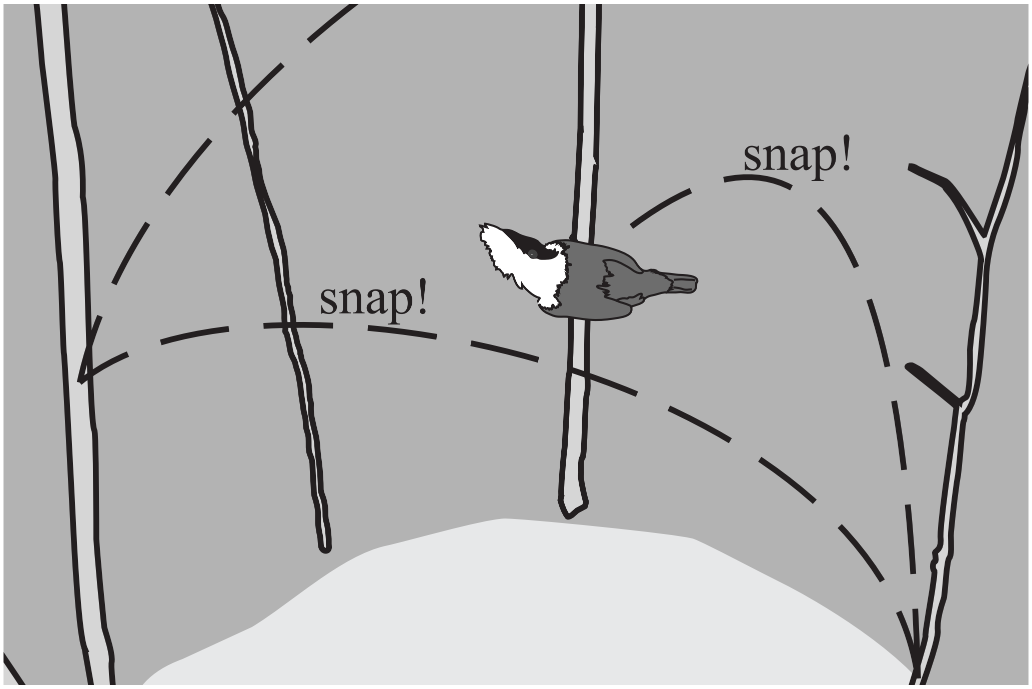

Figure 2.1 Illustration of the courtship dance of a male golden-collared manakin, the jump-snap display. The male jumps between saplings delimiting his court and produces loud wingsnaps in midair. Between jumps, the male freezes shortly on the saplings (on perch) with his golden throat feathers erected (beard up).

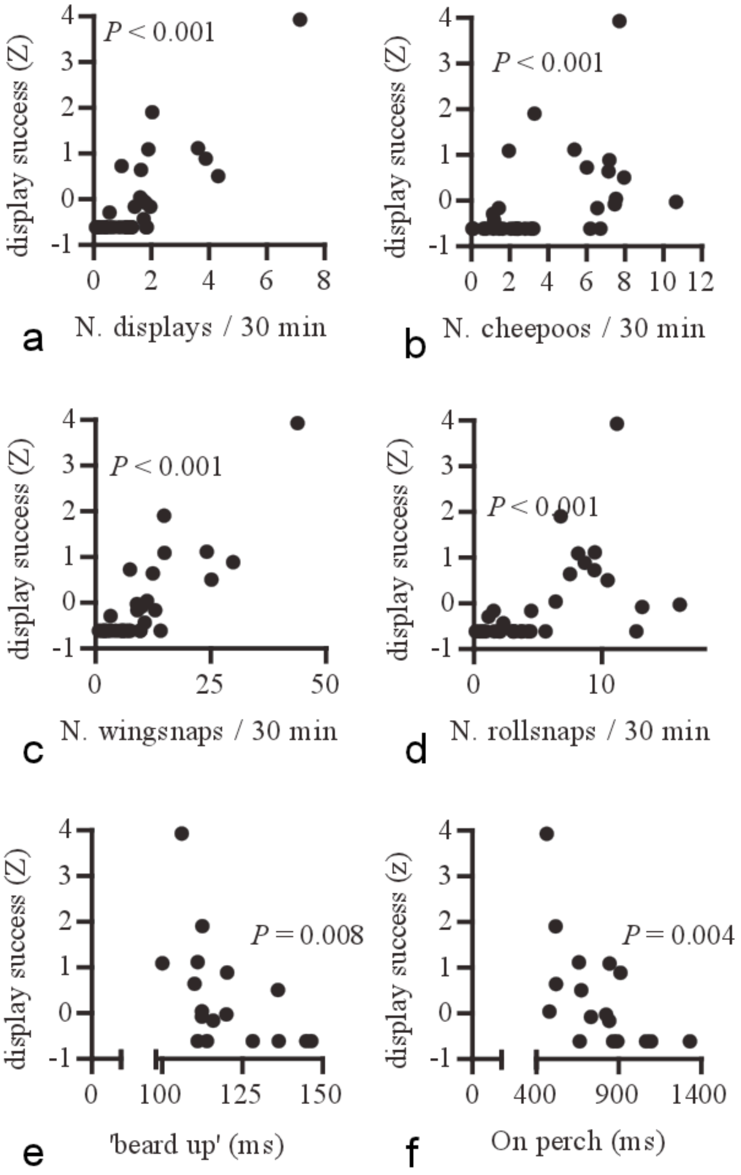

Figure 2.2 (a-d) Scatterplots of display activity variables versus display success, an index of reproductive success. (e-f) Scatterplots of two display choreography variables, 'beard up' and 'on perch', versus display success. Among display activity variables, wingsnap frequency was the best predictor of male display success in a regression analysis. 'On perch' was the best predictor of male display success in a regression analysis including display choreography variables. The P value refers to Spearman non-parametric correlation coefficients. See Table 1 and text for details of the statistical analyses.

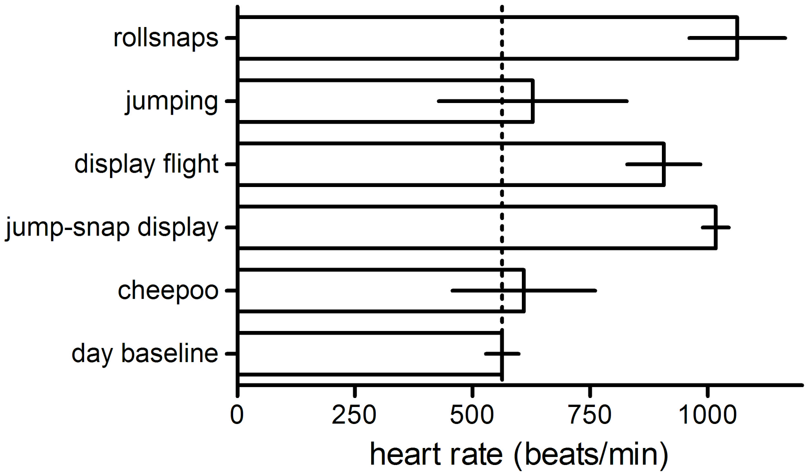

Figure 2.3 The heart rate of male GC manakins doubles during courtship dances. Mean (± SEM) heart rate of males recorded telemetrically during various daily activities. In baseline the bird is perching or moving around the lek; in cheepoo, the male emits vocalizations; in jump-snap display, he jumps between the saplings delimiting his court producing loud wingsnaps; in display flight, he performs a 'noisy' display flight near his court; in jumping, the bird jumps between the saplings of his court but he does not produce wingsnaps; in rollsnap, the bird produce a mechanical trill-like sound using the wings. The dashed line indicates the daytime baseline.

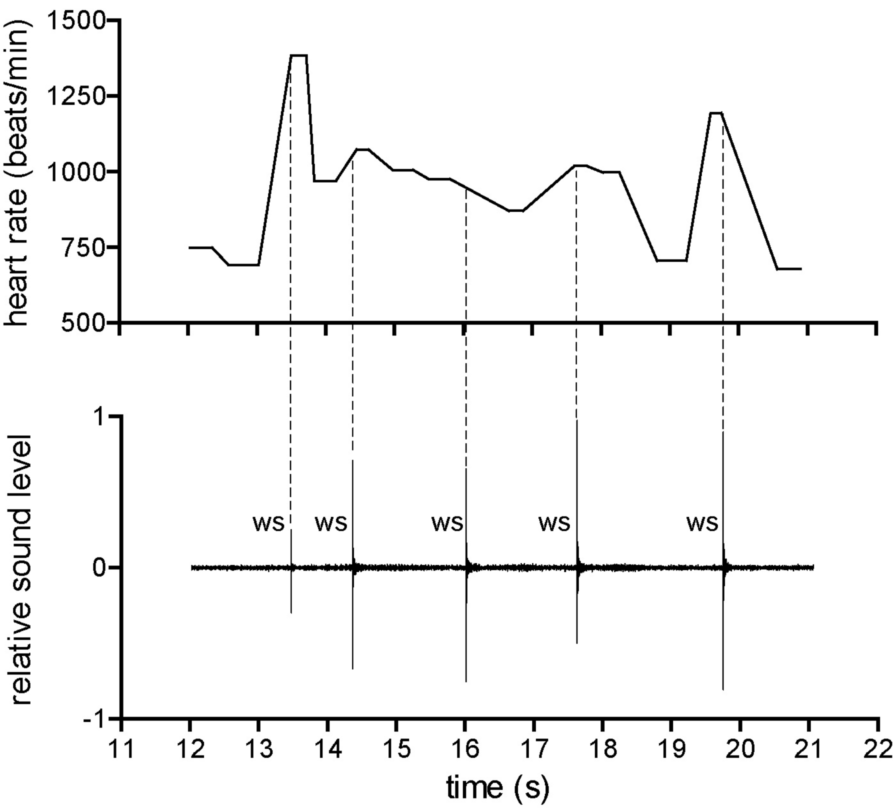

Figure 2.4 The heart rate of male GC manakins increases to known vertebrate maxima during the acrobatic courtship display. The lower panel shows the sound recording of a jump-snap display in the field. Vertical spikes are single wingsnaps (ws), i.e. powerful sounds of the wings produced mid-air as males jump between saplings during their courtship display. The upper panel shows the heart rate of manakins recorded telemetrically during the same display. The dotted vertical lines indicate the occurrence of wingsnaps during the displays. At the beginning of the display, the heart rate rose from ~750 beats/min to ~1000 beats/min, reaching peaks of 1374 beats/min during wingsnaps.

Table 2.1 Correlations matrix (Spearman, non parametric) of display success with courtship and morphological variables. The rightmost column reports the significance of the test after application of serial Bonferroni's correction.

Variable	r_S	N	*P*	*Corrected sig.*
Display activity				
Display frequency	**0.711**	31	**<0.001**	**S**
Wingsnap frequency	**0.756**	31	**<0.001**	**S**
Rollsnap frequency	**0.642**	31	**<0.001**	**S**
Cheepoo frequency	**0.562**	31	**<0.001**	**S**
Display choreography				
Jump duration	0.103	17	0.695	NS
Jump speed	-0.192	17	0.461	NS
On perch	**-0.663**	17	**0.004**	**S**
Beard up	**-0.620**	17	**0.008**	**S**
Wingsnap rate	**0.526**	17	**0.030**	NS
Morphology				
Tarsus length	0.101	27	0.615	NS
Beard length	0.037	27	0.855	NS
Wing length	0.275	27	0.164	NS
Body mass	0.309	27	0.117	NS

2.7 References

Andersson, M. 1994. *Sexual Selection*. Princeton, NJ: Princeton University Press.

Andersson, S. 1992. Female preference for long tails in lekking jackson widowbirds - experimental evidence. *Anim. Behav.* 43, 379-388.

Bevan, R. M., Speakman, J. R. & Butler, P. J. 1995. Daily energy-expenditure of tufted ducks - A comparison between indirect calorimetry, doubly labeled water and heart-rate. *Funct. Ecol.* 9, 40-47.

Bishop, C. M. 1997. Heart mass and the maximum cardiac output of birds and mammals: implications for estimating the maximum aerobic power input of flying animals. *Phil. Trans. R. Soc. B.* 352, 447-456.

Bisson, I.-A., Butler, L. K., Hayden, T. J., Romero, L. M. & Wikelski, M. C. 2009. No energetic cost of anthropogenic disturbance in a songbird. *Proceedings of the Royal Society B: Biological Sciences* 276, 961-969.

Butler, P. J., Green, J. A., Boyd, I. L. & Speakman, J. R. 2004. Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods. *Funct. Ecol.* 18, 168-183.

Byers, J., Hebets, E. & Podos, J. 2010. Female mate choice based upon male motor performance. *Anim. Behav.* 79, 771-778.

Candolin, U. 2003. The use of multiple cues in mate choice. *Biological Reviews* 78, 575-595.

Chapman, F. M. 1935. The courtship of Gould's manakin (*Manacus vitellinus vitellinus*) on Barro Colorado Island, Canal Zone. *Am. Mus. Nat. Hist. Bull.* 68, 472-521.

Charlton, B. D., Reby, D. & McComb, K. 2007. Female red deer prefer the roars of larger males. *Biology Letters* 3, 382-385.

Clark, C. J. 2009. Courtship dives of Anna's hummingbird offer insights into flight performance limits. *Proceedings of the Royal Society B: Biological Sciences* 276, 3047-3052.

Cochran, W. W. & Wikelski, M. 2005. Individual migratory tactics of New World Catharus thrushes: current knowledge and future tracking options from space. In *Birds of two worlds: the ecology and evolution of migration* (ed. R. Greenberg & P. P. Marra), pp. 274-289: John Hopkins University Press.

Darwin, C. 1871. *The descent of man, and selection in relation to sex*. London: Murray.

Fisher, R. A. 1930. *The genetical theory of natural selection*. Oxford: Clarendon Press.

Folstad, I. & Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* 139, 603-622.

Fusani, L., Giordano, M., Day, L. & Schlinger, B. 2007. High-speed video analysis reveals individual variability in the courtship displays of male golden-collared manakins. *Ethology* 113, 964-972.

Hamilton, W. D. & Zuk, M. 1982. Heritable true fitness and bright birds - a role for parasites. *Science* 218, 384-387.

Hoefler, C. D., Persons, M. H. & Rypstra, A. L. 2008. Evolutionarily costly courtship displays in a wolf spider: a test of viability indicator theory. *Behav. Ecol.* 19, 974-979.

Höglund, J., Alatalo, R. V. 1995. Monographs in Behavior and Ecology: Leks. *Monographs in Behavior and Ecology; Leks*, xiii+248p. Princeton University Press.

Höglund, J., Kalas, J. A. & Fiske, P. 1992. The costs of secondary sexual characters in the lekking great snipe (*Gallinago media*). *Behav. Ecol. Sociobiol.* 30, 309-315.

Hunt, J., Brooks, R., Jennions, M. D., Smith, M. J., Bentsen, C. L. & Bussiere, L. F. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature* 432, 1024-1027.

Hurd, P. L. 2004. Conventional displays: Evidence for socially mediated costs of threat displays in a lizard. *Aggressive Behavior* 30, 326-341.

Johnsgard, P. A. 1994. *Arena birds: sexual selection and behavior*. Washington DC: Smithsonian Institution Press.

Kare, M. R. 1965. The special senses: The eye and vision. In *Avian Physiology* (ed. P. D. Sturkie), pp. 407-418. Ithaca, NY: Cornell University Press.

Lasiewski, R. C. 1964. Body Temperatures, Heart and Breathing Rate, and Evaporative Water Loss in Hummingbirds. *Physiological Zoology*, 37, 212–223.

Lill, A. 1974. Sexual behavior of the lek forming white-bearded manakin, *M. manacus trinitatis*. *Z. Tierpsychol.* 36, 1-36.

Maddocks, S. A., Goldsmith, A. R. & Cuthill, I. C. 2001. The influence of flicker rate on plasma corticosterone levels of european starlings, *Sturnus vulgaris*. *Gen. Comp. Endocrinol.* 124, 315-320.

McNitt-Gray, J. L., Hester, D. M., Mathiyakom, W. & Munkasy, B. A. 2001. Mechanical demand and multijoint control during landing depend on orientation of the body segments relative to the reaction force. *J. Biomech.* 34, 1471-82.

Møller, A. P. & Petrie, M. 2002. Condition dependence, multiple sexual signals, and immunocompetence in peacocks. *Behav. Ecol.* 13, 248-253.

Neave, N., McCarty, K., Freynik, J., Caplan, N., Hoenekopp, J. & Fink, B. 2011. Male dance moves that catch a woman's eye. *Biol. Lett.* 7, 221-224.

Nooker, J. K. & Sandercock, B. K. 2008. Phenotypic correlates and survival consequences of male mating success in lek-mating greater prairie-chickens (*Tympanuchus cupido*). *Behav. Ecol. Sociobiol.* 62, 1377-1388.

Prum, R. O. 1990. Phylogenetic analysis of the evolution of display behavior in the neotropical manakins (Aves: Pipridae). *Ethology* 84, 202-231.

Prum, R. O. 1998. Sexual selection and the evolution of mechanical sound production in manakins (Aves: Pipridae). *Anim. Behav.* 55, 977-994.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43, 223-225.

Rowe, C. 1999. Receiver psychology and the evolution of multicomponent signals. *Anim. Behav.* 58, 921.

Shorey, L. 2002. Mating success on white-bearded manakin (*Manacus manacus*) leks: male characteristics and relatedness. *Behav. Ecol. Sociobiol.* 52, 451-457.

Steiger, S. S., Kelley, J. P., Cochran, W. W. & Wikelski, M. 2009. Low metabolism and inactive lifestyle of a tropical rain forest bird investigated via heart rate telemetry. *Physiol. Biochem. Zool.* 82, 580.

Stein, A. C. & Uy, J. A. C. 2006. Plumage brightness predicts male mating success in the lekking golden-collared manakin, *Manacus vitellinus*. *Behav. Ecol.* 17, 41-47.

Uy, J. A. C. & Endler, J. A. 2004. Modification of the visual background increases the conspicuousness of golden-collared manakin display. *Behav. Ecol.* 15, 1003-1015.

Vehrencamp, S. L., Bradbury, J. W. & Gibson, R. M. 1989. The energetic cost of display in male sage grouse. *Anim. Behav.* 38, 885-896.

Zahavi, A. 1975. Mate selection - a selection for a handicap. *J. Theor. Biol.* 53, 205-214.

CHAPTER 3

Female Manakins Challenge Male Motor Skills in a Paired Dance

3.1 Abstract

Synchronized courtship displays are a common feature of species forming long-term pair bonds. In lekking species, on the contrary, there are no pair bonds because partners meet only to copulate. Typically, females of these species do not participate in courtship displays but observe the male's performance and the morphological and behavioral traits that indicate his quality. However, in the neotropical bearded manakins, females join males in their acrobatic courtship displays. I hypothesize that females join males in their displays not only to evaluate more closely their performance but also to challenge their displaying capacities. I filmed at high speed the courtship displays of bearded manakins and compared displays performed by males alone with those performed together with a female. In addition, I studied the coordination between partners during the display. I found that when a female is present, males increase the speed or frequency of several performance parameters that are known to influence mate choice. Additionally, males adjust their rhythm to that of the female as she takes the lead in the duo dance. These results suggest that before choosing a mate for copulation, female manakins challenge the motor skills of prospective males.

3.2 Introduction

Mate choice in animals that form long-term pairs often involves mutual courtship. Examples of spectacular synchronized dances in the avian world include those of the great-crested grebe (Huxley 1914; 1923), trumpeter swan (Cooper 1979) and wandering albatross (Jouventin & Lequette 1990). These coordinated displays are thought to help establish, strengthen, and maintain the pair bond, a requirement for species in which both parents contribute to raising the brood (Wachtmeister 2001). In contrast, males of species with a lek mating systems contribute only sperm to the offspring and females raise their brood alone. These males aggregate and compete for copulations by means of secondary sexual signals (Höglund & Alatalo, 1995; Darwin, 1871). After careful observations, females choose a male for copulation based on secondary sexual traits that indicate his quality as a mate. Otherwise, the female plays no role in the male's courtship display (Andersson 1994). In bearded manakins of the genus *Manacus*, however, females interact with males and join them in the display before choosing their mate (Figure 3.1). Over an extended breeding season lasting up to 7 months, males aggregate in groups of 2-20. Each male clears a court on the forest floor and performs the jump-snap display (Chapman 1935; Schlinger et al. 2008), a series of jumps between the saplings delimiting the court, which are accompanied by loud mechanical sounds produced by clapping wings (wingsnaps; Bostwick & Prum, 2003; Fusani, Giordano, Day, & Schlinger, 2007). Female choice in manakins appears to consist of several stages (e.g. Lill, 1974 and personal observations). Initially, the female is attracted to a lek by the noise of male wingsnaps, rollsnaps and vocalizations. Once at the lek, the female spends time in the lower canopy to observe and, presumably, compare different males. From above, the cleared background of the court enhances the male's plumage and behavioral signals (Stein & Uy 2006). The female eventually selects a male, descends into his court, and the two move within the court, remaining opposite to each other, in what has been described as a ‘duo dance’ (Figure 3.1; Fusani et al. 2007). Often, but not always, these duo dances end with a copulation (Lill 1974; Stein & Uy 2006). In a previous study, I have found that males that perform elements of their courtship display faster (in the order of 10 ms!) obtain more copulations, suggesting that females can distinguish the very rapid and precise movements of a male that likely indicates to her his neuromuscular capabilities and his quality as a mate (Barske et al. 2011).

Courtship success in manakins, defined as the female decision to enter a male’s court, is a good proxy of mating success (Lill 1974; Stein & Uy 2006). Therefore, if females gain sufficient information about male quality by observing his courtship behavior, I was interested in understanding why females then participate in the display before copulating. By joining the male in a duo dance the female could challenge the male and test his maximum performance capacity for those display parameters that are preferred by females. In addition, or alternatively, females could join the male to determine if he can adjust his display to her pace. In both cases, females would gain additional information on male motor skills that are crucial for her choice of a mate.

