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Wendy P. Tori
University of Missouri-St. Louis, toriwen@yahoo.com

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Sexual selection in a species with exploded leks: the White-crowned Manakin (*Pipra pipra*)

by

Wendy P. Tori
M.S. Biology, University of Missouri-St. Louis, 2005
B.S. Biology, Universidad Nacional Agraria La Molina, 1999

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University of Missouri-St. Louis in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

Advisory Committee

Bette A. Loiselle, Ph.D. (Advisor)
John G. Blake, Ph.D.
Patricia G. Parker, Ph.D.
Michael S. Webster, Ph.D.

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Saint Louis, Missouri

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ABSTRACT

Understanding what determines male reproductive success is central to sexual selection theory. Differences in male mating success result from interactions among males to get access to females, and from choices females make among the males they have access to. Male competitive abilities and female mate choice can influence male reproductive success simultaneously and their relative importance varies within and across species.

The main goal of this dissertation was to characterize the processes that shape male reproductive success in an exploded lekking species: the White-crowned Manakin (*Pipra pipra*). Specifically I addressed the question: ***What factors make males attractive and reproductively successful?*** To answer this question, I examined how genetic, ecological and behavioral factors influence female mate choice and male mating success.

First, I examined if females selected males with certain genetic characteristics (i.e., high heterozygosity or compatible genes) to gain indirect fitness benefits. Specifically, I tested if females preferentially mated with unrelated males or males with high overall heterozygosity to increase the genetic diversity of their offspring (avoid inbreeding). My results suggest that females were not preferentially mating with highly heterozygous or unrelated males. Heterozygosity, however, appears to play a role in mate selection. I found that heterozygosity may have influenced territory acquisition (believed to be a pre-requisite of male mating success), and that males with intermediate heterozygosity had higher reproductive success than those with low or high heterozygosity. This suggests that females may optimize heterozygosity levels of their offspring by negotiating a balance between inbreeding and outbreeding costs.

Second, I examined if food resources within territories affected mating success of territory owners. In lekking species, it is usually assumed that territories do not contain resources and that female mate choice is based on male characteristics unrelated to resources within territories. Nevertheless, the validity of these assumptions are unclear for species with exploded leks (such as the White-crowned Manakin), in which males have relatively large territories that may contain resources. I found that food resources did not affect female visitation patterns. Males that sired offspring, however, had more resources within their territories and tended to display for longer periods of time. These results suggest that resources may indirectly affect male mating success by influencing male display characteristics that females select during mate choice.

Lastly, I examined the effect of male behavioral traits and territory characteristics on male mating success. I found that mating success of territorial males was more influenced by male characteristics associated with female mate choice (e.g. advertisement traits) than male-male competition (e.g. male-male interactions or spatial traits). Specifically my results suggest that females use a combination of male behavioral characteristics (i.e., display performance, territory attendance and vocalization rate) during mate selection and that the relative importance of each of these traits may change over time.

Taken together these results suggest that female mate choice is the main mechanism that affects male reproductive success in White-crowned Manakins. Resources were found to influence male traits used during mate selection, and females appear to use a combination of genetic and behavioral traits during mate choice. These results highlight the importance of considering multiple factors and their interactions to understand the processes that determine male reproductive success.

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CHAPTER 1

Do females preferentially mate with highly heterozygous or unrelated males? A test of the genetic compatibility and heterozygosity hypotheses for the White-crowned Manakin (*Pipra pipra*)

INTRODUCTION

In lekking species, females often prefer specific males even when there is no evidence that mates contribute anything other than genes to the offspring (Houle and Kondrashov 2002). This preference for certain males strongly suggests that female mate choice is important and that the most likely basis for this preference is differential genetic quality among males (Ligon 1999). Choosy females may produce offspring of superior genetic quality by mating with males whose genes confer greater attractiveness (Fisherian models) and viability (good gene models) to their offspring. The Fisherian model proposes that mate choice is based on traits that are attractive to females but that do not confer viability advantage to the offspring (arbitrary process, Fisher 1930). By choosing attractive males, females will produce sons that in turn will display superior attractiveness and achieve greater reproductive success. Alternatively, the "good genes" model states that choosy females may produce offspring of superior genetic quality by mating with males whose genes can confer greater viability to the offspring (Andersson 1994, Mays and Hill 2004).

Increased homozygosity through inbreeding is believed to lead to lower fitness (reduced survival: e.g., Allendorf and Leary 1986, Stockley *et al.* 1993, Keller *et al.*

1994, Daniels and Walters 2000, Keller and Waller 2002, Suter *et al.* 2007; reduced pathogen resistance: Whiteman *et al.* 2006; and lower reproductive success: e.g., Keller 1998, Westermeier *et al.* 1998, Kruuk *et al.* 2002, Slate *et al.* 2004), due to the expression of detrimental recessive alleles and loss of potentially beneficial alleles, especially when confronted with environmental changes (Ralls *et al.* 1986, Keller and Waller 2002). In contrast, increased heterozygosity often has been associated with an increase in vigor (fertility, survival, growth, etc.), leading to phenotypically superior individuals (Garten 1976, Baker and Fox 1978, Allendorf and Leary 1986, Tiira *et al.* 2006). The major histocompatibility complex (MHC) is one of many potential candidates for the genetic basis of mate choice. MHC is related to immune response and MHC heterozygosity appears to increase offspring fitness by conferring greater resistance to a greater number of diseases (von Schatz *et al.* 1996, Westerdahl *et al.* 2005, Bonneaud *et al.* 2006). As lekking females are thought to be free to select among males displaying at leks, they may select males in a manner that maximizes the genetic quality of their offspring.