These two hypotheses equally lead to the prediction that males change their courtship performance in the presence of a female in the court. If the male maximized his effort in the presence of a female, one or more behavioral patterns of his display should show directional changes. Because females prefer a faster execution of certain behavioral elements of the display (Barske et al. 2011), I predict that male behaviors that are relevant for mate choice will be performed faster in the presence of a female. In particular, I predict that in the presence of a female, the pause between jumps and the time to restore the beard up posture after a jump will require even less time, and the frequency of wingsnaps will increase. In addition, if males were challenged by females to keep their pace I would predict that females initiate each move of the dance and that the execution of the moves is correlated between partners.

3.3 Methods

Using high-speed videography (MotionMeter camera, RedLake Inc., San Diego, CA, USA; 125 frames per second), displaying males and females were filmed over the course of 10 months (February – May 2006; March 2007; March – April 2008; March 2009; February – April 2010). Videos were recorded in three different sites: La Selva, Costa Rica (10°26'N, 84°00'W; study species: white-bearded manakins, *Manacus candei*); Gamboa, Panama (9°07'N, 79°42'W; study species: golden-collared manakin, *Manacus vitellinus*), Bocas del Toro, Panama (9°19'N, 82°15'W; study species: lemon-collared manakin, hybrid between *M. vitellinus* and *M. candei;* Parsons et al. 1993; Brumfield & Braun 2001; McDonald et al. 2001). Birds in Gamboa were color banded for individual recognition; in the remaining two sites, birds were identified by their courts, which males defend aggressively during the courting season (McDonald et al. 2001). Over the course of over 15 years of observations, it has never been seen that a resident male allows an adult intruder to enter and display in his court without being attacked by the resident male (Schlinger and Fusani, personal communication).

The camera was placed 5 m from the court and controlled remotely. Slow motion analysis of videos was performed using The Observer Video Pro 4.0 (Noldus Information Technology, Wageningen, The Netherlands; Barske et al., 2011, Fusani et al., 2007). The behavioral variables of the courtship display that were quantified are described in Table 3.1. Wingsnap rate, 'on perch' and 'beard up' have been shown to play a primary role in female choice (Barske et al., 2011).

To investigate the effect of a female in male display performance, I analyzed video sequences in which a male displays alone and is subsequently joined by a female. Thus, each display sequence was divided into 3 periods: before the female joins the male (preF), while the female displays with the male (withF), and after the female has left the arena (postF).

Finally, to reveal differences in behavior between males and females, and to ascertain if males adjust their behavior on the movements of the females, I compared the behaviors displayed by both sexes: 'on perch', jump duration and jump interval (Table 3.1). To determine which sex takes the lead in the display, I identified the jumping order and measured latencies between the male and the female at the beginning and end of each synchronous jump.

3.3.1 Statistical analyses

I used SPSS 19 (IBM) for statistical analyses. I used a Mixed Model design to test for female presence as a random repeated measure. Videos were nested within males and to control for species differences, species was included as a fixed factor. A similar model was used to investigate differences between males and females. Here the repeated measure was sex, as males and females in a duo dance are not independent from each other. To understand if there was a correlation between males and females for certain behaviors, I used a linear regression to control for species (species differences will be reported in a separate manuscript). I used Pearson's Chi Square Test to investigate if one of the sexes initiated and ended the jumps before the other one. If needed, data were log transformed to obtain normality. Data in the text are given as Mean ± SEM.

3.4 Results

3.4.1 Effects of female presence

For a total of 26 males I obtained video recordings in which I could compare their behavior before a female entered the arena with that recorded while she was in the arena and that recorded immediately after she departed. Males significantly shortened time ‘on perch’ during female presence, that is, they paused on saplings for 511 ± 187 ms, compared to 848 ± 190 ms before she entered the arena and 1233 ± 226 ms once she had departed ($F_{2,86} = 10.364$, $P < 0.001$, $N = 117$; post hocs: preF vs withF: $P = 0.014$; withF vs postF: $P < 0.001$, $N = 113$; Fig. 3.2a). Jump speed significantly decreased once the female left the arena ($F_{2,94} = 8.098$, $P = 0.001$; post hocs: preF vs postF: $P = 0.001$; withF vs postF: $P = 0.001$; Fig. 3.2b). Wingsnap frequency was significantly affected by female presence (on average being greater in the presence of the female), but when using post-hoc tests with Bonferroni corrections, none of the paired comparisons were significant ($F_{2,92} = 3.551$, $P = 0.033$, $N = 105$; Fig. 3.2c). Paired differences were significant when no correction for multiple comparisons were used (preF vs withF: $P = 0.042$; withF vs postF: $P = 0.027$). I found no significant effects of female presence on ‘beard up’ ($F_{2,80} = 0.046$, $P = 0.955$; $N = 126$; Fig. 3.2d).

3.4.2 Male and female movements and their synchronization

High-speed videos revealed that the movements of the females differ substantially from those of the male: while the males jump (and do not fly) between saplings, using their wings for the production of wingsnaps only, females fly using on average 6 wing strokes to achieve the same distance (Figure 3.1). Males also take extraordinary care in restoring their posture at the end of each jump, with the beard well exposed, while females land in more random positions that are difficult to quantify (Table 3.1, Fusani et al. 2007).

From recordings of 18 males dancing with a female, I was able to obtain 185 measures of the following behaviors that can be directly compared between sexes: jump duration, ‘on perch’, and the interval between two jumps, jump interval, which is the sum of consecutive ‘on perch’ and jump duration units (Table 3.1). Males needs 6% less time than females to cross the arena, in other words, males spend on average 17 ms less time in air (jump duration: $F_{1,355} = 6.241$, $P = 0.013$; Fig. 3.3b). There is no difference in the amount of time spent on saplings between two jumps/flights (on perch: $F_{1,350} = 0.027$, $P = 0.869$; Fig. 3.3a), or in jump interval ($F_{1,349} = 0.323$, $P = 0.570$; Fig. 3.3c).

To understand if males adjust their courtship to the female’s behavior, I correlated male and female jump duration, ‘on perch’ and jump interval (Table 3.1). A significant correlation was found between male and female ‘on perch’ ($R^2 = 0.768$, $N = 185$, $F_{3,184} = 86.813$, $P < 0.001$, Fig. 3.4a) and jump interval ($R^2 = 0.693$, $N = 185$, $F_{3,184} = 136.412$, $P < 0.001$, Fig. 3.4b). No correlation was found for Jump duration ($R^2 = 0.144$, $N = 185$, $F_{3,184} = 1.282$, $P = 0.282$). A significant positive correlation per se does not explain whether the males adjust their movements to the females or vice versa. However, I found that females start each move more often than males (Fig. 3.5). Females were first to take off 90.2% of the time (5.5 ± 0.5 of 6.1 ± 0.6 jumps) leading by 0.16 ± 0.02 s ($X^2_9 = 30.8$, $P < 0.001$), and were more often the first ones to land, 0.10 ± 0.01 s before the male ($X^2_9 = 27.558$, $P = 0.001$). Thus, the female takes the lead in the duo dance.

3.5 Discussion

The presence of a female in a male manakin’s court and her participation in courtship displays affect male performance. With a female present, males ultimately speed up ‘on perch’, a trait correlated with female preference: males that remain less amount of time perched between two jumps have higher mating success (Barske et al., 2011). In addition, jump speed and wingsnap frequency are influenced by female presence. This shows that males ultimately maximize their courtship behavior in this advanced phase of mate choice process when the likelihood of obtaining a copulation is very high (Lill 1974; Stein & Uy 2006). At the same time these results indicate that females challenge males by pacing their courtship. High-speed videography enabled us to discover that the female has the lead role during the duo display, suggesting that the males coordinate their movements between the saplings with those of the females. Females might challenge male manakins by pushing them to the extremes of their neuromuscular capacities in two ways: males ultimately increase certain display parameters including the energy demanding production of wingnsaps while adjusting their jumps to her pace.

Paired courtship, including synchronized dances or song duetting, has been described in many species which have long term relationships and their suggested function includes phenotypic matching as well as strengthening of pair bonds (Wachtmeister 2001). To my knowledge, bearded manakins are the first lekking birds for which a tight male-female courtship synchronization has been described. I cannot rule out that this synchronization plays a role in phenotypic matching, i.e. a mechanism allowing the assessment of genetic relatedness, but as manakins do not form stable pairs, and a few males obtain most of the copulations, it appears unlikely that this is the major driver for the duo dance. Rather, I suggest that in the few seconds of duo dance females increase their knowledge about the quality of the potential mate. Males would benefit from such a mechanism because they would perform at their maxima only to females that have shown a strong interest in them and that are likely candidates for a successful mating (Andersson 1994). Females appear to be able to distinguish subtle differences - tens of milliseconds - in the performance of certain moves of the male's display that require outstanding motor skills (Barske et al. 2011). In addition, I have found previously that male manakin heart rates accelerate up to 1300 beats per minute during courtship displays (Barske et al. 2011). Thus, by challenging the male, females might indirectly test additional physiological capabilities such as cardiovascular potential. Increased signal honesty during female presence has been shown for running agility in the pronghorn (Byers 1997), complexity of calls in the tungara frog (*Physalaemus pustulosus;* Akre & Ryan, 2011)*,* color darkness in minnows (*Phoxinus phoxinus*, Kekäläinen et al., 2010), and carotenoid-based bill color in zebra finches (Gautier et al. 2008).

Male adjustments to female behavior have been observed in other lekking species in which the female does not participate in the display. In bowerbirds, for example, males adjust their display intensity based on female signals of receptivity to copulation. It has been proposed that by doing so females minimize the risk of forced copulation, as males adjust their courtship intensity until they observe female crouching behavior, an indicator of her disposition to copulation (Patricelli et al. 2002; Patricelli et al. 2006). As I have never observed forced copulations in manakins this latter argument is an unlikely explanation for why male manakins adjust to females. Other studies have shown that females can signal receptivity using pheromones (Maxwell et al. 2010), acoustic signals (*Sylvia communis*; Balsby & Dabelsteen, 2002), and behavioral signals (e.g., *Mnesarete pudica*; Guillermo-Ferreira & Bispo, 2012). A female manakin signals receptivity to copulation by joining the male in the display and encouraging the male to invite her to mate with a specific behavior (Lill 1974, Cocoon et al.

2012). However, it seems unlikely that the hectic duo dance in which the female engages has the sole function of signaling her sexual receptivity. Copulation occurs on one particular sapling of the arena, the mating sapling. If females simply wanted to signal their receptivity, they could directly land on that sapling without engaging in the courtship display before.

It is also possible that males change their courtship performance to improve their position in relation to that of the female. Presumably, females observe displaying males from different angles when they are in close proximity and the male might adjust his visual display signals to exploit the female sensory system. For example, fiddler crabs adjust angle and intensity of claw waving when approaching females (How et al. 2008). Adjustment of song amplitude according to male-female distance has been suggested for zebra finches, *Taeniopygia guttata* (Brumm & Slater 2006). When the female manakin is above the court, the male enhances his visibility by displaying against a homogeneous background, obtained by clearing the court of any leaf matter (Uy & Endler 2004). This seems to increase the contrast between the collar and beard color, a trait correlated with body condition and involved in mate choice (Stein & Uy 2006). By coordinating his moves with the female, a male might maintain an optimal position in respect to her, increasing the visibility of the bright color of his beard and collar. In this way, males might maximize their multimodal signals towards the female (Candolin 2003).

These findings emphasize some degree of plasticity of male manakin courtship behaviors. Unlike static traits, such as morphological traits including plumage color and ornaments, dynamic behavioral traits might be more difficult to accurately quantify. As shown here, male courtship performance may vary significantly in the presence or absence of the female. The recent advances in high-speed videography to examine rapidly performed courtship displays will no doubt improve out understanding of the functions of these remarkable behaviors.

3.6 Figures and Tables

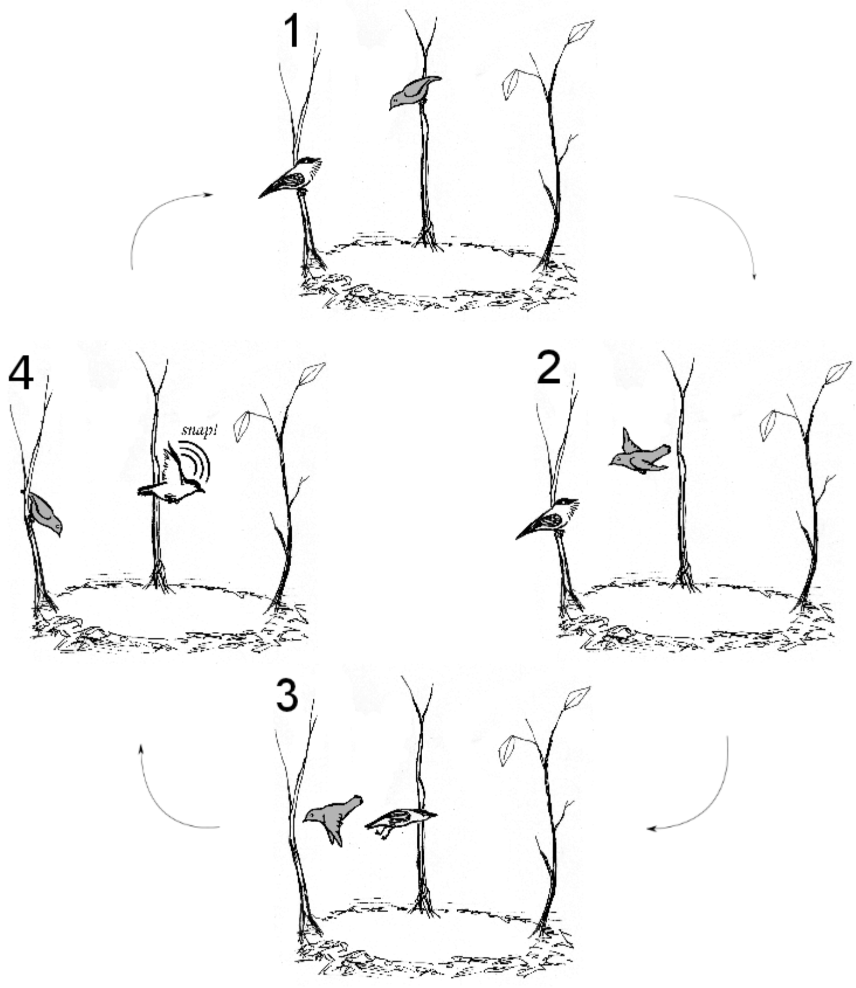

Figure 3.1 Male and female golden-collared manakins in the ‘duo dance’. To attract females, males perform courtship displays between small saplings surrounding a cleared arena. In some cases, a female joins the male in a duo dance which usually starts as the female takes off first. The female flies from one sapling to the next using, on average, 6 wingstrokes. The male follows ~ 160 ms after the female, by jumping, not flying, across the arena, often producing a wingsnap in midair. After ~100ms that the female has landed, the male lands gracefully with his beard of elongated throat feathers exposed towards the centre of the arena.

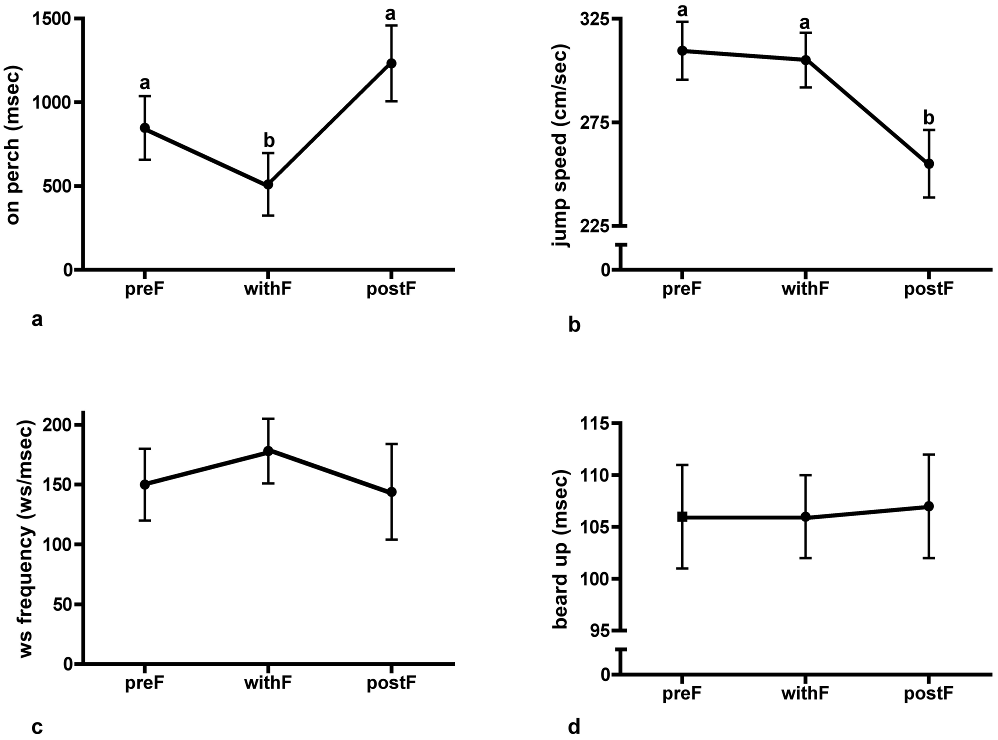

Figure 3.2 Male manakin courtship performance before, during and after the presence of a female. (a) On perch significantly decreased during female presence ($F_{2,86} = 10.364$, $P < 0.001$, $N = 117$; post hocs: preF vs withF: $P = 0.014$; withF vs postF: $P < 0.001$, $N = 113$). (b) Jump speed significantly decreased once the female left the arena ($F_{2,94} = 8.098$, P = 0.001; post hocs: preF vs postF: $P = 0.001$; withF vs postF: $P = 0.001$). (c) Wingsnap frequency was significantly affected by female presence, but differences between groups were not significant ($F_{2,92} = 3.551$, $P = 0.033$, $N = 105$). (d) I found no significant difference for beard up ($F_{2,80} = 0.046$, $P = 0.955$; $N = 126$).

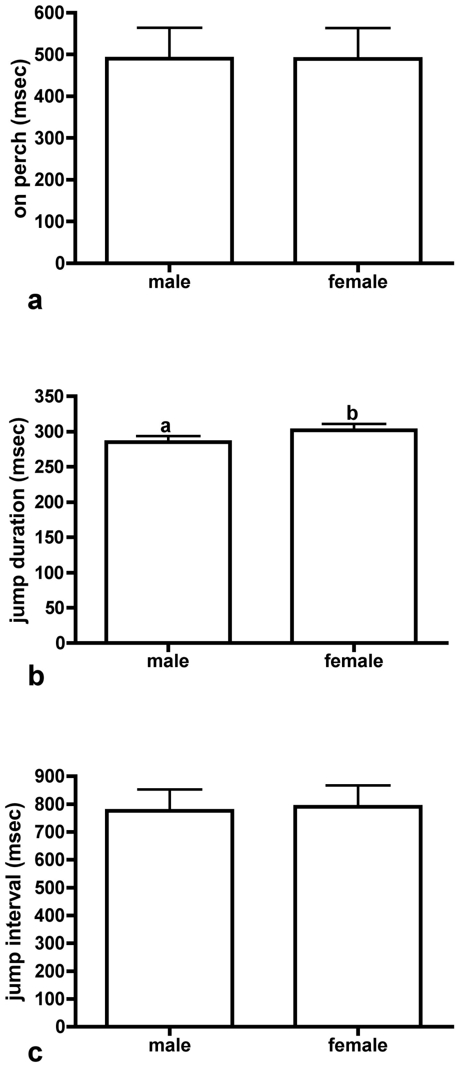

Figure 3.3 Comparisons of behaviors performed by both males and females in the manakin courtship display: 'on perch', jump duration and the interval between two jumps, jump interval, which is the sum of consecutive 'on perch' and jump duration units (Table 3.1). (a) There was no difference in amount of time spent on saplings during two jumps/flights (On perch: $F_{1,350} = 0.027$, $P = 0.869$). (b) Males jump faster than females (Jump duration: $F_{1,355} = 6.241$, $P = 0.013$). (c) There was no difference in the jump interval between males and females ($F_{1,349} = 0.323$, $P = 0.570$).

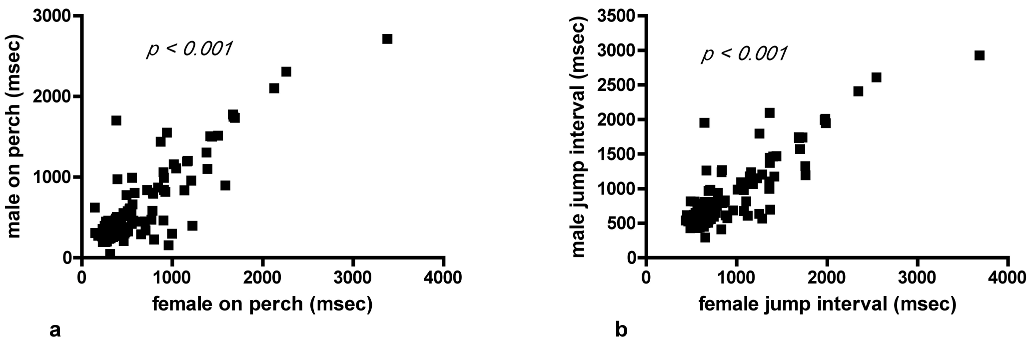

Figure 3.4 Synchronization of courtship between males and females. (a) There was a significant correlation between males and females for ‘on perch’ (Linear Regression: $R^2 = 0.768$, $N = 185$, $F_{3,184} = 86.813$, $P < 0.001$) as well as for (b) jump interval (Linear Regression: $R^2 = 0.693$, $N = 185$, $F_{3,184} = 136.412$, $P < 0.001$).

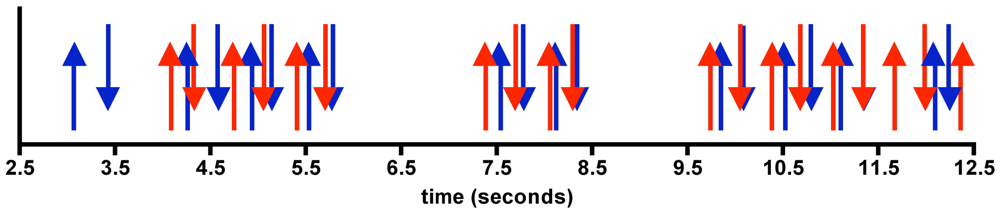

Figure 3.5 Females lead the 'duo dance'. Females usually lead the duo dance as shown in this scheme describing a representative example of male and female jumps along a timeline (seconds). The male (blue arrows) brings himself in position and performs a jump (upward arrow = starts the jump; downward arrow = lands). During the duo displays, the male and the female perform almost simultaneous jumps. The female (red arrows) is the first to initiate the synchronized jumps. The sequence stops when the female leaves the court (last red upward arrow).

Table 3.1 Elements of the courtship display of manakins

element name	**element description**	**male**	**female**
On Perch	time spent on a sapling between two jumps	yes	yes
Jump/Flight Duration	time spent in air	yes	yes
Jump/Flight Speed	distance between two saplings divided by Jump/flight duration	yes	yes
Wingsnap Frequency	number of wingsnaps per second	yes	no
Beard Up	time required for the bird to resume his statuary posture with the erected beard at the end of the jump, from the moment in which the feet touch the landing sapling to the freezing of the posture	yes	no
Jump Interval	interval between two jumps, thus the sum of jump duration and on perch	yes	yes

(see Barske et al., 2011; Fusani et al., 2007)

3.7 References

Akre, K. L., & Ryan, M. J. 2011. Female tungara frogs elicit more complex mating signals from males. *Behavioral Ecology*, 22, 846–853.

Andersson, M. 1994. *Sexual Selection.* Princeton, NJ: Princeton University Press Princeton.

Balsby, T. J. S., & Dabelsteen, T. 2002. Female behavior affects male courtship in whitethroats, Sylvia communis: an interactive experiment using visual and acoustic cues. *Animal Behavior*, 63, 251–257.

Barske, J., Schlinger, B., Wikelski, M., & Fusani, L. 2011. Female choice for male motor skills. *Proceedings of the Royal Society B: Biological Sciences*, 278, 3523–3528.

Bostwick, K. S., & Prum, R. O. 2003. High-speed video analysis of wing-snapping in two manakin clades (Pipridae: Aves). *Journal of Experimental Biology*, 206, 3693–3706.

Brumfield, R. T., & Braun, M. J. 2001. Phylogenetic relationships in bearded manakins (Pipridae : *Manacus*) indicate that male plumage color is a misleading taxonomic marker. *The Condor*, 103, 248–258.

Brumm, H., & Slater, P. J. B. 2006. Animals can vary signal amplitude with receiver distance: evidence from zebra finch song. *Animal Behavior*, 72, 699–705.

Byers. 1997. *American Pronghorn. Social Adaptations and the Ghosts of Predators Past.* Chicago.

Candolin, U. 2003. The use of multiple cues in mate choice. *Biological Reviews*, 78, 575–95.

Chapman, F. 1935. The courtship of Gould’s manakin (Manacus vitellinus vitellinus) on Barro Colorado Island, Canal Zone. *Bulletin of American Museum of Natural History*, 68, 471–525.

Cooper. 1979. Trumpeter Swan nesting behavior. *Wildfowl*, 30, 55–71.

Darwin, C. 1871. *The descent of man, and selection in relation to sex.* London, UK: John Murray.

Fusani, L., Giordano, M., Day, L. B., & Schlinger, B. A. 2007. High-Speed Video Analysis Reveals Individual Variability in the Courtship Displays of Male Golden-Collared Manakins. *Ethology*, 113, 964–972.

Gautier, P., Barroca, M., Bertrand, S., Eraud, C., Gaillard, M., Hamman, M., Motreuil, S., Sorci, G., & Faivre, B. 2008. The presence of females modulates the expression of a carotenoid-based sexual signal. *Behavioral Ecology and Sociobiology*, 62, 1159–1166.

Guillermo-Ferreira, R., & Bispo, P. C. 2012. Male and female interactions during courtship of the Neotropical damselfly Mnesarete pudica (Odonata: Calopterygidae). *Acta Ethologica*, 15, 173–178.

How, M. J., Hemmi, J. M., Zeil, J., & Peters, R. 2008. Claw waving display changes with receiver distance in fiddler crabs , Uca perplexa. *Animal Behavior*, 75, 1015–1022.

Huxley, J. S. 1914. The courtship-habits of the great crested grebe (Podiceps cristatus); with an addition to the theory of sexual selection. *Proceedings of the Zoological Society of London*, 35, 491–562.

Huxley, J. S. 1923. Courtship activities in the red throated diver (Colymbus stellatus Pontopp.); together with a discussion of the evolution of courtship in birds. *Journal of the Linnean Society (Zoology)*, 35, 253–292.

Höglund, J., & Alatalo, R. V. 1995. *Leks.* Princeton, NJ: Princeton University Press.

Jouventin, P., & Lequette, B. 1990. The Dance of the Wandering Albatross Diomedea exulans. *Emu*, 90, 122–131.

Kekäläinen, J., Valkama, H., Huuskonen, H., & Taskinen, J. 2010. Multiple Sexual Ornamentation Signals Male Quality and Predicts Female Preference in Minnows. *Ethology*, 116, 895–903.

Lill, A. 1974. Sexual behavior of the lek-forming White-bearded Manakin (Manacus manacus trinitatis Hartert). *Zeitschrift Für Tierpsychologie*, 36, 1–36.

Maxwell, M. R., Barry, K. L., & Johns, P. M. 2010. Examinations of Female Pheromone use in Two Praying Mantids , Stagmomantis limbata and Tenodera Aridifolia Sinensis (Mantodea : Mantidae). *Entomological Society of America*, 103, 120–127.

McDonald, D. B., Clay, R. P., Brumfield, R. T., & Braun, M. J. 2001. Sexual Selection on Plumage and Behavior in an Avian Hybrid Zone : Experimental Tests of Male-Male Interactions. *Evolution*, 55, 1443–1451.

Parsons, T. J., Olson, S. L., & M. J. Braun. 1993. Unidirectional spread of secondary sexual plumage traits across an avian hybrid zone. *Science*, 260, 1643–1646.

Patricelli, G. L., Coleman, S. W., & Borgia, G. 2006. Male satin bowerbirds, Ptilonorhynchus violaceus, adjust their display intensity in response to female startling: an experiment with robotic females. *Animal Behavior*, 71, 49–59.

Patricelli, G. L., Uy, A. C., Walsh, G., & Borgia, G. 2002. Male displays adjusted to female's response. *Nature*, 415, 279–280.

Schlinger, B. A., Day, L. B., & Fusani, L. 2008. Behavior, natural history and neuroendocrinology of a tropical bird. *General and comparative endocrinology*, 157, 254–258.

Stein, A. C., & Uy, J. A. 2006. Plumage brightness predicts male mating success in the lekking golden-collared manakin , Manacus vitellinus. *Behavioral Ecology*, 17, 41–47.

Uy, J. A. C., & Endler, J. A. 2004. Modification of the visual background increases the conspicuousness of golden-collared manakin displays. *Behavioral Ecology*, 15, 1003–1010.

Wachtmeister, C. 2001. Display in monogamous pairs: a review of empirical data and evolutionary explanations. *Animal Behavior*, 61, 5, 861–868.

CHAPTER 4

Courtship of Golden-collared Manakins *(Manacus vitellinus*) – Sprints, not a Marathon

4.1 Abstract

In lek mating systems, females choose mates by their attractiveness, often through indicators of quality, which males exhibit via their intense participation in courtship activities. In temperate regions, displaying seasons are generally relatively brief (1-2 months), but in the tropics, courtship seasons may be prolonged, raising questions about how males sustain elevated energetic costs of lek behavior demonstrated to exist in temperate regions. Daily, over the course of a nearly 7-month long breeding season, male golden-collared manakins (*Manacus vitellinus*) of Panamanian rainforests perform vigorous acrobatic courtship displays that markedly elevate heart rates suggesting that lekking is a metabolically demanding activity. Typically, animals of tropical lowland forests like manakins, exhibit a 'slow pace of life' metabolic strategy. Thus, it is of interest if manakin lekking behavior is indeed metabolically costly or whether the birds retain a low daily energy expenditure, as seen in other tropical species. To assess these questions, I calibrated manakin heart rate to metabolic rate, examined daily lek activity and, using telemetry, obtained heart rates of individual wild, lekking male manakins. Although metabolic rates peak during courtship displays, I found that males actually invest minimal time per day performing displays. As a consequence, the daily energy expenditure of male manakins is comparable to other lowland tropical species. I conclude that courtship in tropical lekking manakins is energetically more similar to a series of sprints than to a marathon, with short, intense bursts of courtship that employ extraordinary neuromuscular, cardiovascular, and respiratory capabilities.

4.2 Introduction

In many species, breeding males provide no direct benefits to partners or parental care but aggregate in leks in order to court females for purposes of mating (Höglund & Alatalo 1995). Under these conditions, females may be able to choose a partner among many males, presumably discriminating attractiveness such as male ornaments, behavior, or position in the lek, which might act as indicators of quality (Andersson 1994; Höglund & Alatalo 1995). Such strong male-male competition and/or female choice can lead to the evolution of exaggerated secondary sexual phenotypic and/or behavioral traits (Fisher 1930; Zahavi 1975). In temperate regions, the displaying season for lekking species may last only 1-2 months and animals are thought to expend considerable energy for their courtship. Note that by 'courtship' I refer to the extended period animals spend on their lek during their breeding season and this needs to be distinguished from single 'courtship displays'. In birds, examples of high energetic investment in courtship are given by the great snipe (*Gallinago media;* Höglund et al. 1992) and the sage grouse (*Centrocercus urophasianus;*, Vehrencamp et al. 1989)*,* where males increase their metabolic rate on the leks, reaching elevated levels over many hours resulting in substantial weight loss across the breeding season. The large investment in courtship, however, can be rewarded by high reproductive success, as a few males monopolize most matings (Höglund & Alatalo 1995; Vitousek et al. 2008).

Many species of lekking birds live in the tropics (Höglund & Alatalo 1995), but studies of their metabolic investment in courtship is scarce. Compared to birds breeding at high latitudes, lowland tropical birds tend to develop slowly, invest less in reproduction each year, display slow senescence and extended longevity and display a low metabolic rate; a series of life history traits that collectively reflect a tropical 'slow pace of life' (Ricklefs 1968; Snow & Lill 1974; Ricklefs

1976; Wiersma et al. 2007a; Williams et al. 2010; Weathers 1979; Hails 1983; Weathers 1997; Wikelski et al. 2003; Wiersma et al. 2007b). Note that by tropical, I refer here, and for the remainder of this study, to the wet, lowland forest environment, as different metabolic life-history strategies appear to apply to tropical animals living at high elevations or in deserts (Anderson & Jetz 2005).

Golden collared manakins (*Manacus vitellinus*; hereafter GC manakins) are tropical passerine birds that are known for their elaborate courtship displays. GC manakins live in lowland forests of Panama. During a lengthy breeding season (up to 7-months in duration), adult males spend much of the day at their leks engaged in social and courtship-related activities including male-male and male-female interactions that include performance of courtship displays. These displays involve vigorous and rapid jumping between young saplings interspersed with individual, loud snaps (Chapman 1935; Schlinger et al. 2008; Fusani & Schlinger 2012). The individual 'wingsnaps' and the high frequency 'rollsnaps' are produced as the wings strike one another after being lifted rapidly over the birds' back (Bostwick & Prum 2003; Fusani et al. 2007). When displaying, heart rates are profoundly elevated in males, even briefly exceeding 1300 beats per minute (Barske et al. 2011), one of the highest heart rates recorded for any avian/mammalian species (Lasiewski 1964; Jürgens et al. 1996). This elevated heart rate suggests that male manakins expend considerable energy in the performance of courtship activities. Outside of courtship, however, GC manakins exhibit life history traits typical of the 'slow pace' of tropical life producing small clutches (1-2 eggs; Ricklefs 1976; Skutch 1985) and reportedly living as long as 14 years (Snow & Lill 1974; Karr et al. 1990; Brawn et al. 1995). Thus, it is of interest whether during the breeding season, male GC manakins were able to sustain a tropical slow pace of life in the face of the putative energy demands of courtship or if net energy expenditure is increased through courtship behavior and makes manakins an exception to the general rule that tropical birds have a lower energy expenditure than temperate birds.

Efforts to assess organismal energy metabolism do so using various measures that describe overlapping and distinct states of metabolism. Basal metabolic rate (BMR) describes energy expenditure at rest under thermoneutral and postabsorbtive conditions whereas resting metabolic rate (RMR), does not require the animal to be in a postabsorbitve state (Brody 1945; Kleiber 1961). A measure of an animal's peak metabolic rate (PMR), or the maximum attainable rate, is usually experimentally induced through exercise or cold (Wiersma et al. 2007a). Finally, field metabolic rate (FMR) or daily energy expenditure (DEE), are used to assess the average metabolic rate of an animal under natural conditions performing normal behaviors. Whereas the tropical 'slow pace of life' is associated with a relatively low BMR and PMR (Weathers 1979; Hails 1983; Weathers 1997; Wikelski et al. 2003; Wiersma et al. 2007b; Williams et al. 2010), there is insufficient data to derive a definitive FMR measure for lowland tropical birds, though a trend towards decreased metabolic rate is described (Anderson & Jetz 2005).

To understand energy demands of discrete behaviors, one can determine the absolute amount of energy required for the behavior, giving importance to the length of the behavioral bout (measured in Joules, 'joule cost'). Alternatively, the energy demand can be seen as the increase in energetic demand per unit time, where the emphasis lies in the peak achieved (measured in Watts or Joules per sec, 'power'; Clark 2012). These measures are usually expressed in relation to BMR or RMR. These distinctions are important to efficiently tell apart behaviors consisting in rapid bursts of high energy, such as a sprint, from behaviors that like a marathon involve a lower power output for extended time periods. For example, during a chase, cheetahs display a 50-fold increase in their resting metabolic, but only for a very short time (Taylor 1974). This behavior then requires high power but low Joule cost. By contrast, while running, a marathoner displays a minimal increase in metabolic rate but for an extended time period. This involves a high Joule cost but less power (Gill et al. 2009; Hawkes et al. 2011). Both types of behaviors might bring the animal to its physiological limits, but each requires specialized physiological and morphological adaptations, one for bouts of peak metabolic rate and one for extended metabolic demands (reviewed in Speakman 2000; Piersma 2011) . Such distinctions are important for the study of courtship behavior. Bear in mind that ‘Joule cost’ has a different meaning than ‘cost of a trait’ in an evolutionary context (Fisher 1930; Zahavi 1975), wherein a phenotypic trait used in sexual selection produces a negative influence or ‘cost’ on the owner’s fitness (Kotiaho 2001).

Previous studies of the GC manakin obtained estimates of BMR that suggest they maintain metabolic rates comparable to other tropical species that lack vigorous courtship displays (Vleck & Vleck 1979; Bartholomew et al. 1983; Wiersma et al. 2007b). As the relationship between BMR and DEE is not clearly defined (Nagy 2005), I sought to obtain diurnal and nocturnal measures of energy expenditure of wild lekking males during their peak months of courtship. I used heart rate telemetry in combination with respirometry to estimate energy expenditure from heart rate recordings of wild birds. Further, using audio recording of individual leks as well as focal observations of individual males I obtained measures of daily activity (this study and (Barske et al. 2011). By analyzing the above data in combination with previously collected heart rate data recorded during discrete GC manakin behaviors (Barske et al. 2011) as well as of those obtained from a non-lekking tropical bird of similar size, the spotted antbird (*Hylophylax naevioides*; Steiger et al. 2009), I was able to offer new insight into the energetics of tropical courtship.

4.3 Methods

Studies were conducted during the dry season (January - April) in forests around Gamboa, Panama (9° 7' N, 79° 42' W) and in facilities maintained by the Smithsonian Tropical Research Institute (STRI). All procedures for animal use were approved by UCLA Chancellor's Animal Research Committee, STRI and the Panamanian Autoridad Nacional del Ambiente.

4.3.1 Transmitter attachment

Heart rates were recorded via telemetry using miniature transmitters as described previously for other small birds (Cochran & Wikelski 2005; Steiger et al. 2009; Bisson et al. 2009; Sapir et al. 2010) including GC manakins (Barske et al. 2011). Using mistnets, 8 adult male GC manakins were captured from five independent leks. Animals were brought back to the laboratory facilities where transmitters were attached and respirometry calibration was performed. Birds were given colored leg-bands for individual identification. On 4 birds, transmitters were attached in the field and birds were released (within ~10mins) at the site of capture. Each transmitter (~1g; Sparrow systems, Fisher, IL, USA) has a 9 cm flexible wire antenna and 2 wire electrodes and was fixed using glue to trimmed feathers on the center of the bird's back. The two electrodes are inserted beneath the skin (for further details, see Cochran & Wikelski 2005 and Steiger et al. 2009).

4.3.2 Calibration of heart rate versus O_2 consumption

Heart rate was calibrated against O_2 consumption in 4 of the 8 male GC manakins. These males were transferred to a lab facility within 10 km of their capture site, fitted with a transmitter and placed in an open flow respirometer consisting of an air-tight chamber that enabled simultaneous measurement of O_2 inflow and CO_2 outflow. Energy metabolism is assumed to be equivalent to

O_2 consumption, as aerobic respiration restocks depleted ATP. Fick's equation relates heart rate to O_2 consumption using stroke volume (the amount of blood pumped per heart beat) and the arterio-venous difference in O_2 content (or tissue O_2 extraction) the product of which is defined as O_2 pulse (OP; Fick 1870). If OP is constant or varies in a predictable manner, a calibration between O_2 consumption and heart rate can define this relationship (Butler et al. 2004; Green 2011). If CO_2 production is known, the respiratory quotient (RQ, the ratio of O_2 consumption and CO_2 production) can be calculated in order to estimate metabolic rate following Walsberg & Hoffman (2005). Heart rate is not a measure of absolute energy expenditure as a potential error is associated with the metabolic rate estimates based on the RQ obtained in the calibration equation (Green 2011). Instantaneous O_2 consumption was calculated using the equation of Bartholomew et al. (1981), followed by Withers (1977) to obtain the rate of O_2 consumption (for detailed description of the method see Bisson et al. (2009). For each bird, the respiratory exchange ratio was determined at 7-8 different heart rates.

4.3.3 Heart rate telemetry

Heart rate transmitters emit a continuous, amplitude modulated carrier signal that is frequency modulated by heart muscle potentials. Transmitters attached in this way fall off after 10-14 days eliminating the need for recapture of the birds (Raim 1978). Using a Yagi antenna, connected to an AR8000 receiver (AOR Ltd. Tokyo, Japan), heart rates were recorded every ten minutes for 30 seconds as sound files (mp3) on a digital recorder connected to the receiver (mp3 recorder, Edirol R09, Roland Inc.). In this way, heart rate measures were obtained randomly with respect to behavior, to obtain an accurate reflection of the bird's daily commitment to different energetic demands. Sound files were analyzed using CoolEdit 2000 software (Syntrillium Software, Phoenix, Arizona). Band pass filters (Fast Fourier transform filters) were applied to increase the signal-noise ratio between heart rate frequency and carrier frequency. Heart rate was measured by identifying 10 consecutive amplitude spikes and then averaging the time intervals between each spike pair (Sapir et al. 2010); skeletal muscle potentials were irregular and easily distinguished from iterative potentials emanating from the heart.

4.3.4 Courtship activity

In a previous study, I found that courtship displays and mechanically produced sounds (wingsnaps and rollsnaps) are associated with extremely high heart rates, hence, they are the most energetically demanding behaviors performed on the lek (Barske et al. 2011). Wingsnap frequency is a significant predictor of mating success (Barske et al. 2011). I estimated average on-lek social activity across the day from audio recordings. Note that during the study period, sunrise occurred between 06:03 - 06:34 with sunset occurring between 18:10-18:35. Average values are taken for the representation in Figure 4.1. Given twilight periods, for statistical analyses, all subsequent measures labeled as ‘diurnal’ refer to the time between 06:30 to 17:30. A microphone attached to a digital recorder with > 12 hrs of battery-life was placed in a central position within 5 different leks (with 4 ± 1 adult courting males) from which I obtained three1-day audio recordings. To estimate daily social and courtship activity, audio files were analyzed using CoolEdit 2000 software. I divided the file into 1-hour intervals, starting at 6:30, I counted wingsnaps and rollsnaps between 06:30 – 07:30, which represented activity at 07:00 and so on. To estimate activity of individual males on a lek, I divided the hourly sums of mechanical sounds produced by the number of males occupying that lek. In addition, I used data from previously obtained video recordings (N =17) and observations (N = 31) of individual males, to estimate mating success in relation to the time spent by males performing courtship displays (Barske et al. 2011). High-speed videos (N =87) of 17 courting males were used to measure the average duration of a courtship display. With focal observations I measured the number of displays and rollsnaps performed by males as well as their mating success.