There are two potential ways by which females may increase the levels of genetic heterozygosity in their offspring. First, females may choose sires with whom they are genetically compatible; that is, genetically dissimilar males or those males with whom they share the fewest alleles across loci (compatibility hypothesis, e.g., Tregenza and Wedell 2000, Freeman-Gallant *et al.* 2006, Oh and Badyaev 2006, Kempenaers 2007). By pairing with males with different genotypes at variable loci, females will produce heterozygous young, which likely will have fitness advantages over homozygotes (Mays and Hill 2004). The second mechanism by which females can increase genetic heterozygosity of offspring is by mating with highly heterozygous males (heterozygosity hypothesis, Brown 1997, Sauermann *et al.* 2001, Hoffman *et al.* 2004, Widdig *et al.* 2004, Hoffman *et al.* 2007, Kempenaers 2007, Rubenstein 2007, but see Lehmann *et al.* 2007). Heterozygosity appears to underlie the superiority of males with respect to disease resistance (Coltman *et al.* 1999, Reid *et al.* 2003, Reid *et al.* 2007, Whiteman *et al.* 2006), display performance (Reid *et al.* 2005, Marshall *et al.* 2003, Seddon *et al.* 2004) and general condition (Allendorf and Leary 1986, Stockley *et al.* 1993), which may lead to greater phenotypic competitive abilities (mating advantage, Hoffman *et al.* 2004). Although the superiority of a male due to heterozygosity is not heritable, a female that mates with a highly heterozygous male will increase her chances of having heterozygous offspring, as rare alleles are more common in heterozygotes (Charlesworth 1988, Brown 1997, Mays and Hill 2004). According to the heterozygosity hypothesis, male phenotypic expression is expected to correlate with individual heterozygosity, providing mate choice cues. Males with higher overall heterozygosity will be more attractive to females and, as a consequence, female mate choice will result in variance in male reproductive success associated with male heterozygosity.

Here, we test the compatibility and heterozygosity hypotheses for female mate choice in the White-crowned Manakin (*Pipra pipra*). We measured male mating success using behavioral observations (female visits) and molecular techniques (paternity analysis of offspring using microsatellites). Then, we examined the relationship of these measures of male reproductive success to individual heterozygosity and to their degree of genetic similarity to females who laid eggs fertilized by focal males. Additionally, we examined the relationship between heterozygosity and spatial and behavioral characteristics of males, such as territory attendance, vocalization rate, courtship display time, territory size and position, to examine if these traits act as “honest signals” of male heterozygosity.

METHODS

Study area

Tiputini Biodiversity Station (TBS) is located approximately 300 km ESE of Quito on the north bank of the Tiputini River in eastern Ecuador (~0º 38' S, 76º 08' W). It encompasses approximately 650 hectares of largely undisturbed tropical rainforest, which includes primarily terra firme forest, but also flooded forest, palm swamps, and areas of natural regrowth. TBS is within the 1.5 million hectare Yasuní Biosphere Reserve and boasts extremely high species diversity (Pitman *et al.* 2002, Valencia *et al.* 2004, Blake 2007). This study was conducted on one 100 hectare plot (ca. 1 km * 1 km, hereafter called the Harpia plot) at TBS and in its adjacent forest (in areas identified as adequate habitat for *P. pipra* 400 meters north and west of the plot; total area sampled was approximately 180 ha).

Study species:

The White-crowned Manakin is a small bird (average weight of males: 10.8 g; females: 13.4 g, unpublished data) in the family Pipridae. It is distributed from Costa Rica to eastern Brazil (Ridgely and Greenfield 2001). They are sexually dimorphic, where adult males are black with white crowns and napes, and females are olive-green with blue-grey heads (del Hoyo *et al.* 2004). Juvenile males fledge the nest with plumage coloration similar to females, and usually show some signs of male plumage (i.e., white feathers in crown and black feathers in body) after their first year (i.e., end of first breeding season).

Males acquire full adult plumage late in their second year (Ryder and Durães 2005). White-crowned Manakins display on exploded leks, so males are in auditory but not visual contact. Territories are defended by individual adult males and range from 234 – 1003 m^2 in size (Tori unpublished data). Males have one advertisement call and one whistle call. Courtship displays are performed individually and include forward and back flights among several horizontal display perches and slow butterfly-flights (deep and slow wing beats) around females following them from perch to perch (Snow 1961). In addition, territorial males often interact with males from neighboring territories (Castro-Astor *et al.* 2007). Male-male coordinated interactions take place in the absence of females and have different display elements than courtship displays. These interactions may act as a mechanism to establish and maintain dominance hierarchies among males (Tori, unpublished data).