4.3.5 Statistical analyses

SPSS 19 (IBM Inc., Chicago, IL) was used for statistical analyses. Calibration relationships between heart rate and O_2 consumption of birds in the respirometer were determined by least square regressions. A Generalized Linear Model (GLM) with metabolic rate as the dependent variable, individual as a random factor and heart rate as a covariate, was used to determine the overall relationship between heart rate and O_2 consumption based on the data collected for the four birds during calibration. The standard error of the estimate (SEE) and the coefficient of variability (COV = 100*SEE/estimate) were calculated after Green (Green 2011). To investigate the pattern of courtship activity, I used a GLM with hour of the day as the repeated measure (daytime) and leks as the between subject factor. To investigate the pattern of heart rate across the day, I used a GLM with hour of day as the repeated measure (daytime). Heart rates were log transformed to obtain normality. Post-hoc tests with Bonferroni corrections were used to identify differences of activity or heart rate between different time points. Differences between antbirds and GC manakins were investigated using One-way ANOVAs. If not indicated differently, values in the text are given as Mean ± SEM.

4.4 Results

4.4.1 Patterns of daily courtship activity

Activity levels varied significantly across the day and also between leks (daytime: $F_{3.5,34.6} = 9.159$, $P<0.001$; lek: $F_{4,10} = 5.643$, $p = 0.012$; interaction: $F_{13.8,34.6} = 1.743$, $p = 0.266$; Figure 4.2a-e). Thus, despite differences between leks in the magnitude of activity, leks were similar with respect to their patterns of daily courtship. Activity was generally elevated in the morning, declining to a nadir at 12:00, after which activity levels increase significantly to a peak at 14:00 and then declined significantly by 17:00 (Table 4.1).

4.4.2 Patterns of daily heart rate

Telemetry transmitters added ~ 5.5 % of a bird's body mass. I think that the transmitters have minimal impact on the birds as I have observed males with transmitters courting and copulating with females (see the electronic supplementary material, video S1, of Barske et al. 2011). Nevertheless, I cannot rule out the possibility that they have some impact on the birds, including increasing energy expenditure leading to an overestimation of natural energy expenditure (reviewed in Barron, 2010).

For six birds I measured heart rate for 24 hrs and in two additional birds I collected recordings during daylight hours (61 ± 12 diurnal recordings versus 17 ± 4 nocturnal recordings). When comparing hourly individual averages, I found a significant effect of daytime on heart rate ($F_{10,40} = 6.180$, $p < 0.001$; Figure 4.1). Heart rates rose steeply after 05:00, reaching a significant peak of 596 ± 37 beats min^{-1} at 07:00, where they remained for another hour (Table 4.1). Following this early-morning peak, heart rates significantly decreased to 394 ± 14 beats min^{-1} at

13:00. For the remainder of the afternoon, heart rates are slightly elevated with a peak ~ 18:00 (500 ± 16 beats min^{-1}).

At sunset, heart rates markedly decline with nocturnal rates generally low, decreasing to a min of 290 ± 8 beats min^{-1} at 04:00. This result corresponds with previous findings of low nocturnal metabolic activity in GC manakins to an extent that the birds appear to enter a state of hypothermia (Bartholomew et al. 1983). Nevertheless, GC manakins retain the capacity to be reactive (Bartholomew et al. 1983), and I observed discrete surges in heart rate (~ 700 beats min^{-1}) in one bird during the course of the night, likely a reaction to disturbance.

Taken together, these data allow us to calculate mean diurnal (active) heart rate of 450 ± 15 beats min^{-1}, calculated from 6:00 to 18:30 and a mean nocturnal heart rate of 332 ± 23 beats min^{-1}. Overall, the mean daily heart rate (24-h-Period) was 398 ± 13 beats min^{-1}.

4.4.3 Calibration of heart rate versus O_2 consumption

Heart rate calibration measurements spanned 382 to 943 beats min^{-1} , coinciding roughly with the range of heart rates recorded in the field (195 to 979 beats min^{-1}). During courtship displays, heart rates can exceed these ranges (Barske et al., 2011), so there may be some error in estimating metabolic rate at those elevated heart rates. The RQ was on average 0.88 ± 0.01, which lies within the predicted range of 0.7 – 1 (Kleiber 1961).

A linear relationship between O_2 consumption and heart rate of captive birds is required to estimate energy expenditure from heart rates recorded in the wild. I found such a relationship in GC manakins (Figure 4.3, Table 4.2), confirming what had been observed previously in other small birds (e.g. Steiger et al. 2009; Bisson et al. 2009; Sapir et al. 2010) . When comparing the regressions obtained from the four birds, I found that intercepts were significantly different between individual regressions ($F_{4, 28} = 11.77$, $p < 0.001$) but the slopes were the same (Table 4.2). Following Green et al. (Green et al. 2001), I obtained a final regression equation, using the slope from the pooled data with an average of the individual intercepts to predict energy expenditure in the wild:

$MR = (f_H * 0.148) - 19.796$

where MR is the metabolic rate in kJ d^{-1} and f_H is the heart rate in b min^{-1}. Equation (11) from Green et al. (2001) was used to calculate the SE of the estimated MR. The error associated with the variation between GC manakins (d^2) was 48.5, the error associated with the scatter around the regression lines (e^2) was 0.000081.

4.4.4 Daily energy consumption

From the average daily heart rate I estimated DEE to be 39.1 ± 2.5 kJ d^{-1} (Table 4.2), a reliable estimate given the relatively low 4-6% coefficients of variation (COV) I obtained for each estimate. The lowest recorded nocturnal heart rate was used to estimate minimal MR, calculated to be 14.1 ± 4.7 kJ d^{-1}.

4.4.5 Comparison between heart rates and DEE between antbirds and manakins

Heart rates measures and estimates of DEE obtained in the present study were compared with those obtained using the same method in another tropical bird of similar size, the spotted antbird (17.6 g; Steiger et al., 2009). Data were obtained from 6 adult male birds. Heart rates were recorded continuously and measurements were obtained every 5 min for 24 hours. Surprisingly, manakins have significantly lower day, night, and 24-hour heart rates than do spotted antbirds, but no difference was found in DEE between the two species (Table 4.3).

4.4.6 Energetic requirements for the courtship display

Male GC manakin heart rates rise during the performance of courtship displays and rollsnaps to 1017 ± 28 and 1062 ± 102 beats min^{-1}, respectively (Barske et al. 2011). For courtship displays, this rate corresponds to 131 kJ d^{-1} (5.5 kJ h^{-1}) with a COV of 3% (corrected from Barske et al. 2011) indicating that courtship display requires 1.2 W above the minimal MR. This corresponds to the PMR induced by exercise measured by Wiersma et al. (2007). Although overall lek activity lasts for many hours per day, males vary greatly in the total number of displays they perform per day, ranging from 1.6 to 140, producing an average of 32 ± 5 displays per day (N = 31). Importantly, each display lasts for only 9.5 ± 0.5 sec (N= 17), thus on average, males actually engage in courtship displays for 5 ± 1 min per day (0.25- 22.2 mins per day; data from Barske et al. 2011). This means that they spend on average 0.46 kJ in courtship displays, which corresponds to 1.2 % of their DEE. In addition, males produce on average 10.8 ± 1.5 rollsnaps hr^{-1}, thus expending an additional 0.04 kJ. Thus, the performance of the two metabolically most demanding courtship behaviors requires on average 0.5 kJ per day. If I assume that the power required for a courtship display is similar across males, then, the least active males would spend ~ 0.1 % of their DEE in courtship displays compared with ~ 5.2 % of the most active ones (Figure 4.4). As is the case for the majority of lekking species, the few extremely active males obtain most of the copulations (Stein & Uy 2006; Barske et al. 2011). Although I have no evidence for a link between the energetic investment in courtship and mating success in GC manakins, it is likely that from least active to most active and successful males, GC manakins expend a minimal amount of energy in courtship.

4.5 Discussion

By all measures, GC manakins have a low DEE, as expected for tropical birds. This conclusion, based on heart rate measures, is confirmed by several independent analytic approaches. First, using the same procedures in the same habitat, I found no difference between DEE of GC manakins and the spotted antbird, a non-lekking Panamanian bird with a low DEE (Steiger et al. 2009). Second, when I applied a multivariate model designed previously to estimate avian energy expenditure of tropical birds (Anderson & Jetz 2005), I obtained an estimate of manakin DEE of 38.8 kJ d^{-1}. This value is noticeably similar to the estimate I obtained through heart rate telemetry of 39.1 kJ d^{-1} and confirms a DEE typical for tropical life. Third, according to Nagy et al. (1999), a passeriform bird of 18.1 g should possess a DEE of 74.5 kJ d^{-1}, a value nearly two-fold higher than my estimate. Finally, Garamszegi et al. (2006) obtained an average mass corrected DEE of songbirds from arid and mesic climates of 9 kJ d^{-1} g^{-1}, which is, again, about twice that of the GC manakin (4.5 kJ d^{-1} g^{-1}; mcDEE = DEE/$M^{0.75}$).

Estimates of metabolic rate, derived from heart rate measures of wild male GC manakins, provide new information on DEE during the peak courtship period of a tropical bird. As heart rate is not an absolute measure of an individual's energy turnover, it stands as a proxy for energy expenditure (Butler et al. 2004). With this caveat in mind, it appears nonetheless that despite performance of high power courtship displays, male GC manakins maintain low DEE, comparable to that of other tropical birds.

For a proper interpretation of the data I need to consider both the advantages and the limitations of heart rate telemetry for estimating the metabolic rate of wild animals. Notably, heart rate measures provide excellent temporal resolution such that estimates of metabolic rate are obtained in the order of seconds to minutes as compared to more traditional techniques such as the doubly-labeled water method, that provides estimates across days (Bevan et al. 1994; 1995; Green et al. 2001; Butler et al. 2004). Moreover, the telemetry methodology I used is minimally invasive, even when applied to small birds, and does not require recapture of the subjects (Wikelski et al. 1999; Steiger et al. 2009; Sapir et al. 2010; Hawkes et al. 2011). Limitations of this method include the possible impact of the transmitter weight on the behavior and metabolism. There are also limits to the signal detection range as well as the difficulty of observing the animals, that, in dense forests, confound simultaneous behavioral observations during collections of heart rate measures, especially for behaviors that last for only seconds at a time. To overcome these limitations, I combined heart rate data from one group of birds with estimates of activity from additional birds. In addition, to relate DEE to energy expended during courtship behaviors, I combined this work with data from a third group of birds, for which I obtained heart rates during focal sampling of behavioral measures (Barske et al., 2011). From these data I know that heart rates double during courtship displays that involve the production of mechanical sounds (wingsnaps and rollsnaps), whereas other behaviors, like jumping on the arena or producing non-mechanical cheepoo sounds, elevate heart rate minimally (Barske et al., 2011). Thus, I focused the analysis on behaviors with the greatest power requirements. Despite these experimental limitations, I am confident that these estimates can be generalized across individuals and leks, and improvements in data collection would not substantially alter the conclusions I have reached.

Compared to temperate breeding birds BMR is lower in tropical birds, including the GC manakin (Vleck and Vleck, 1979; Bartholomew et al. 1983; Wiersma et al., 2007). These data on the metabolic rate of wild manakins obtained from heart rate measures compares favorably with these previous estimates obtained from studies of captive birds. Because life-history variables such as environmental condition and feeding cannot be controlled in wild birds, minimal MR is the best estimate of RMR and BMR, measures that can be obtained directly in captivity. Using the equation of Aschoff and Pohl (1970), a manakin-sized bird of the temperate zone is predicted to have a BMR of 26 kJ d^{-1}, a value twice the minimal MR of 14 kJ d^{-1} that I estimated by use of the heart rate method. Lower than predicted levels of BMR in GC manakins have also been described previously by Vleck and Vleck (1979; 21 kJ d^{-1}) and Bartholomew et al. (1983; 20 kJ d^{-1}). Thus, from my measures and from those of previous studies, I can conclude that GC manakins fall in the life strategy classification of "slow pace of life" typical for tropical birds (Ricklefs 1976; Wiersma et al. 2007).

I was surprised to discover that overall daily courtship activity in GC manakins lacks high Joule costs, that is, it is not as energetically demanding as I had expected. Although individual courtship displays *per se* have high energy demands, reaching peak metabolic rates 9-fold higher than RMR and 3-fold higher than DEE (Wiersma et al. 2007a; Barske et al. 2011) males actually spend minimal time per day in courtship performance, using on average only ~ 1.5 % of their DEE on courtship dances. Collectively, males on a lek produce considerable noise in order to attract females and engage in courtship bouts in hopes of mating. Yet, each individual invests minimal energy in the lekking enterprise. Even the most active male, performing as many as 140 courtship displays per day, invests only 5% of his DEE, because each display only lasts ~ 9.5 secs. This strategy of low energetic costs could explain why manakins are able to display for 6 or more months of the year and retain a lengthy life-span.

In contrast to GC manakins, other species engage in energetically costly courtship involving high Joule costs (Höglund et al. 1992; Kotiaho et al. 1998; Kotiaho 2000; Thomas 2002). For example, each day, sage grouse males perform on average 1680 displays during one or two bouts per day (Wiley 1973; Gibson & Bradbury 1985). Thus, each bout of displaying (totalling about 1.4 hrs per day) functions more like an endurance exercise or marathon that, by the end of the breeding season, produce significant weight loss in the performers (Vehrencamp et al. 1989). Presumably, these added energy demands impose limits on the duration of the season of courtship.

Courtship enables females to assess male quality for purposes of mating (Byers et al. 2010). Presumably, males that engage in courtship that exacts high Joule cost do so, at least in part, to demonstrate their capacity to withstand the added energetic demands of courtship behavior (Fisher 1930; Zahavi 1975; Andersson 1994). The females might then choose males based on their behavioral and/or physiological adaptations to accumulate and efficiently metabolize the energy reserves required for courtship activity. Rather than being limited by total energy, male GC manakins appear to be performance-limited, that is the courtship display may be designed to demonstrate physiological and morphological qualities associated with their rapid, acrobatic performances. For example, male manakins have hypertrophied skeletal muscles and fibres modified for increased muscular contraction (Lowe, 1942; Schultz et al. 2001). It is conceivable that the high heart rates achieved during courtship performance reflect optimal cardiovascular and respiratory functions that female’s indirectly assess via the courtship displays.

In conclusion, these data indicate that one tropical bird species with a lek mating system does not experience the extreme energetically demanding periods of courtship that have been described in some temperate-breeding birds. Despite being present on the lek for much of the day, GC manakins appear to periodically show off their morphological and physiological proficiency in sprint-like display bouts across the day while maintaining a tropical ‘slow pace of life’ metabolic strategy (Irschick et al. 2007; Byers et al. 2010; Clark 2012). Many species of neotropical manakins engage in lekking behavior, with a variety of patterns of timing and frequency of courtship (Bostwick 2000; Duval 2007; Shorey 2002). Additional research is required to determine if the low Joule cost strategy of GC manakins is common to other tropical lekking birds.

4.6 Figures and Tables

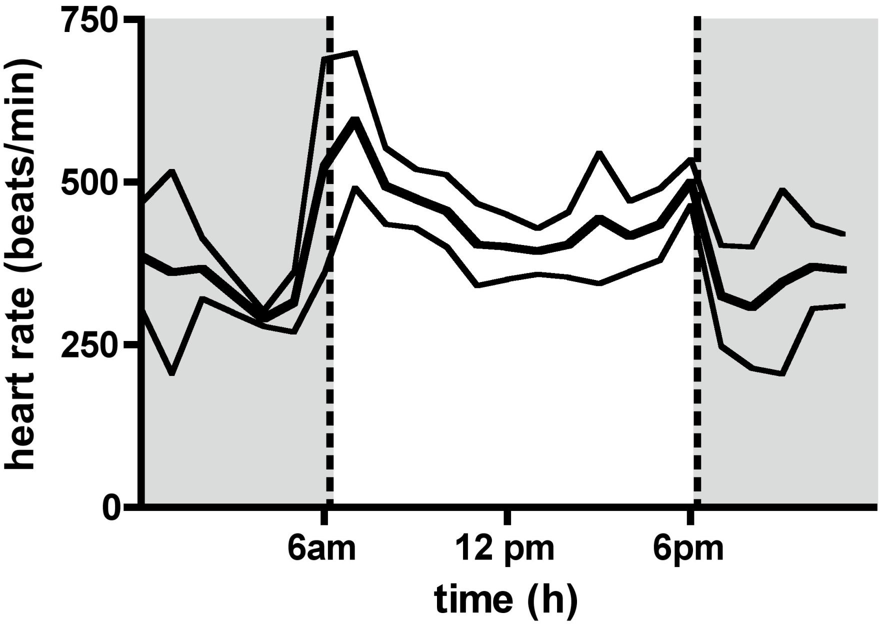

Figure 4.1 Daily heart rate pattern. The thick line represents the hourly mean heart rates of 8 male GC manakins. Hourly averages were calculated for the 30 minutes before and after the time point. During the night, I obtained heart rate measurements of only 6 manakins. No measure was taken at 3:00 in the morning, thus I averaged values of 2:00 and 4:00 to complete the graph. Thin lines represent the 95% confidence intervals (1 standard deviation). Shaded areas indicate night-time (18:12 until 06:15).

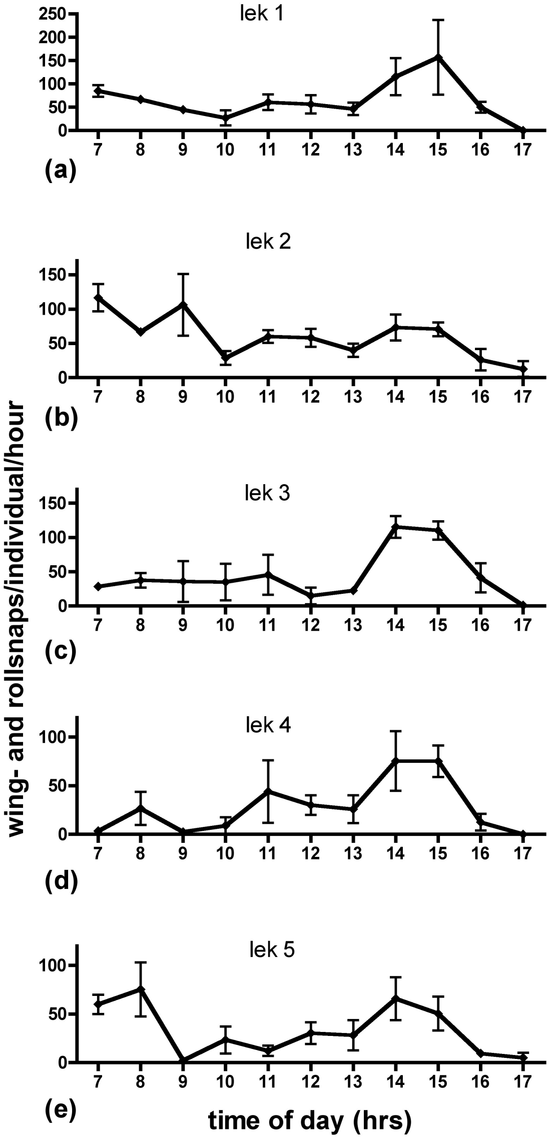

Figure 4.2 Diurnal pattern of courtship activity of male GC manakins. The number of mechanically produced sounds (wingsnaps and rollsnaps), a measure of manakin social and courtship behavior at the lek were recorded for 5 different leks in 3x 1-day recordings. Hourly means (±SE) per male are represented and were calculated including 30 minutes before and after the time point. Values were then divided by the number of males (4 ± 1) on the corresponding lek that was determined with direct behavioral observations. Figures 1a-e display the average number of sounds produced per male on each of the 5 leks.

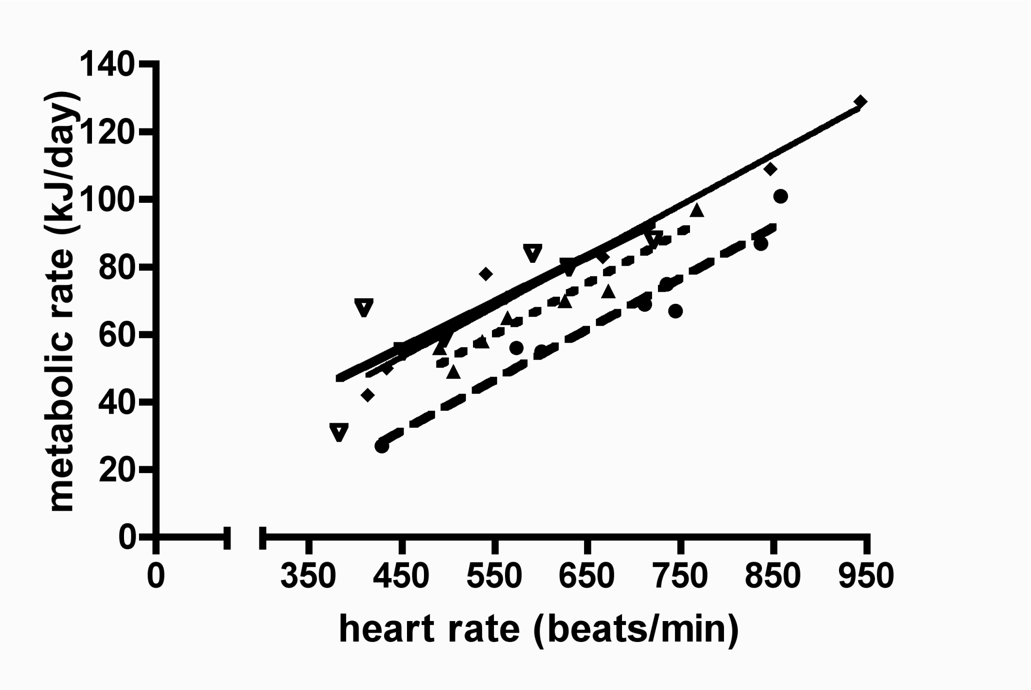

Figure 4.3 Relationship between heart rates and metabolic rates. Least square regressions of heart rates against metabolic rates for 4 birds determined by calibration measurements: bird E (p < 0.000), bird G (p = 0.013), bird H (p < 0.000) and bird I (p< 0.000). See Table 4.1 for statistical details.

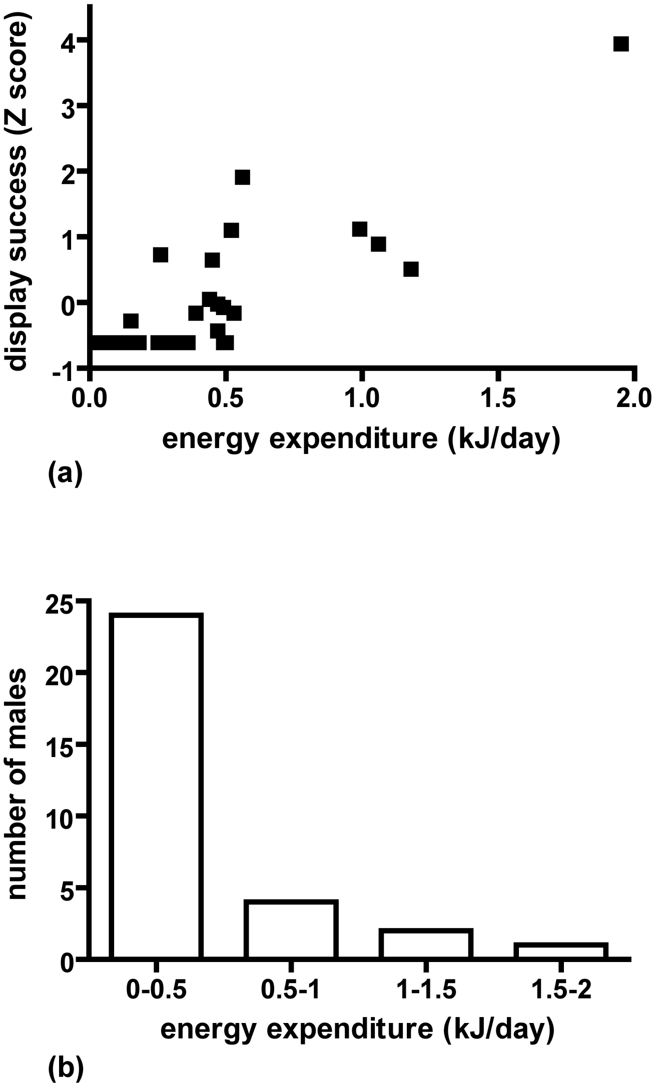

Figure 4.4 Differences in energy expenditure for courtship between lekking males. (a) The figure shows the display success - a measure of the number of matings and of female visits - of 31 lekking males versus individual energy expenditure that was estimated from average metabolic rate during display and amount of displays performed. Display success and number of displays performed are calculated from data published previously (Barske et al. 2011). (b) Frequency distribution of energy expenditure among different males.

Table 4.1 Significant results of Bonferroni corrected post-hocs comparing activity and heart rate levels between different hours of the day (7:00 – 17:00). The shaded area indicates heart rates and arrows refer to the hours on the top. For example heart rate is lower at 13:00 compared to 8:00. The non-shaded area of the table refers to activity and arrows refer to hours on the left.

		heart rate										
		7	8	9	10	11	12	13	14	15	16	17
activity	7	■								↓		
	8		■					↓				
	9			■								
	10				■							
	11					■						
	12						■					
	13							■				
	14						↑	↑	■			
	15									■		
	16										■	
	17	↓	↓				↓		↓	↓		■

Table 4.2 Parameters of Least Square Regressions for heart rate against metabolic rate of four male golden-collared manakins. Animals equipped with a heart rate transmitter were put in a respirometer and heart rate was measured simultaneously to O2 consumption and CO2 production.

id	N	a	b	r^2	p
E	7	-13.8	0.15	0.97	< 0.001
G	7	-4.76	0.14	0.71	0.017
H	7	-22.55	0.15	0.93	< 0.001
I	8	-35.18	0.15	0.94	< 0.001

(id: birds used for calibrations; N: number of measurements that have been taken at different heart rates; a: intercept; b: slope)

Table 4.3 Heart rates measures and estimates of Daily Energy Expenditure (DEE) obtained with heart rate telemetry were compared between two tropical birds: the golden-collared manakin (18.1g) and the spotted antbird (17.6g). The first two columns represent mean values for manakins and antbirds for following variables: average diurnal heart rate (day), average minimal heart rate (night), average daily heart rate (24-h) and average DEE. Measures given as mean with standard error and sample number in parenthesis. The last two columns represent the results of the Anovas comparing the different variables between the two species. Manakins have lower heart rates for all three measures, but no difference is found in DEE. Also when comparing mass corrected DEE, I did not find a significant difference.

	manakin	antbird	F	p
day (b/min)	450 ± 15 (8)	601 ± 24 (6)	30.629	0.000
night (b/min)	332 ± 23 (6)	384 ± 15 (6)	90.419	0.000
24-h (b/min)	403 ± 10 (6)	492 ± 12 (6)	20.480	0.001
DEE (kJ/d)	39.1 ± 2.5 (6)	37.4 ± 2.4 (6)	0.371	0.556

Data on antbirds are taken from (Steiger et al. 2009).

4.7 References

Anderson, K. J., & Jetz, W. 2005. The broad-scale ecology of energy expenditure of endotherms. *Ecology Letters*, 8, 310–318.

Andersson, M. 1994. *Sexual Selection.* Princeton, NJ: Princeton University Press Princeton.

Barske, J., Schlinger, B., Wikelski, M., & Fusani, L. 2011. Female choice for male motor skills. *Proceedings of the Royal Society B: Biological Sciences*, 278, 3523–3528.

Bartholomew, G. A., Vleck, C. M., & Bucher, T. L. 1983. Energy metabolism and nocturnal hypothermia in two tropical passerine frugivores, Manacus vitellinus and Pipra mentalis. *Physiological Zoology*, 56, 370–379.

Bartholomew, G. A., Vleck, D., & Vleck, C. M. 1981. Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *Journal of Experimental Biology*, 90, 17–32.

Bevan, R. M., Speakman, J. R., & Butler, P. J. 1995. Daily energy expenditure of Tufted Ducks: a comparison between indirect calorimetry, doubly labelled water and heart rate. *Functional Ecology*, 9, 40–47.

Bevan, R. M., Woakes, A. J., & Butler, P. J. 1994. The use of heart rate to estimate oxygen consumption of free-ranging Balck-browed Albatrosses Diomedea melanophrys. *Journal of Experimental Biology*, 193, 119–137.

Bisson, I.-A., Butler, L. K., Hayden, T. J., Romero, L. M., & Wikelski, M. C. 2009. No energetic cost of anthropogenic disturbance in a songbird. *Proceedings of the Royal Society B Biological Sciences*, 276, 961–9.

Bostwick, K. S., & Prum, R. O. 2003. High-speed video analysis of wing-snapping in two manakin clades (Pipridae: Aves). *Journal of Experimental Biology*, 206, 3693–3706.

Brawn, J. D., Karr, J. R., Nichols, J. D., Ecology, S., & Jan, N. 1995. Demography of Birds in a Neotropical Forest : Effects of Allometry, Taxonomy, and Ecology. *Ecology*, 76, 41–51.

Brody, S. 1945. *Bioenergetics and Growth.* New York: Hafner.

Butler, P. J., Green, J. A., Boyd, I. L., & Speakman, J. R. 2004. Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods. *Functional Ecology*, 18, 168 – 183.

Byers, J., Hebets, E., & Podos, J. 2010. Female mate choice based upon male motor performance. *Animal Behavior*, 79, 771–778.

Chapman, F. 1935. The courtship of Gould's manakin (Manacus vitellinus vitellinus) on Barro Colorado Island, Canal Zone. *Bulletin of American Museum of Natural History*, 68, 471–525.

Clark, C. J. 2012. The role of power versus energy in courtship: what is the "energetic cost" of a courtship display? *Animal Behavior*, 84, 269–277.

Cochran, W. W., & Wikelski, M. 2005. Individual migratory tactics of New World Catharus thrushes: current knowledge and future tracking options from space. In: *Birds of Two Worlds: The Ecology and Evolution of Migration*, (Ed. by P. Marra & R. Greenberg), pp. 274–289. Baltimore: Johns Hopkins University Press.

Fick, A. 1870. Uber die Messung des Blutquantums in der Herzventrikeln. *Sitz. Physik. Med. Ges.*, 2,

Fisher, R. A. 1930. *The genetical theory of natural selection.* Oxford: Oxford Press.

Fusani, L., Giordano, M., Day, L. B., & Schlinger, B. A. 2007. High-Speed Video Analysis Reveals Individual Variability in the Courtship Displays of Male Golden-Collared Manakins. *Ethology*, 113, 964–972.

Fusani, L., & Schlinger, B. 2012. Proximate and ultimate causes of male courtship behavior in Golden-collared Manakins. *Journal of Ornithology*, 153, 119–124.

Gibson, R. M., & Bradbury, J. W. 1985. Sexual selection in lekking sage grouse: phenotypic correlates of male mating success. *Behavioral Ecology and Sociobiology*, 18, 117–123.

Gill, R. E., Tibbitts, T. L., Douglas, D. C., Handel, C. M., Mulcahy, D. M., Gottschalck, J. C., Warnock, N., McCaffery, B. J., Battley, P. F., & Piersma, T. 2009. Extreme endurance flights by landbirds crossing the Pacific Ocean: ecological corridor rather than barrier? *Proceedings of the Royal Society B Biological Sciences*, 276, 447–57.

Green, J. A. 2011. The heart rate method for estimating metabolic rate: review and recommendations. *Comparative Biochemistry and Physiology, Part A*, 158, 287–304.

Green, J., Butler, P. J., Woakes, A. J., Boyd, I. L., & Holder, R. 2001. Heart rate and rate of oxygen consumption of exercising Macaroni Pinguins. *Journal of Experimental Biology*, 204, 673–684.

Hails, C. J. 1983. The metabolic rate of tropical birds. *Condor*, 85, 61–65.

Hawkes, L. A., Balachandran, S., Batbayar, N., Butler, P. J., Frappell, P. B., Milsom, W. K., Tseveenmyadag, N., Newman, S. H., Scott, G. R., Sathiyaselvam, P., Takekawa, J. Y., Wikelski, M., & Bishop, C. M. 2011. The trans-Himalayan flights of Bar-headed Geese (Anser indicus). *Proceedings of the National Academy of Sciences*, 108, 9516–9519.

Höglund, J., & Alatalo, R. V. 1995. *Leks.* Princeton, NJ: Princeton University Press.

Höglund, J., Kålås, J. A., & Fiske, P. 1992. The costs of secondary sexual characters in the lekking Great Snipe (Gallinago media). *Behavioral Ecology*, 30, 309–315.

Irschick, D. J., Herrel, A., Vanhooydonck, B., & Damme, R. V. 2007. A functional approach to sexual selection. *Functional Ecology*, 21, 621–626.

Jürgens, K. D., Fons, R., Peters, T., & Sender, S. 1996. Heart and respiratory rates and their significance for convective oxygen transport rates in the smallest mammal, the Etruscan shrew Suncus etruscus. *The Journal of experimental biology*, 199, 2579–84.

Karr, J. R., Nichols, J. D., Klimkiewicz, M. K., & Brawn, J. D. 1990. Survival Rates of Birds of Tropical and Temperate Forests: Will the Dogma Survive? *The American naturalist*, 136, 277–291.

Kleiber, M. 1961. *The fire of life. An introduction to animal energetics.* New York.

Kotiaho, J. S. 2000. Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. *Behavioral Ecology and Sociobiology*, 48, 188–194.

Kotiaho, J. S. 2001. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biological reviews of the Cambridge Philosophical Society*, 76, 365–76.

Kotiaho, J. S., Alatalo, R. V., Mappes, J., Nielsen, M. G., Parri, S., & Rivero, A. 1998. Energetic Costs of Size and Sexual Signalling in a Wolf Spider. *Proceedings of the Royal Society B: Biological Sciences*, 265, 2203–2209.

Lasiewski, R. C. 1964. Body Temperatures, Heart and Breathing Rate, and Evaporative Water Loss in Hummingbirds. *Physiological Zoology*, 37, 212–223.

Nagy, K. a. 2005. Field metabolic rate and body size. *The Journal of experimental biology*, 208, 1621–5.

Piersma, T. 2011. Why marathon migrants get away with high metabolic ceilings: towards an ecology of physiological restraint. *The Journal of experimental biology*, 214, 295–302.

Raim, A. 1978. A Radio Transmitter Attachment for Small Passerine Birds. *Bird-Banding*, 49, 326–332.

Ricklefs, R. E. 1968. Patterns of growth in birds. *Ibis*, 110, 419–451.

Ricklefs, R. E. 1976. Growth rates of birds in the humid New World tropics. *Ibis*, 118, 179–207.

Sapir, N., Wikelski, M., McCue, M. D., Pinshow, B., & Nathan, R. 2010. Flight modes in migrating European Bee-eaters: heart rate may indicate low metabolic rate during soaring and gliding. *PloS one*, 5, e13956.

Schlinger, B., Day, L. B., & Fusani, L. 2008. Behavior, natural history and neuroendocrinology of a tropical bird. *General and comparative endocrinology*, 157, 254–258.

Schultz, J. D., Hertel, F., Bauch, M., & Schlinger, B. A. 2001. Adaptations for rapid and forceful contraction in wing muscles of the male golden-collared manakin: sex and species comparisons. *Journal Of Comparative Physiology A*, 187, 677–684.

Skutch, F. 1985. Clutch size, nesting success, and predation on nests of neotropical birds, reviewed. *Ornithological Monographs*, 36, 575–594.

Snow, D. W., & Lill, A. 1974. Longevity records for some neotropical land birds. *The Condor*, 76, 262–267.

Speakman, J. R. 2000. *The Cost of Living : Field Metabolic Rates of Small Mammals.*

Steiger, S. S., Kelley, J. P., Cochran, W. W., & Wikelski, M. 2009. Low metabolism and inactive lifestyle of a tropical rain forest bird investigated via heart-rate telemetry. *Physiological and Biochemical Zoology*, 82, 580–589.

Taylor, C. R. 1974. Running in cheetahs, gazelles, and goats: energy cost and limb configuration. *American Journal Of Physiology*, 227, 848–850.

Thomas, R. J. 2002. The costs of singing in nightingales. *Animal Behavior*, 63, 959–966.

Vehrencamp, S. L., Bradbury, J. W., & Gibson, R. M. 1989. The energetic cost of display in male Sage Grouse. *Animal Behavior*, 38, 885–896.

Vitousek, M. N., Rubenstein, D. R., Nelson, K. N., & Wikelski, M. 2008. Are hotshots always hot? A longitudinal study of hormones, behavior, and reproductive success in male marine iguanas. *General and comparative endocrinology*, 157, 227–32.

Vleck, C. M., & Vleck, D. 1979. Metabolic rate in five tropical bird species. *Condor*, 81, 89–91.

Walsberg, G. E., & Hoffman, T. C. M. 2005. Direct calorimetry reveals large errors in respirometric estimates of energy expenditure. *The Journal of experimental biology*, 208, 1035–43.

Weathers, W. W. 1979. Climatic adaptation in avian standard metabolic rate. *Oecologia*, 42, 81–89.

Weathers, W. W. 1997. Energetics and thermoregulation by small passerines of the humid, lowland tropics. *The Auk*, 114, 341–353.

Wiersma, P., Chappell, M. A., & Williams, J. B. 2007a. Cold- and exercise-induced peak metabolic rates in tropical birds. *Proceedings of the National Academy of Sciences*, 104, 10866–10871.

Wiersma, P., Muñoz-Garcia, A., Walker, A., & Williams, J. B. 2007b. Tropical birds have a slow pace of life. *Proceedings of the National Academy of Sciences*, 104, 9340–9345.

Wikelski, M., Lynn, S., Breuner, J. C., Wingfield, J. C., & Kenagy, G. J. 1999. Energy metabolism, testosterone and corticosterone in White-crowned Sparrows. *Journal Of Comparative Physiology A*, 185, 463–470.

Wikelski, M., & Ricklefs, R. E. 2001. The physiology of life histories. *Trends in Ecology & Evolution*, 16, 479–481.

Wikelski, M., Spinney, L., Schelsky, W., Scheuerlein, A., & Gwinner, E. 2003. Slow pace of life in tropical sedentary birds: a common-garden experiment on four Stonechat populations from different latitudes. *Proceedings of the Royal Society B: Biological Sciences*, 270, 2383–2388.

Wiley, R. H. 1973. The strut display of male sage grouse: a "fixed" action pattern. *Behavior*, 129–152.

Williams, J. B., Miller, R. A., Harper, J. M., & Wiersma, P. 2010. Functional linkages for the pace of life, life-history, and environment in birds. *Integrative and comparative biology*, 50, 855–68.

Withers, P. C. 1977. Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. *Journal of applied physiology*, 42, 120–123.

Zahavi, A. 1975. Mate Selection-A Selection for a Handicap. *Journal of Theoretical Biology*, 53, 205–214.

CHAPTER 5

Cardiovascular Biology of an Acrobatic Bird (*Manacus vitellinus*)

5.1 Abstract

Plasticity of the heart has been observed in accordance to different environments and behaviors posing different metabolic demands on the organism. Courtship behavior in birds has been shown to be highly energetically demanding, but nothing is known about underlying cardiovascular changes to enable such behaviors. While performing a highly acrobatic courtship dance, the heart rate in golden-collared manakins increases up to 1300 beats per minute, among the highest heart rates ever recorded in the avian and mammalian world. I hypothesize that male manakins have increased cardiovascular functioning in order to perform courtship displays with such extreme demands on the heart. Using histological and molecular techniques, I compared the heart structure and the expression of the genes involved in Ca^{2+} handling, action potential duration as well as the steroid signaling pathway of manakins and zebra finches, a bird of similar size, which courtship consists of mostly song, with heart rates reaching around 700 beats per minute. I found that the LV wall was significantly thicker and the LV chamber size was significantly smaller in manakins compared to zebra finches. The expression of ryanodine receptor was significantly higher, whereas the expression of AKT was significantly lower in manakins compared to zebra finches. I also found high androgen sensitivity in the manakin heart, suggesting a steroid dependent pathway in manakin cardiovascular functioning.

5.2 Introduction

With its crucial function of supplying the body with nutrients and oxygen, the heart displays considerable long- and short-term plasticity as energetic demands vary with the environment or with different life-history stages. As they are successful in a diversity of environments and periodically perform unusually energetically demanding behaviors, birds provide superb models to explore cardiovascular physiology. For example, because colder environments require higher metabolic rates and lower relative air pressure demands increased oxygen transport that requires a relatively more massive heart, avian heart size varies with latitude and with altitude (Norris & Williamson 1955; Brush 1966; Koester 1976). Heart size can also change seasonally such as the increases in heart mass observed prior to migration in semipalmated sandpipers *Calidris pusilla* (Driedzic et al. 1993), eared grebes *Podiceps nigricollis* (Jehl 1997) and barnacle geese *Branta leucopsis* (Bishop et al. 1996) and decreases seen during migration in garden warblers *Sylvia borin* (Bauchinger et al. 2005). Little is known about the mechanisms underlying this plasticity in avian heart mass.

Most comparative studies have investigated heart weight as a proxy for function. To better understand how cardiac tissues change to match changing cardiovascular demands, investigators have focused their attention largely on humans and rodents; less is known about attributes of the avian heart across a changing landscape of energetic demands (Brush 1966; DiDio 1966; Froelicher 1972; Szabo et al. 1986; Junker et al. 1994; Bishop 1997; Allah et al. 2010). In common with these taxa, the avian heart has four chambers, two atria and two ventricles. From the right ventricle, the blood is pumped to the lungs with the left ventricle responsible for the systemic blood supply. Thus, the muscle responsible for the systemic blood supply includes the free left ventricular wall (LV wall) and the interventricular septum (IVS). During exercise or other ways of increased oxygen demand, the heart might beat faster which in turn increases blood pressure. Increased blood pressure leads to an increased afterload, the tension created on the LV wall during blood ejection. According to Laplace's law an increased pressure increases the tension on the myocardial wall during ejection, which responds by thickening. The heart of an athlete can experience physiological hypertrophy, an increase in cardiac muscle mass, that serves to maintain or increase function (reviewed in (Howard & Baker 1991; Frey & Olson 2003; Fagard 2003; Mihl et al. 2008; Bernardo et al. 2010). In contrast, pathological hypertrophy, which results from conditions such as hypertension, can lead to heart failure (Iemitsu et al. 2003; Maron 2005). Depending on the initiating stimulus, cardiac hypertrophy is often classified as either eccentric hypertrophy characterized by increased muscle and chamber size caused by a volume overload during endurance training or as concentric hypertrophy which involved only an increase in muscle size caused by a pressure overload.

Much like migratory behavior, avian courtship displays have been shown to pose strong metabolic demands on individual males (Vehrencamp et al. 1989; Höglund et al. 1992). It is unknown, however, if cardiovascular adaptations are needed to meet such demands. Manakins are tropical sub-oscines, a family of almost 50 species, most of them having complex courtship displays (Prum 1990). Among them is the golden-collared manakin (*Manacus vitellinus*; hereafter manakin). In this species, males perform a highly acrobatic, fast-paced and noisy courtship display (Chapman 1935; Schlinger et al. 2008). Males also produce loud snapping sounds (wing- and roll-snaps) as they singly, or repeatedly thrust their wings over their heads causing the wings to collide and produce the snaps (Chapman 1935; Schlinger et al. 2001). Within their lek breeding system, each male manakin establishes an arena on the forest floor where he performs his display jumping between saplings. Using miniature telemetry on wild courting males, I have previously found that their heart rates accelerate up to 1300 beats per min, among the highest heart rates ever recorded in birds and mammals (Barske et al. 2011). Heart rates also rise when males wing- and roll-snap. Wingsnaps are produced mid-air during the courtship dance, one snap per jump involving on average 9 snaps per display. Rollsnaps, a fast series of ~ snaps at 50—70 Hz is produced when perched. Although these behaviors last only a few seconds, males produce on average 32 displays and 136 rollsnaps per day over a 7-month long breeding season (Chapter 4). Some males perform as many as 140 displays and 315 rollsnaps per day (Chapter 4). Heart rates rise and fall rapidly in parallel with behavior, similar to an athlete's cardiovascular functioning, which is able to rapidly increase to meet momentary increased demands and then return to baseline once the acute demand has ceased (Fagard 2003; Barske et al. 2011). Manakins appear to have optimized their cardiovascular function because their average daily heart rate is ~ 450 beats per min, significantly lower than the spotted antbird *Hylophylax naevioides* (~600 beats per min) a bird of same size and habitat but which lacks a vigorous courtship display (Steiger et al. 2009; Chapter 4). Thus, with each stroke, the manakin heart may maximize the amount of oxygen pumped to the body. This could indicate that cardiac output is increased with an increase in stroke or ejection volume, which could be associated with cardiac hypertrophy. This in turn could be evoked through the extremely high heart rates as explained above.

Based on these observations, I hypothesize that adult male manakins have increased cardiovascular functioning. In particular, I predict that the myocardium, especially the LV muscle, is hypertrophied in male manakins with cellular and molecular properties that allow for faster cardiac contraction. Moreover, as the androgen testosterone (T) activates courtship behavior in manakins (Fusani et al. 2007; Day et al. 2007) and as the mammalian myocardium is known to be androgen sensitive (Marsh et al. 1998), I hypothesize that T assists in regulating cardiac function in male manakins.

To investigate male manakin myocardium, I compare hearts from reproductively active manakins with those of a species of similar size, the zebra finch *Taeniopygia guttata*, which lacks an acrobatic courtship performance. Males of this species use song to attract females, a behavior that is a much less energetic than male manakin courtship. In zebra finches, song accelerates heart rates up to ~700 beats per minute from baseline estimated ~ 600 beats per minute (Morris 1954; Cooper & Goller 2006; Zollinger et al. 2011). When housed with conspecifics, zebra finches sing on average for 3.2 mins/day (they sing between 7 – 50 phrases per hour and each phrase last 0.8 sec). Based on the differences in cardiovascular demand posed by courtship in manakins and zebra finches, I compared male reproductively active birds, which have relatively similar T levels (Adkins-Regan et al. 1990; Fusani et al. 2007), but very different cardiovascular demands. I compared cardiac morphometrics and investigated expression levels of several candidate genes associated with cardiac hypertrophy, rapid cardiac contractions, and increased cardiac metabolism (Table 5.1; reviewed in Bernardo et al. 2010; see methods). I also examined the potential role of steroid hormones on the manakin heart investigating expression levels of genes involved in sex-steroid signaling (Table 5.1; Fusani et al. 2007). These included androgen receptors (AR), ERα and ERβ as well as the enzyme aromatase that converts T into estradiol and can be expressed in cardiac tissues (Grohe et al. 1998; Marsh et al. 1998; Babiker et al. 2002; Wiik et al. 2005). Steroid receptors are nuclear receptors, thus once they bind to the respective hormones they transfer into the nucleus and act as transcription factors (Bourguet et al. 2000). Thus, to investigate if T effected gene expression I assessed expression of several genes in hearts collected from non-breeding or reproductively inactive manakins with naturally low levels of T (Schlinger et al. 2008) with non-breeding manakins treated with T.

5.3 Methods

Animal use procedures were approved by Animal Care and Use Committee of the University of California, Los Angeles (UCLA), the Smithsonian Tropical Research Institute and the Panamanian Autoridad Nacional del Ambiente. Using mistnets, manakins were collected during their courtship period (March-April) on their leks around Gamboa, Panama (Latitude: 9° 7' 0 N, Longitude: 79° 42' 0 W; Fig. 5.1a and 5.1c). Mistnets were controlled every 10 min. At capture animals were sacrificed by transection of the neck after 3 sec exposure to isoflurane at an on-site temporary field laboratory. The heart was isolated by cutting arteries and veins, the pericardial membranes were removed and the organ was dried briefly on a kimwipe tissue. Hearts were either fixed in paraformaldehyde for histological analyses or flash-frozen on dry ice for gene expression studies (see below). Hearts of reproductively active zebra finches were collected in an identical way from our colony at UCLA, where animals are housed in same-sex aviaries (6’ x 6’ x 4’) in visual and acoustic contact with the opposite sex (Fig. 5.1b and 5.1d). After capture, zebra finches were subjected to the same procedures described above for manakins.

5.3.1 Androgen effects on gene expression

To test the effects of the androgen on myocardial gene expression in manakins, I experimentally elevated T in non-breeding male manakins. Animals were captured between late July to early September, when T levels are low (Schlinger et al. 2008). Males were either implanted with T implant (n = 3) or a blank implant (n=4). Implants were prepared in the lab at UCLA. Silastic tubing (Dow Corning Corporation, Michigan, USA) of 12 mm was filled with T crystals (Steraloids Inc, New Hampshire, USA) and sealed at both ends with silicone adhesive (Dow Corning Corporation, Michigan, USA) as described previously (Fuxjager et al. 2012). Implants were inserted subcutaneously through a 1-2 mm incision on the upper back. For three weeks males were housed individually in small cages (32 cm x 629 cm) and fed papaya ad libitum. It has been shown in prior studies that the same method of T-treatment in manakins increased circulating T to levels normally observed in reproductively active males captured at the onset of the breeding season without impacting overall health of the birds (Day et al. 2006; Day et al. 2007). Hearts were collected as described above.

5.3.2 Histological Studies

The heart was washed and subsequently stored in 4% paraformaldehyde at 4 °C over night, followed by a night at 4 °C in 20% sucrose solution. Finally the heart was frozen and stored in OTC (Sakura Finetek Europe B.V., The Netherlands) at -80 °C. Back at UCLA, hearts were cut using a cryostat (CM3050 S; Leica AG) into cross sections of 5 or 10 µm starting from the apex. Visualization with Hematoxylin Eosin Staining (H&E) was applied to measure cardiac parameters. Following protocol was used for the staining: 3 min in Hematoxylin (Mayer's Hematoxylin, Sigma-Aldrich, Mo, USA), rinse in ddH$_20$, 5 min in H_2O, 12 dips in acid ethanol, 1 min in H_2O (repeated twice), 2 min in ddH$_2$O, 30 sec in Eosin (aqueous Eosin Y, Sigma-Aldrich, Mo, USA), 3 min in 70% Ethanol (repeated twice), followed by 1 min dehydration steps in 95% and 100% ethanol and 1 min Xylene.

5.3.3 Morphometric Analyses

H&E stained slides were photographed under a light microscope (Nikon) with constant (20x) magnification. Using Image J (Schneider et al. 2012), I measured the heart cross sectional area, septal thickness, ventricle and cavity areas and wall thickness of the left ventricle (LV) and right ventricle (RV) (Fig. 5.1e and 1f). Heart areas were determined by tracing the outlines of the heart, cavities and ventricles (the LV included the septum). The wall thickness was measured at several sites on a perpendicular line to the tangent to the outline of the corresponding area. These measurements were performed on 3 sections per heart and average values were computed for comparisons.

5.3.4 Gene Expression Studies

I used two different comparison groups for the gene expression study. Group 1 investigated species differences and compared breeding male manakins (N=4) and zebra finches (N=6). Group 2 investigated the effects of T treatment on non-breeding manakins (N=4) with non-breeding manakins treated with T (N = 3). I investigated expression levels of several candidate genes responsible for increased cardiac function including genes involved in muscle growth and contractility described below (Table 5.1; reviewed in Bernardo et al., 2010).

Candidate genes involved in Ca^{2+} handling

In brief, the process of cardiac contraction is as follows: triggered through an action potential, Ca^{2+} enters the cell through voltage gated L-type Ca^{2+} channels; this in turn activates ryanodine receptors (RYR), which causes the release of Ca^{2+} from the sarcoplasmic reticulum (SR); Ca^{2+} allows for the binding between actin and myosin and causes the fiber to contract; to release the contraction, Ca^{2+} needs to be removed from the cytoplasm. The Ca^{2+} sarcoplasmic/endoplasmatic ATPase (SERCA) pumps Ca^{2+} back into the SR. Inside the SR, calsequestrin (CSQ) binds Ca^{2+} and enhances Ca^{2+} release (Knollmann 2009). As I was interested to understand if manakin hearts have physiological adaptations for increased heart rates, I investigated differences in SERCA, RYR as well as CSQ in zebra finch and manakin hearts.

Candidate genes involved in hypertrophy

Genes involved in cardiac growth are the insulin like growth factor 1 (IGF1) and the protein kinase A (AKT). IGF1 activates intracellular signaling pathways that induce cell growth (Scheinowitz et al. 2003) and Akt1 mediates myocyte growth (DeBosch et al. 2006).

Additional candidate genes

Additional genes include KCNQ, which encodes for Kv7.1, a voltage gated potassium channel protein important for repolarization, thus termination of the action potential (Robbins 2001). Increased rate of contraction has a faster energy turnover and thus such a muscle might have more mitochondria, which could be indicated by higher levels of TFAM, the mitochondrial transcription factor A (Li et al. 2011). It has also been shown that in athletes there is a shift to fatty acid oxidation of which the peroxisome proliferator-activated receptor alpha (PPARα) is a marker, as it increases the transcription of genes involved in fatty acid oxidation (Rimbaud et al. 2009). Lastly, there might be an increased demand for oxygen. Myoglobin is responsible for oxygen transport in muscles (Saunders & Fedde 1991).

RNA extraction and reverse transcription

Using Trizol (Invitrogen, Carlsbad, CA), RNA was extracted from both ventricles and septum combined. RNA integrity and concentration was determined through gel electrophoresis and nanodrop (Thermo Scientific, Wilmington, DE, USA). Following DNAse treatment (Promega, Madison, WI), 1μg RNA was reverse transcribed to obtain doubled stranded cDNA using Superscript II Reverse Transcriptase (Invitrogen,) in 50 min at 42°C, followed by 15 min at 70°C. A successful reaction was determined through band visualization by gel electrophoresis. The remaining cDNA was used to amplify genes of interest with primers designed from the annotated zebra finch genome on UCSC Genome database (Supplementary Table 5.1; http://genome.ucsc.edu/; Fujita et al., 2010). Each 25 ul of PCR reaction contained 0.375mM of deoxynucleotide triphosphate, 0.4 μM of each primer (forward and reverse), 50 ng of cDNA template, 0.06 ng of DNA taq polymerase (Bioline, Randolph, MA), and buffer. Reactions were carried out at 95 °C for 5 min, 39 cycles of 95 °C for 30 sec, 64 °C for 30 sec, 72 °C for 1 min, followed by 72 °C for 10 min. To identify if targets have been amplified successfully, PCR products were run on gel electrophoresis to confirm the length of the product and were sequenced (Genewiz Inc., La Jolla, CA, USA) to confirm results by blasting the amplicon against the zebra finch genome (Kent 2002). From these products I designed species-specific primers for RT PCR to quantify expression. All primers were designed using Primer3Plus (http://www.primer3plus.com/; Untergasser et al. 2007; Supplementary Information Table 5.2).

Real time PCR

RT PCR was performed on an ABI 7300 sequence detection system, using SYBR Green Master Mix kits (Applied Biosystems Inc., Foster City, CA) with 5ng of cDNA template and 0.3 - 0.9 mM (depending on primers used) of each primer (forward and reverse) per reaction. The reactions consisted in following steps: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min, with an additional dissociation stage consisting of 95 °C for 15 sec, 60 °C for 30 sec, and 95 °C for 15 sec. Reaction efficiencies were between 90 %-110

%, correlation coefficients were greater than 0.95 and dissociation curves were used to verify the absence of contamination. Samples were run in duplicate and the deltaCT method was used to measure relative expression of each gene of interest compared to the housekeeping control gene. Species specific genes were run on samples from both species and if no differences in expression levels between primers were encountered, just one primer set was used for both species (Supplementary Table 5.2).

5.3.5 Statistical analyses

Statistical analyses were performed in SPSS 19 (IBM Inc., Chicago, IL). To investigate differences between species for dependent morphometric variables I used Manovas to control for multiple comparisons. Manovas were also used to investigate species differences or differences caused by T treatment in three groups of genes: hypertrophy, Ca^{2+} handling and steroid receptor genes. I used T tests to investigate differences in expression of genes not belonging to one of these groups. Significance was set at $p \leq 0.05$. Data were log or square root transformed to achieve normality. Values are given in mean ± SE.

5.4 Results

5.4.1 Morphometrics

Manakin males weigh about 25% (4.5 g) more than zebra finches and their heart is 36 % (0.1 g) heavier, thus HW/BW ratio is increased in manakins (Table 5.2). I measured morphometric parameters using cross sections of the whole heart, at the point of its largest diameter. While there was no significant difference in the heart cross sectional area between the two species ($F_{1,8} = 0.669$, $p = 0.437$; Fig. 5.2a), examinations revealed clear differences in the LV wall thickness and the LV chamber size. The septum and LV wall were significantly thicker in manakins (25 % and 22 % respectively; septum: $F_{1,8} = 10.513$, $p = 0.012$; LV wall: $F_{1,8} = 9,867$, $p = 0.014$; Fig. 5.2c). In contrast, zebra finches have a 70 % larger LV cavity ($F_{1,8} = 6.443$, $p = 0.035$; Fig. 5.2b). No significant differences were found in LV area, RV area, wall or cavity (LV area: $F_{1,8} = 1.806$, $p =0.216$; RV area: $F_{1,8} = 0.330$, $p =0.582$; RV wall: $F_{1,8} = 0.045$, $p = 0.837$; RV cavity: $F_{1,8} = 1.600$, $p = 0.242$).

5.4.2 Gene expression

Species comparison

I found a significant difference in expression level in genes associated with Ca^{2+} handling between the species ($F_{3,6} = 13.493$, $p =0.004$). Among the genes that are associated with Ca^{2+} handling, RYR transcript levels were significantly higher in manakain than zebra finches ($F_{1,8} = 27.292$, $p = 0.001$; Fig. 5.3a), and there were no significant differences in the expression of SERCA and CSQ (SERCA: $F_{1,8} = 0.904$, $p = 0.370$;Fig. 5.3b ; CSQ: $F_{1,8} = 3.972$, $p = 0.081$; Fig. 5.3c). AKT was expressed at significantly higher levels in zebra finches compared to manakins (hypertrophy genes: $F_{2,7} = 21.966$, $p = 0.001$; AKT: $F_{1,8} = 40.372$, $p < 0.001$; Fig.

5.3e). There were no significant species differences for IGF1 ($F_{1,8}$ = 2.474, p = 0.154; Fig. 5.3d), MYB, KCNQ, TFAM or PPARα (MYB: t_8 = 1.859, p = 0.100; Fig.5.3f; KCNQ: t_8 = -1.818, p = 0.107; Fig. 5.3g; TFAM: t_8 = -0.264, p = 0.799; Fig. 5.3h; PPARα: $F_{1,8}$ = 0.361, p = 0.727; Fig. 5.3i).

Hormone sensitivity

There was a significant difference in steroid receptor expression between male manakins and zebra finches ($F_{2,7}$ = 27.628, p < 0.001). The expression of androgen receptor was significantly greater in the heart of male manakins compared to zebra finches ($F_{1,8}$ = 38.974; p < 0.001; Fig. 5.4a). There was no difference in the expression of ERα ($F_{1,8}$ = 1.475; p = 0.259; Fig. 5.4b). ER β and aromatase appear not to be expressed in the manakin or zebra finch heart at detectable levels.

Androgen effects on gene expression

There was no difference in AR or ERα expression between any of the three manakin groups (breeding, non-breeding, non-breeding with T treatment; $F_{4,16}$ = 0.527, p = 0.750,Fig. 5.5), so treating non-breeding males with T should evoke similar responses to what I would expect with naturally elevated T levels in breeding males. Overall, there was no difference in expression levels with T treatment on Ca^{2+} handling genes ($F_{3,3}$ = 4.970, p = 0.110). When looking at the single Anovas, SERCA was significantly upregulated by T ($F_{1,5}$ = 12.130, p =0.018, Fig. 5.6a) but there was no influence of T on RYR ($F_{1,5}$ = 2.962, p =0.146, Fig. 5.6b) or CSQ ($F_{1,5}$ = 0.552, p = 0.491; Fig. 5.6c). T had no effect on hypertrophy gene expression levels ($F_{2,4}$ = 0.430, p = 0.677; IGF1: $F_{1,5}$ = 0.194, p = 0.678, Fig. 5.6d; no difference in AKT: $F_{1,5}$ = 1.049, p =0.353,

Fig. 5.6e). There were also no significant differences for MYB, TFAM, PPARα or KCNQ (MYB: $t_5 = -0.107$, $p = 0.919$, Fig. 5.6f; KCNQ: $t_5 = 0.031$, $p = 0.976$, Fig. 5.6g; TFAM: $t_5 = -0.314$, $p = 0.766$, Fig. 5.6h; PPARα: $F_{1,5} = 0.482$, $p = 0.650$, Fig. 5.6i).

5.5 Discussion

Our studies suggest the existence of morphological and molecular attributes of the male manakin heart that may help sustain the increased cardiovascular demands of their acrobatic courtship displays. Moreover, these data suggest that the manakin heart is a target of androgenic hormones that might regulate the expression of cardiac genes that improve myocardial performance.

To identify cardiovascular specializations I compared wild reproductively active male golden-collared manakins to captive held zebra finches. These birds are relatively closely related, both belonging to the order Passeriformes, are of similar size, and demonstrate similar levels of circulating T in adult breeding males (Adkins-Regan et al. 1990; Fusani et al. 2007). Compared to manakins, zebra finches do not display an energy intensive courtship display (see above). These comparisons, however, must be taken with caution because the captive finches obviously experience many different demands that surely impact their cardiovascular needs. Nevertheless, I believe these studies, and comparisons, are useful and lay the groundwork for more extensive studies of cardiovascular adaptations in other avian species.

Two lines of evidence suggest that the manakin has a relatively massive (heavier) heart. First, relative to body weight, the manakin heart tends to be heavier than that of the zebra finch, this even though there is no difference in cross-sectional area. Second, when compared to a variety of other tropical birds of similar size (Hartman 1955), the manakin heart is amongst the heaviest (Table 5.3). Differences in methodology may contribute to the differences I observed, so additional studies are needed to confirm my observations. Compared to the manakin, the zebra finch has an enlarged LV chamber size that seems to contribute to their relatively enlarged cross-sectional area but adds no weight. By contrast, the manakin has a significantly thicker LV wall and septum so that although the heart is overall smaller, it has greater muscle mass and is, therefore, heavier. Presumably, the pronounced thickness of the manakin LV muscular wall resists stress and/or enables increased contractile strength when heart rate and cardiovascular output are challenged during the performance of courtship displays and mechanical sound production.

Manakin and zebra finch gene expression profiles of Ca^{2+} handling and hypertrophy genes are significantly different. Importantly, mRNA expression of the ryanodine receptor gene was greater in manakins as compared to zebra finches. Because of its crucial role enabling fast release of Ca^{2+} from the sarcoplasmic reticulum elevated ryanodine receptor expression could assist with the rapid contractility of courting male manakins. Rapid handling of Ca^{2+} may be especially important in heart of manakins that appear to exhibit concentric hypertrophy. Muscular thickness is negatively correlated with cardiac contraction speed (Baggish et al. 2008) so Ca^{2+} may need to be shuttled all the more rapidly in the massive muscle of the manakin LV. I will return to this point below.

AKT plays a key role in regulating the signaling pathways involved in the physiological cardiac growth in the mammalian hearts (DeBosch et al. 2006). However, in the heart of manakins I found that AKT transcript levels were lower compared to zebra finches and that IGF-1 expression levels were similar. It is possible that signaling pathways not involving AKT or IGF-1 are recruited to sustain the more massive LV of the manakin heart.

I found that AR is expressed at high levels in the manakin heart, suggesting it is an important target of androgen hormones like testosterone. I could not identify expression of the estrogen synthetic enzyme aromatase so despite evidence that ERα (but not ERβ) are expressed in the manakin heart, it is likely that sex-steroid actions on the manakin heart are largely mediated through the androgen signaling pathway. T acting though AR may also increase the rate of Ca^{2+} shuttling required for the rapidly contracting heart of courting male manakins (Vicencio et al. 2006). The genes investigated in this study did not show significant differential expression through T treatment. Though when not controlling for multiple comparisons, I found a trend of upregulation of the expression of SERCA in non-breeding males treated with T. Following myocardial contraction, SERCA speeds relaxation, the rate-limiting step for contraction speed, by shuttling Ca^{2+} back into the SR. Thus, T may increase the rate of cardiac contractions by increasing the rate of myocardial contraction by upregulating SERCA expression.

To my knowledge this is the first study to demonstrate sex-steroid receptor expression and steroid-dependent gene expression in the avian heart. Cardiac performance of other bird species may be regulated by sex steroids as well.

Sexual selection is widely accepted to drive the evolution of exaggerated secondary sexual traits such as the peacock's tail (Darwin 1871; Andersson 1982). Such traits are morphological or behavioral features unique to their bearer and might be associated to underlying anatomical or physiological changes (e.g. Nottebohm et al. 1976; Ryan & Drewes 1990; Riede et al. 2010). Traits such as these often arise in males under the influence of the sex steroid testosterone (reviewed in Fusani 2008). In polygamus species, like manakins, where males strongly compete for copulations with females, sexually selected traits often reach extremes, and this includes behavior. Metabolic demands of courtship have been investigated in several species, but no studies have investigated the anatomical and physiological adaptations that accommodate these metabolic demands (Vehrencamp et al. 1989; Höglund et al. 1992; Eberhardt 1994; Chappell et al. 1995; Chappell et al. 1997; Oberweger & Goller 2001; Dearborn et al. 2005). To my knowledge, this is the first study reporting cardiovascular adaptations that might allow for, or result from, the performance of elaborate male courtship behavior. Similar investigation of other species will certainly provide more insight into the cardiovascular performance of courting males and open a new investigation line for physiolofical costs in sexual selection.

5.6 Figures and Tables

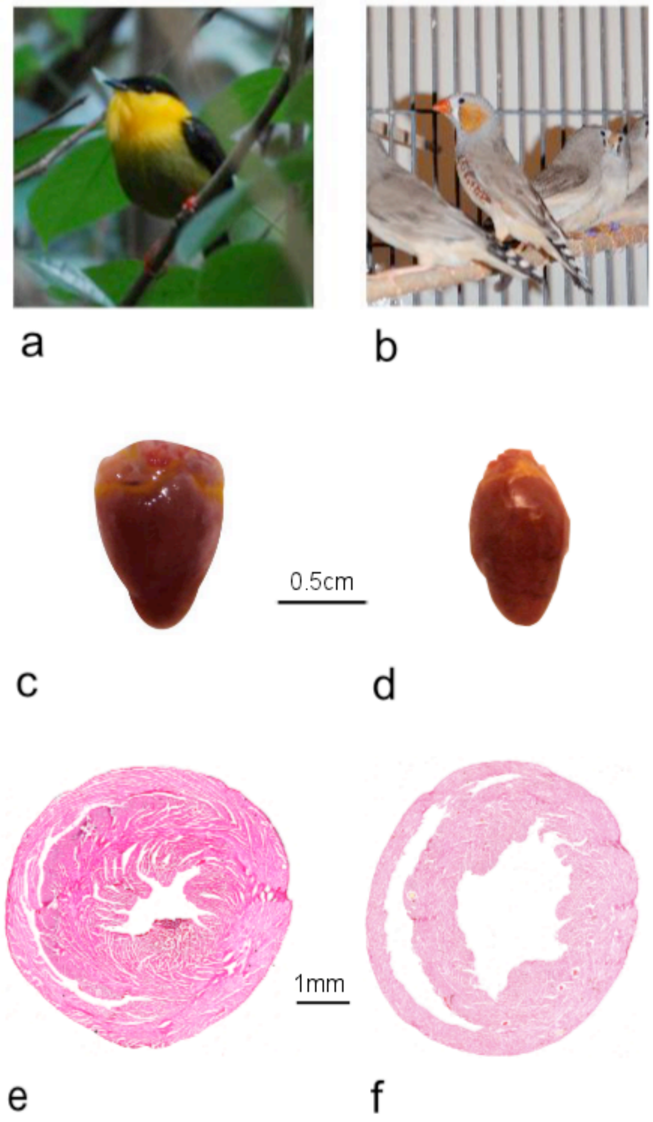

Figure 5.1 Male manakin and zebra finch body and heart (a) Adult male golden-collared manakin living in tropical forests of Panama; (b) Adult male zebra finch living in captivity in flight cages at UCLA; (c) manakin heart and (d) zebra finch heart; (e) cross sectional area of manakin heart; (f) cross sectional area of zebra finch heart.

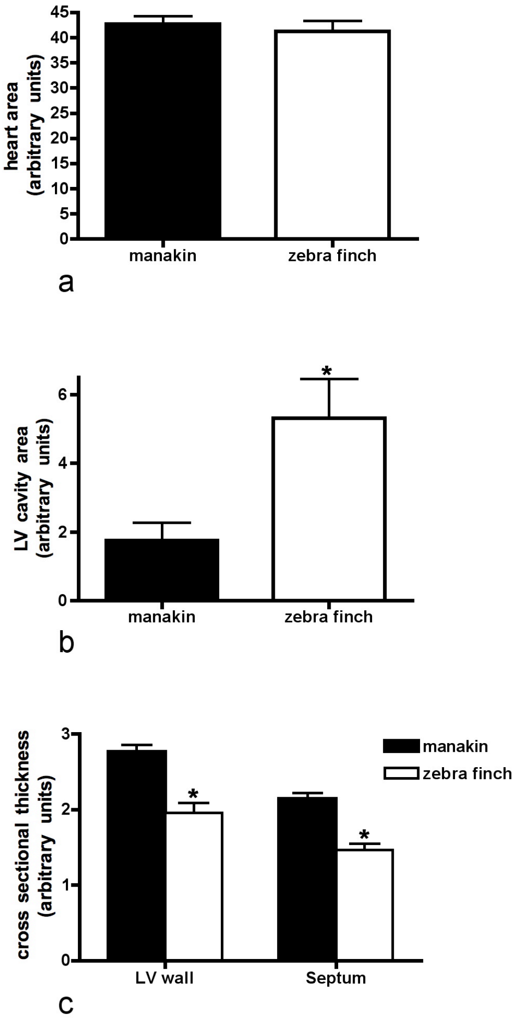

Figure 5.2 Comparison between manakin and zebra finch cardiac cross sections. (a) No differences in cross-sectional area of the heart were found between the two species ($F_{1,8} = 0.669$, $p = 0.437$); (b) LV cross-sectional cavity area is significantly increased in the zebra finch heart ($F_{1,8} = 6.443$, $p = 0.035$); (c) LV posterior wall thickness and septum were increased in the manakin heart (LV wall: $F_{1,8} = 9{,}867$, $p = 0.014$; Septum: $F_{1,8} = 10.513$, $p = 0.012$).

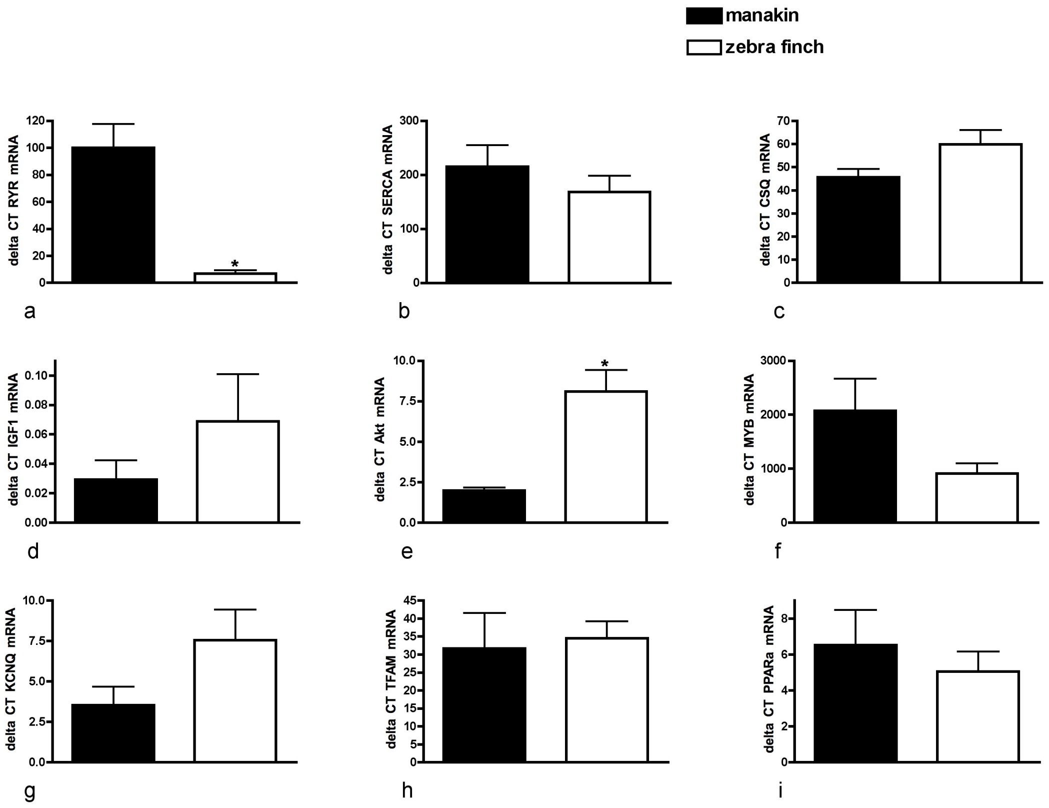

Figure 5.3 Gene expression differences between species. (a-c) There was a significant difference in expression level in genes associated to Ca^{2+} handling between the species ($F_{3,6} = 13.493$, =0.004). Manakins had significantly higher expression levels of RYR than zebra finches ($F_{1,8} = 27.292$, p = 0.001; Fig. 5.3a), but no differences were encountered in SERCA and CSQ expression (SERCA: $F_{1,8} = 0.904$, p = 0.370;Fig. 5.3b ; CSQ: $F_{1,8} = 3.972$, p = 0.081;Fig. 5.3c). (d-e) Against expectations, the hypertrophy marker AKT was increased in zebra finches compared to manakins (hypertrophy genes: $F_{2,7} = 21.966$, p = 0.001; AKT: $F_{1,8} = 40.372$, $p < 0.001$; Fig. 5.3e; IGF1: $F_{1,8} = 2.474$, p = 0.154; Fig. 5.3d). (f-i) There were no significant species differences for MYB, KCNQ, TFAM or PPARα (MYB: $t_8 = 1.859$, p = 0.100; Fig.5.3f; KCNQ: $t_8 = -1.818$, p = 0.107; Fig. 5.3g; TFAM: $t_8 = -0.264$, p = 0.799; Fig. 5.3h; PPARα: $F_{1,8} = 0.361$, p = 0.727; Fig. 5.3i).

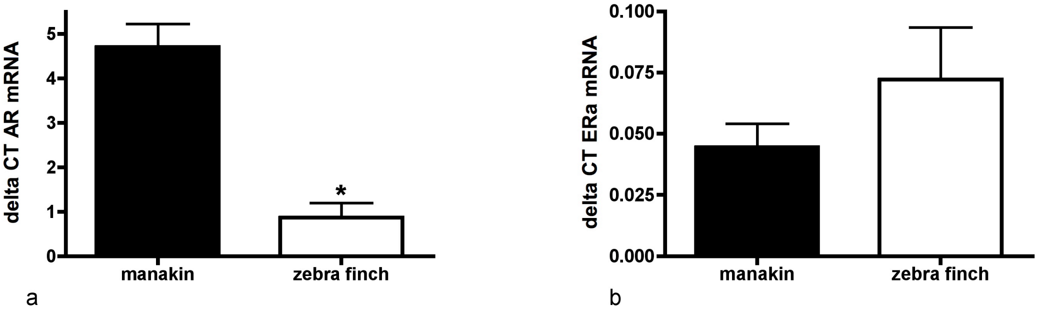

Figure 5.4 Steroid receptor mRNA expression levels in manakins and zebra finches. There was a significant difference in steroid receptor expression between male manakins and zebra finches ($F_{2,7} = 27.628$, $p < 0.001$). (a) There was a significant increase in androgen receptor mRNA expression in manakins compared to zebra finches ($F_{1,8} = 38.974$; $p < 0.001$). (b) There was no difference in ERα ($F_{1,8} = 1.475$; $p = 0.259$).

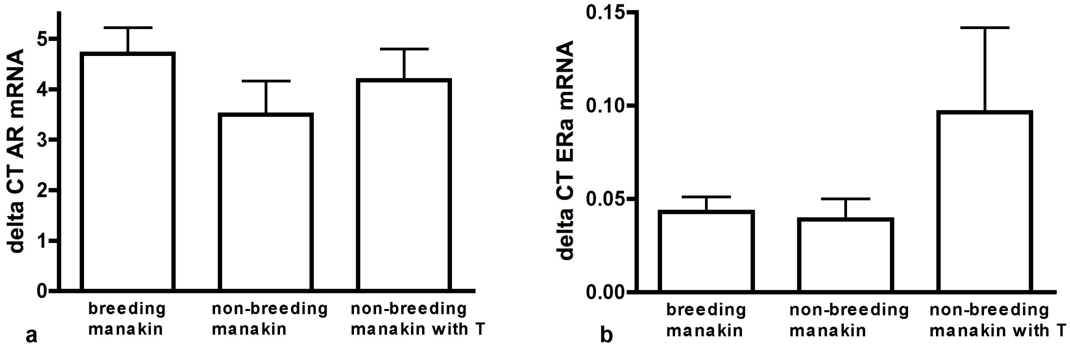

Figure 5.5 Steroid receptor mRNA expression levels in the three manakin groups. There was no difference in AR (a) or ERα expression (b) between any of the three manakin groups (breeding, non-breeding, non-breeding with T treatment; $F_{4,16} = 0.527$, $p = 0.750$).

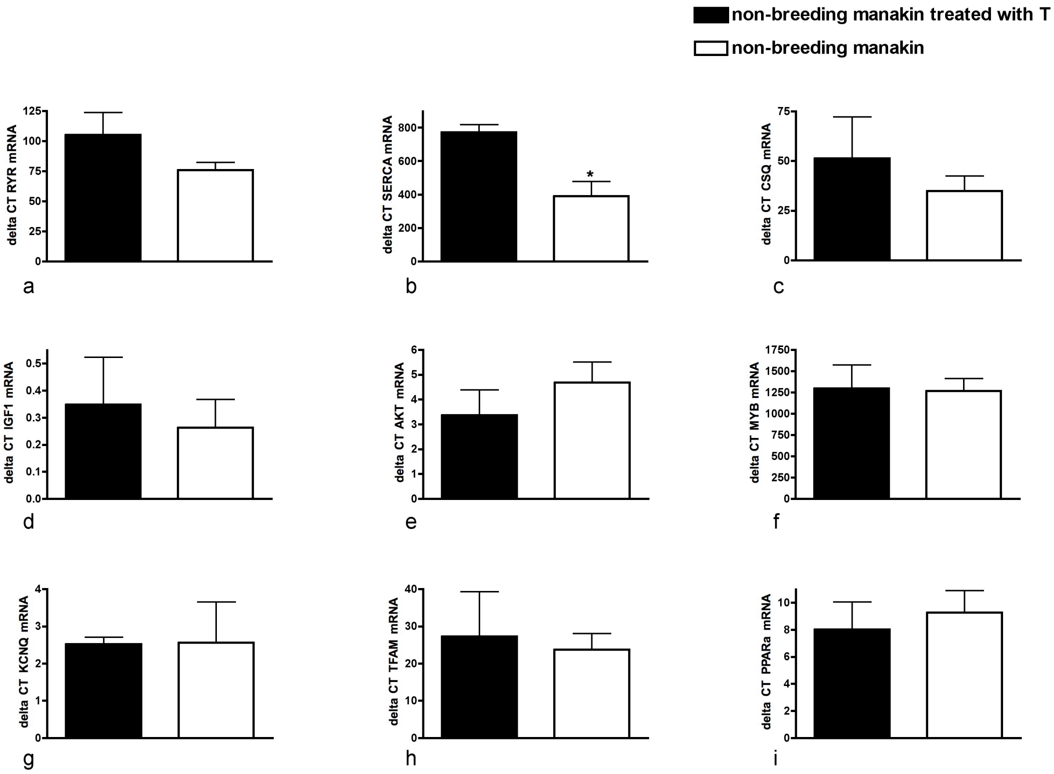

Figure 5.6 Differences in gene expression due to T treatment. (a-c) Overall, there was no difference in expression levels with T treatment in Ca^{2+} handling genes ($F_{3,3} = 4.970$, $p = 0.110$). With a single ANOVA, SERCA was significantly upregulated by T ($F_{1,5} = 12.130$, $p = 0.018$; Fig. 5.6b). There was no influence of T on RYR ($F_{1,5} = 2.962$, $p = 0.146$; Fig. 5.6a) or CSQ ($F_{1,5} = 0.552$, $p = 0.491$; Fig. 5.6c). (d-e) There was no significant effect of T on the hypertrophy genes ($F_{2,4} = 0.430$, $p = 0.677$; IGF1: $F_{1,5} = 0.194$, $p = 0.678$, Fig. 5.6d; AKT: $F_{1,5} = 1.049$, $p = 0.353$, Fig. 5.6e). (f-i) There were no significant differences for MYB, TFAM, PPARα or KCNQ (MYB: $t_5 = -0.107$, $p = 0.919$, Fig. 5.6f; KCNQ: $t_5 = 0.031$, $p = 0.976$, Fig. 5.6g; TFAM: $t_5 = -0.314$, $p = 0.766$, Fig. 5.6h; PPARα: $F_{1,5} = 0.482$, $p = 0.650$, Fig. 5.6i).

Table 5.1 Candidate genes for cardiac hypertrophy and increased contractility. RYR (ryanodine receptor) causes the release of Ca^{2+} from the sarcoplasmic reticulum. SERCA (Ca^{2+} sarcoplasmic/endoplasmatic ATPase) pumps Ca^{2+} back into the SR. Inside the SR, CSQ (calsequestrin) binds Ca^{2+} and enhances Ca^{2+} release. IGF1 (Insulin like growth factor 1) activates intracellular signaling pathways that induce cell growth and Akt1 (protein kinase B) mediates myocyte growth. KCNQ (Kv7.1, a voltage gated potassium channel protein) is important for repolarization, thus termination of the action potential. TFAM (mitochondrial transcription factor A) is correlated to the number of mitochondria. PPARα (peroxisome proliferator-activated receptor alpha) increases the transcription of genes involved in fatty acid oxidation. MYB (myoglobin) is responsible for oxygen transport in muscles. I investigated steroid sensitivity of the manakin heart by means of AR expression (androgen receptor). AROM (aromatase) is the enzyme responsible for converting T into estrogen. I also looked at ERα (estrogen receptor alpha) and ERβ (estrogen receptor beta).

Categories	Full name	Short names
Hypertrophy	Insulin like Growth Factor 1	IGF1
	Protein Kinase B	AKT
Ca^{2+} handling	Ryanodine Receptor	RYR
	Sarco/endoplasmic reticulum Ca^{2+}-ATPase	SERCA
	Calsequestrin	CSQ
Repolarization	Potassium voltage-gated channel subfamily KQT member	KCNQ
Mitochondria	Transcription factor A	TFAM
Metabolism	Peroxisome proliferator-activated receptor alpha	PPARα
O_2 transport	Myoglobin	MYB
Steroid pathway	Androgen Receptor	AR
	Aromatase	AROM
	Estrogen Receptor alpha	ERα
	Estrogen Receptor beta	ERβ

Table 5.2 Cardiac morphology of the manakin and zebra finch. The animals were weight within 5 minutes from capture. Heart weights were obtained once the arteries and veins have been cut off and the heart was dried on a kimwipe. Other measurements were taken from 10um cross sections which had been stained with Hematoxylin and Eosin. Values are Mean ± SE and the number of birds are given in the parenthesis. Statistics are give in the text and significant differences between manakin and zebra finches are indicated by *(p<0.05).

	manakin	zebra finch
Heart weight, HW (g)	0.28 ± 0.06 (2)	0.18 ± 0.02 (4)
Body weight, BW (g)	18.03 ± 1.28 (2)	13.49 ± 2.19 (4)
HW/BW %	1.58 ± 0.45 (2)	1.33 ± 0.08 (4)
Heart cross-sectional area	49.72 ± 1.80 (4)	52.67 ± 2.64 (6)
LV cross-sectional area	36.67 ± 0.49 (4)	39.02 ± 1.37 (6)
RV cross-sectional area	13.70 ± 1.77 (4)	15.12 ± 1.63 (6)
LV cross-sectional cavity area	2.05 ± 0.59 (4)*	6.79 ± 1.45 (6)
RV cross-sectional cavity area	2.76 ± 1.46 (4)	4.88 ± 0.96 (6)
LV posterior wall thickness	3.22 ± 0.11(4)*	2.50 ± 0.17 (6)
RV wall thickness	0.73 ± 0.06 (4)	0.71 ± 0.04 (6)
Septum	2.37 ± 0.1 (4)*	1.77 ± 0.13 (6)

Table 5.3 Body weights (BW) and heart weight to body weight ratios (HW/BW) for a selection of neotropical birds. The data for the Golden-collared manakin are taken from my study and the data on the 7 remaining species were taken from Hartman (1955). The selection criteria were following: collected in Panama, body weight plus or minus 3 grams of the manakins. Male manakins have the highest HW/BW of the 8 species.

Species name	Sex	BW	HW/BW	Habitat
Golden-collared manakin (*Manacus vitellinus)*	Male	18.0	1.58	Lowland
Ruddy Treerunner (*Margarornis rubiginosus)*	Male	16.1	0.97	Highlands
Plain Antvireo (*Dysithamnus mentalis)*	Mixed	14.5	1.16	Avoid lowlands
Dusky-capped flycatcher (*Myiarchus tuberculifer)*	Mixed	19.9	0.81	Broad distribution
Buff-breasted wren (*Cantorchilus leucotis)*	Male	20.1	0.84	Lowlands
Golden-hooded tanager (*Tangara larvata)*	Male	18.0	1.05	From sea level – 1500m
Common Bush tanager (*Chlorospingus ophthalmicus)*	Male	19.8	0.89	Highlands
Rufous-collared sparrow (*Zonotrichia capensis)*	Mixed	17.0	0.84	Broad distribution

Supplementary Table 5.1 PCR primers generated from zebra finch genome. GAPdH (Glyceraldehyde 3-phosphate dehydrogenase) is the house-keeping gene. RYR (ryanodine receptor) causes the release of Ca^{2+} from the sarcoplasmic reticulum. SERCA (Ca^{2+} sercoplamsic/endoplasmatic ATPase) pumps Ca^{2+} back into the SR. Inside the SR, CSQ (calsequestrin) binds Ca^{2+} and enhances Ca^{2+} release. IGF1 (Insulin like growth factor 1) activates intracellular signalling pathways that induce cell growth and Akt1 (protein kinase B) mediates myocyte growth. KCNQ (Kv7.1, a voltage gated potassium channel protein) is important for repolarization, thus termination of the action potential. TFAM (mitochondrial transcription factor A) is correlated to the number of mitochondria. PPARα (peroxisome proliferator-activated receptor alpha) increases the transcription of genes involved in fatty acid oxidation. MYB (myoglobin) is responsible for oxygen transport in muscles. AR (androgen receptor), AROM (aromatase), the enzyme responsible for converting T into estrogen and ERα (estrogen receptor alpha) and ERβ (estrogen receptor beta) are part pf the steroid signaling pathway.

gene	Forward Primer (5’-3’)	Reverse Primer (5’-3’)	Product Size (bp)
GAPdH	CAACATCAAGTGGGCAGATG	GGAACAGAACTGGCCTCTCA	700
RYR	GAATGGGGAAGGAAGAGGAG	CATGTCAGGTTCCACCACAG	971
SERCA	GCTGAAGGAGAAATGGGGCT	CCTGCACACAGACATCTGGT	939
CSQ	GGTCTCCCAGAAGCAGTTCC	TCGTCGTCGTCATCGTCATC	1011
IGF-1	TCCTACATCCATTTCTTCTACCTTG	ACATTCATTCTTCATTCTTGTGGAT	415
AKT	TCAGATCTGGCTCTCCCAGT	TCCATGCTGTCATCTTGGTC	1024
KCNQ	TATGCAGATGCCCTGTGGTG	GTCCTCCACTCTGTTCAGCC	897
TFAM	AGGCTGCAGCTTTGAAAGAG	CAGGTCTTCACGTCCCAGTT	317
PPARα	GATCGCAACTGCAAAATTCA	GGACGATCTCCACAGCAAAT	749
MB	GGCTCAGTGACCAGGAATGG	AGCCTTCTTCATTGCAGCCT	401
AR	TGACGTGTGGGAGCTGCAAA	GGCCATCCACTGGAATAATACTGA	741
AROM	AGGGAACGCCTGCAACTACTACAA	TCATCTCCAGCACACACTGGTTCA	644
ERα	TGTCCCTGACAGCAGAACAG	GTAGCCAGCAGCATGTCAAA	370
ERβ	CCGGGAGCAGAGAAACATTA	CAGTTTCAGCTCTCGGAACC	848

Supplementary Table 5.2 qPCR Primers generated from sequence obtained with PCR primers.

GAPdH (Glyceraldehyde 3-phosphate dehydrogenase) is the house keeping gene. RYR (ryanodine receptor) causes the release of Ca^{2+} from the sarcoplasmic reticulum. SERCA (Ca^{2+} sercoplamsic/endoplasmatic ATPase) pumps Ca^{2+} back into the SR. Inside the SR, CSQ (calsequestrin) binds Ca^{2+} and enhances Ca^{2+} release. IGF1 (Insulin like growth factor 1) activates intracellular signalling pathways that induce cell growth and Akt1 (protein kinase B) mediates myocyte growth. KCNQ (Kv7.1, a voltage gated potassium channel protein) is important for repolarization, thus termination of the action potential. TFAM (mitochondrial transcription factor A) is correlated to the number of mitochondria. PPARα (peroxisome proliferator-activated receptor alpha) increases the transcription of genes involved in fatty acid oxidation. MYB (myoglobin) is responsible for oxygen transport in muscles. AR (androgen receptor), AROM (aromatase), the enzyme responsible for converting T into estrogen and ERα (estrogen receptor alpha) and ERβ (estrogen receptor beta) are part pf the steroid signaling pathway.

gene	Species	Forward Primer (5’-3’)	Reverse Primer (5’-3’)	Produc Size (bp)
GAPdH	ZF	TGACCTGCCGTCTGGAAAA	CCATCAGCAGCAGCCTTCA	70
RYR	MAN	TAGGAGAATGCTTGGCTGCT	GGCAAATTGAGAACTGCTCTG	130
SERCA	ZF	ACCAGTGAAGGCAACACACA	AGCTGCATGCCTTTCCTTT	133
	MAN	GGAAGTGGCAGTTGGAGACA	TTACAGCACGAGGATCAGGC	149
CSQ	ZF	AGCACAGAAGAGCCACCTTG	GGCAACTTGCTTCAGGATTT	149
	MAN	GCTGAAGAAGACGACCCAGA	AGGGTCAATCCAGACAATGC	105
IGF1	MAN	AGGAGGCTGGAGATGTACTGTG	GCACTTCCTTTTGTGCTTTTG	103
AKT	ZF	AGGCACTTTTGGAAAGGTCA	GCGTGTGCGCTACTTCATC	113
	MAN	GGCATCCATTCCTAACAGCT	AAAAGACGCGCTCTCTTGAC	120
KCNQ	ZF	CAGGCTTTGCCCTAAAGGTA	CTGCAGCATAGCATCTCCAT	108
	MAN	GCTGCAGAAAACCCAGACTC	ACCATCACCGAATTTCTTTGG	107
TFAM	ZF	TCAATCAGGGCAAAAAGAGAA	AGCTTTGCCACAGGTGAACT	122
	MAN	ATGCATGGCAAAAACTCTCC	GCTTCCCACGACTTCATTTC	100
PPARα	MAN	ACCAATAACAATCCGCCATT	TCCTTGTTCTGAATCCCATTG	104
MB	ZF	GAGACCCTTGATCGCTTTGA	GCCTCATGATTACCCTTTGC	140
	MAN	TGATGCAATGAAAGGCTCTG	GACAGGGATTTTGTGCTTGG	148
AR	ZF	ATGAGTACCGCATGCACAAA	AACTCCTGGGGTGTGATCTG	100
AROM	MAN	GCAAATCCTCAAGTGTGTCC	CAGTGTGCTGGGTTGTTGT	129
ERα	MAN	CTGGGCAAAAAGAGTTCCAG	AAAGCTTTCCTGGGTGTTCC	131
ERβ	MAN	AGCTTTTGCGCCTGTTTTTA	CAGTGACTATGCTTCGGGATAC	146

5.7 References

Adkins-Regan, E., Abdelnabi, M., Mobarak, M., & Ottinger, M. A. 1990. Sex Steroid Levels in Developing and Adult Male and Female Zebra Finches (Poephila guttata). *General and Comparative*, 78, 93–109.

Allah, R., Dehkordi, F., & Parchami, A. 2010. The Pattern Comparison of Changes of Heart Macro-Structure in the Fowl. 5, 291–293.

Andersson, M. 1982. Sexual selection , natural selection and quality advertisement. 82, 375–393.

Babiker, F. A., Windt, L. J. D., Eickels, M. V., Grohe, C., Meyer, R., & Doevendans, P. A. 2002. Estrogenic hormone action in the heart : regulatory network and function. *Cardiovascular Research*, 53, 709–719.

Baggish, A. L., Wang, F., Weiner, R. B., Elinoff, J. M., Tournoux, F., Boland, A., Picard, M. H., Hutter, A. M., & Wood, M. J. 2008. Training-specific changes in cardiac structure and function: a prospective and longitudinal assessment of competitive athletes. *Journal of applied physiology (Bethesda, Md. : 1985)*, 104, 1121–8.

Barske, J., Schlinger, B., Wikelski, M., & Fusani, L. 2011. Female choice for male motor skills. *Proceedings of the Royal Society B: Biological Sciences*, 278, 3523–3528.

Bauchinger, U., Wohlmann, A., & Biebach, H. 2005. Flexible remodeling of organ size during spring migration of the garden warbler (Sylvia borin). *Zoology (Jena, Germany)*, 108, 97–106.

Bernardo, B. C., Weeks, K. L., Pretorius, L., & McMullen, J. R. 2010. Molecular distinction between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies. *Pharmacology & therapeutics*, 128, 191–227.

Bishop, C. M. 1997. Heart mass and the maximum cardiac output of birds and mammals: implications for estimating the maximum aerobic power input of flying animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 352, 447–456.

Bishop, C. M., Butler, P. J., El Haj, a. J., Egginton, S., & Loonen, M. J. J. E. 1996. The morphological development of the locomotor and cardiac muscles of the migratory barnacle goose (Branta Leucopsis). *Journal of Zoology*, 239, 1–15.

Bourguet, W., Germain, P., Gronemeyer, H., & Ad, A.-. 2000. Nuclear receptor ligand-binding domains : three-dimensional structures , molecular interactions and pharmacological implications. *Trends in Pharmacological Sciences*, 21, 381–388.

Brush, A. 1966. Avian Heart Size and Cardiovascular Performance. *The Auk*, 83, 266–273.

Chapman, F. 1935. The courtship of Gould's manakin (Manacus vitellinus vitellinus) on Barro Colorado Island, Canal Zone. *Bulletin of American Museum of Natural History*, 68, 471–525.

Chappell, M. A., Zuk, M., Johnson, R. S., & Kwan, T. H. 1997. Mate Choice and Aerobic Capacity in Red Junglefowl. *Behavior*, 134, 511–529.

Chappell, M. a., Zuk, M., Kwan, T. H., & Johnsen, T. S. 1995. Energy cost of an avian vocal display: crowing in red junglefowl. *Animal Behavior*, 49, 255–257.

Cooper, B. G., & Goller, F. 2006. Physiological insights into the social-context-dependent changes in the rhythm of the song motor program. *Journal of neurophysiology*, 95, 3798–809.

Darwin, C. 1871. *The descent of man, and selection in relation to sex.* London, UK: John Murray.

Day, L. B., Fusani, L., Hernandez, E., Billo, T. J., Sheldon, K. S., Wise, P. M., & Schlinger, B. 2007. Testosterone and its effects on courtship in Golden-collared Manakins (Manacus vitellinus): seasonal, sex, and age differences. *Hormones and behavior*, 51, 69–76.

Day, L. B., McBroom, J. T., & Schlinger, B. a. 2006. Testosterone increases display behaviors but does not stimulate growth of adult plumage in male golden-collared manakins (Manacus vitellinus). *Hormones and behavior*, 49, 223–32.

DeBosch, B., Treskov, I., Lupu, T. S., Weinheimer, C., Kovacs, A., Courtois, M., & Muslin, A. J. 2006. Akt1 is required for physiological cardiac growth. *Circulation*, 113, 2097–104.

Dearborn, D. C., Anders, A. D., Williams, J. B., Dearborn, C., & Anders, D. 2005. Courtship display by Great Frigatebirds, Fregata minor: an energetically costly handicap signal? *Behavioral Ecology and Sociobiology*, 58, 397–406.

DiDio, L. J. A. 1966. Myocardial Ultrastructure and Electrocardioqrams of the Hummingbird under Normal and Experiment a1 Conditions. *Anat. Rec*, 159, 335–352.

Driedzic, R., Crowe, A. N. D. H. L., Hicklin, W., & Sephton, H. 1993. Adaptations in pectoralis muscle , heart mass , and energy metabolism during premigratory fattening in semipalmated sandpipers (Calidris pusilla). *Canadian Journal of Zoology*, 71, 1602–1608.

Eberhardt, L. S. 1994. Oxygen consumption during singing by male Carolina Wrens (Thryothorus Ludovicianus). *The Auk*, 111, 124–130.

Fagard, R. 2003. Athlete's heart. *Heart*, 89, 1455–1462.

Frey, N., & Olson, E. N. 2003. Cardiac hypertrophy: the good, the bad, and the ugly. *Annual review of physiology*, 65, 45–79.

Froelicher, V. F. 1972. Animal studies of effect of chronic exercise on the heart and atherosclerosis: a review. *American heart journal*, 84, 496–506.

Fusani, L. 2008. Testosterone control of male courtship in birds. *Hormones and Behavior*, 54, 227–233.

Fusani, L., Day, L. B., Canoine, V., Reinemann, D., Hernandez, E., & Schlinger, B. A. 2007. Androgen and the elaborate courtship behavior of a tropical lekking bird. *Hormones and Behavior*, 51, 62 – 68.

Fuxjager, M. J., Schultz, J. D., Barske, J., Feng, N. Y., Fusani, L., Mirzatoni, A., Day, L. B., Hau, M., & Schlinger, B. A. 2012. Spinal Motor and Sensory Neurons Are Androgen Targets in an Acrobatic Bird. *Endocrinology*, 153, 1–12.

Grohe, C., Kahlert, S., Loebbert, K., & Vetter, H. 1998. Expression of oestrogen receptor alfa and beta in rat heart: role of local oestrogen synthesis. *Journal of Endocrinology*, 156, 1–7.

Hartman, A. . 1955. Heart Weight in Birds. *The Condor*, 57, 221–238.

Howard, E. M., & Baker, K. M. 1991. Cardiac Hypertrophy. *Circulation*,

Höglund, J., Kålås, J. A., & Fiske, P. 1992. The costs of secondary sexual characters in the lekking Great Snipe (Gallinago media). *Behavioral Ecology*, 30, 309–315.

Iemitsu, M., Miyauchi, T., Maeda, S., Sakai, S., Fujii, N., Miyazaki, H., Kakinuma, Y., Matsuda, M., & Yamaguchi, I. 2003. Cardiac hypertrophy by hypertension and exercise training exhibits different gene expression of enzymes in energy metabolism. *Hypertension research : official journal of the Japanese Society of Hypertension*, 26, 829–37.

Jehl, J. R. 1997. Cyclical changes in body composition in the annual cycle and migration of the Eared Grebe Podiceps nigricollis. *Journal of Avian Biology*, 28, 132–142.

Junker, J., Sommer, J. R., S, M., Meissnedl, G., Hill, C., & Carolina, N. 1994. Extended Junctional Sarcoplasmic Reticulum of Avian Cardiac Muscle Contains Functional Ryanodine Receptors . 269, 1627–1634.

Kent, W. J. 2002. BLAT---The BLAST-Like Alignment Tool. *Genome Research*, 12, 656–664.

Knollmann, B. C. 2009. New roles of calsequestrin and triadin in cardiac muscle. *The Journal of physiology*, 587, 3081–7.

Koester, F. 1976. Ueber die Hoehenanpassung von Crotopbaga ani und Tyrannus melancholicus in den Anden Kolumbiens. *Journal of Ornithology*, 117, 75–99.

Li, L., Mühlfeld, C., Niemann, B., Pan, R., Li, R., Hilfiker-Kleiner, D., Chen, Y., & Rohrbach, S. 2011. Mitochondrial biogenesis and PGC-1α deacetylation by chronic treadmill exercise:

differential response in cardiac and skeletal muscle. *Basic research in cardiology*, 106, 1221–34.

Maron, B. J. 2005. Distinguishing hypertrophic cardiomyopathy from athlete's heart: a clinical problem of increasing magnitude and significance. *Heart (British Cardiac Society)*, 91, 1380–2.

Marsh, J. D., Lehmann, M. H., Ritchie, R. H., Gwathmey, J. K., Green, G. E., & Schiebinger, R. J. 1998. Androgen Receptors Mediate Hypertrophy in Cardiac Myocytes. *Circulation*, 98, 256–261.

Mihl, C., Dassen, W. R. M., & Kuipers, H. 2008. Cardiac remodelling: concentric versus eccentric hypertrophy in strength and endurance athletes. *Netherlands heart journal*, 16, 129–33.

Morris, D. 1954. The Reproductive Behavior of the Zebra Finch (Poephila guttata), with Special Reference to Pseudofemale Behavior and Displacement Activities. *Behavior*, 6, 271–322.

Norris, R. A., & Williamson, F. S. L. 1955. Variation in Relative Heart Size of Certain Passerines with Increase in Altitude. *The Wilson Bulletin*, 67, 78–83.

Nottebohm, F., Arnold, A. P., & Url, S. 1976. Sexual Dimorphism in Vocal Control Areas of the Songbird Brain Sexual Dimorphism in Vocal Control Areas of the Songbird Brain. *Science*, 194, 211–213.

Oberweger, K., & Goller, F. 2001. The metabolic cost of birdsong production. *The Journal of experimental biology*, 204, 3379–88.

Prum, R. O. 1990. Phylogenetic analysis of the evolution of display behavior in the neotropical Manakins (Aves:Pipridae). *Ethology*, 84, 202–231.

Riede, T., Fisher, J. H., & Goller, F. 2010. Sexual dimorphism of the zebra finch syrinx indicates adaptation for high fundamental frequencies in males. *PloS one*, 5, e11368.

Rimbaud, S., Sanchez, H., Garnier, a., Fortin, D., Bigard, X., Veksler, V., & Ventura-Clapier, R. 2009. Stimulus specific changes of energy metabolism in hypertrophied heart. *Journal of Molecular and Cellular Cardiology*, 46, 952–959.

Robbins, J. 2001. KCNQ potassium channels: physiology, pathophysiology, and pharmacology. *Pharmacology & therapeutics*, 90, 1–19.

Ryan, M. J., & Drewes, R. C. 1990. Vocal morphology of the Physalaemus pustulosus species group (Leptodactylidae): morphological response to sexual selection for complex calls. *Biological Journal of the Linnean Society*, 40, 37–52.

Saunders, D., & Fedde, M. 1991. Physical Conditioning: Effect on the myoglobin concentration in skeletal and cardiac muscle of bar-headed geese. *Comparative Biochemistry and Physiology, Part A*, I, 349–352.

Scheinowitz, M., Kessler-Icekson, G., Freimann, S., Zimmermann, R., Schaper, W., Golomb, E., Savion, N., & Eldar, M. 2003. Short- and long-term swimming exercise training increases myocardial insulin-like growth factor-I gene expression. *Growth hormone & IGF research*, 13, 19–25.

Schlinger, B. A., Day, L. B., & Fusani, L. 2008. Behavior, natural history and neuroendocrinology of a tropical bird. *General and comparative endocrinology*, 157, 254–258.

Schlinger, B. A., Schultz, J. D., & Hertel, F. 2001. Neuromuscular and endocrine control of an avian courtship behavior. *Hormones and Behavior*, 280, 276 –280.

Schneider, C. a, Rasband, W. S., & Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671–675.

Steiger, S. S., Kelley, J. P., Cochran, W. W., & Wikelski, M. 2009. Low metabolism and inactive lifestyle of a tropical rain forest bird investigated via heart-rate telemetry. *Physiological and Biochemical Zoology*, 82, 580–589.

Szabo, E., Viragh, S., & Challice, C. E. 1986. The Structure of the Atrioventricular Conducting System in the Avian Heart. *Perfusion*, 9, 1–9.

Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R., & Leunissen, J. a M. 2007. Primer3Plus, an enhanced web interface to Primer3. *Nucleic acids research*, 35, W71–4.

Vehrencamp, S. L., Bradbury, J. W., & Gibson, R. M. 1989. The energetic cost of display in male Sage Grouse. *Animal Behavior*, 38, 885–896.

Vicencio, J. M., Ibarra, C., Estrada, M., Chiong, M., Soto, D., Parra, V., Diaz-Araya, G., Jaimovich, E., & Lavandero, S. 2006. Testosterone induces an intracellular calcium increase by a nongenomic mechanism in cultured rat cardiac myocytes. *Endocrinology*, 147, 1386–95.

Wiik, A., Gustafsson, T., Esbjörnsson, M., Johansson, O., Ekman, M., Sundberg, C. J., & Jansson, E. 2005. Expression of oestrogen receptor alpha and beta is higher in skeletal muscle of highly endurance-trained than of moderately active men. *Acta physiologica Scandinavica*, 184, 105–12.

Zollinger, S. A., Goller, F., & Brumm, H. 2011. Metabolic and respiratory costs of increasing song amplitude in zebra finches. *PloS one*, 6, e23198.

CHAPTER 6

Conclusion

Using a highly integrated approach, my work offers several new insights on the evolution of sexual selection, from perspectives that capture both proximate and ultimate mechanisms. I have combined the study of animal behavior with cardiovascular biology and endocrinology. Using miniature heart rate transmitters, I found that heart rates of males accelerate to exceptionally high levels during the performance of courtship displays. Moreover, I was able to explore instantaneous metabolic demands of the manakin courtship display. It is unlikely that the high energetic demands imposed on male manakins during single courtship displays would have been discovered with more conservative methods like doubly labeled water, as shown in the relatively low average heart rates across the day. These results provide support for the idea that energetic demands can be of two kinds: marathon-like (relatively high energetic demand over an extended period of time) or sprint-like (extremely high energetic demand over short period of time; Clark 2012). With this in mind, it is clear that investigating animal behaviors like these with rapidly improving new technologies will shed new light on the evolution of behavior, especially the highly elaborate courtship behaviors of some species.

Testosterone is known to be crucial in regulating sexual behavior across a broad range of taxa. My study shows for the first time that the avian heart expresses androgen receptors with additional evidence for functional androgen receptor action as indicated by changes in expression levels of a gene involved in Ca^{2+} handling. My data show that male manakins possess an increased cardiac muscle. It remains unknown whether T plays any role in male manakin cardiac morphology, whether this increase in myocardial size results from the direct action of T, or indirectly by T induced activation of behavior. Nevertheless, these studies pave the way for future studies that can more fully assess the physiological costs of sexual selection. What we have discovered for the cardiovascular system may well apply in significant ways to other physiological systems.

By using high-speed videography, I also found how females of a lekking species actively engage in courtship. After having identified that females are able to distinguish males by tens of milliseconds in certain moves, we now have evidence that they additionally challenge male performance capabilities by taking the lead role in the final 'duo dance' before choosing a mate for copulation.

I have shown for the first time how selection operates to stimulate the production of rapid and precision movements of male golden-collared manakins and how optimal cardiovascular function co-occurs to enable the performance while minimizing the metabolic demands. Integrating physiology and behavior in the study of sexual selection opens the door for examination of many new questions on costs/benefits of sexually selected traits and hopefully advances in technology will reduce invasiveness even further to increase studies on animals in the wild.

6.1 References

Clark, C. J. 2012. The role of power versus energy in courtship: what is the “energetic cost” of a courtship display? *Animal Behavior*, 84, 269–277.