Mist-netting

White-crowned Manakins were sampled by systematic mist-netting activities at 96 permanent net sites in the Harpia plot during March 2001, January and March 2002-2006, as well as by target-netting at leks during January-April 2004-2006. Captured birds were banded, individually marked with uniquely numbered aluminum - and color- leg bands and blood samples were taken for molecular genetic analysis. During the course of this study, all known adult males in the Harpia plot and its adjacent forest (400 meters north and west of the plot) were banded.

Offspring sampling

Nests were located via systematic searches of the study area from December to April 2004-2005, 2005-2006, corresponding to the main breeding season in the region.

Systematic searches were supplemented by radio-tracking females that were captured in breeding condition. Radio transmitters (Holohil Systems Ltd.) weighing 0.51 g (model BD-2N) were attached using a Rappole harness (Rappole and Tipton 1991). Females were then tracked to find the location of their nests. We replaced the eggs with plaster replicas and incubated the eggs ex-situ to avoid losing the genetic sample to nest predation. After hatching, blood samples were taken and chicks were immediately returned to their original nest to be raised by their mother (for details see Tori *et al.* 2006). In an effort to increase our offspring sample size, we captured and assigned paternity of juvenile males (green-plumaged individuals sexed as males in the lab, n = 34) and fledglings (green-plumaged individuals with yellow gapes and brown eyes, n = 3). We only assigned parentage of juvenile males, since females do not change plumage color and we were not able to identify their age.

Lek and territory location

Mapping activities were conducted in February and April 2002-2004, December to April 2004-2005 and November to April 2005-2006. Data were used to identify the number and location of leks (n = 7) and territories (n = 63) on the study area. Territories were defined as defended areas where individual males display and advertise for females. Leks were defined as discrete assemblages of male territories over space, in which neighboring males were in auditory contact (i.e., exploded leks). Males captured within the study area that were never observed to own a territory were considered non-territorial males (n = 29 males).

Behavioral variables

We conducted focal observations of 37 territorial males at 4 selected leks within the Harpia plot. Territorial males (n = 26) from three additional leks in the Harpia plot vicinity were not observed and therefore were not included in the analysis of behavioral traits. Observations were conducted during January–March 2005 and December 2005–March 2006. The basic sample unit was 2-hour focal observation periods. We observed each male for a minimum of 12 hours (mean 18 hours) during their peak of daily activity (07:00-09:00, 12:00-14:00 and 14:30-16:30, Durães *et al.* unpublished data). When possible, simultaneous observations took place at two or more leks. During observations, we recorded the following variables:

1) Territory attendance: mean number of minutes a male spent in his territory during the 2-hour observation period (unit: minutes / 2-hr observation).

2) Vocalization rate*:* mean number of advertisement calls during the 2-hour observation period (units: number of advertisement calls / 2-hr observation).

3) Number of coordinated interactions: mean number of male-male synchronized display behaviors during the 2-hour observation period (unit: number of coordinated interactions / 2-hr observation). Male-male coordinated interactions vary in their length and elaboration, but for this analysis all coordinated interactions were considered equivalent (equally weighted).

4) Number of aggressive interactions during courtship display: mean number of aggressive chases during courtship display across the 2-hour observation period (unit: number of aggressive interactions during courtship display / 2-hr observation).

Aggressive interactions here are defined as chases of a territorial male toward intruding neighboring male during female visitation to that territory.

5) Courtship display time: mean number of seconds males spent displaying across the 2-hour observation period (units: seconds displaying / 2-hr observation).

6) Standardized female visits: number of female visits standardized by 12-hours of observation. To estimate this value, we calculated the average number of female visits observed during 2 hour observations and multiplied it by 6 (unit: number of female visits / 12-hr).

Spatial variables

During focal observations, we marked perches that territorial males used to sing from (advertisement calls and whistles), rest and interact with other males. Afterwards, all perches were mapped and geo-referenced. Territory size was calculated by building a minimum convex polygon around advertisement call perches (AP) using the Animal Movement Extension, ArcView v.3. 2 (Hooge and Eichenlaub 1997). To be conservative, we excluded from the analysis coordinated interaction (CP), whistling (WP) and resting perches (RP). CP and WP were excluded because they were in peripheral areas usually used by more than one male and RP were excluded because perching was not considered enough evidence to suggest territoriality (most RP perches, however, were within the MCP area). Territory and lek centers were determined using the centroid polygon script in ARCGIS v. 9.1 (i.e., geometric center, ESRI 2005).

Genetic analyses

DNA was isolated from blood samples via a phenol-chloroform extraction method, followed by a cleaning step of dialysis in 1 X $TNE_{2.}$ DNA yield was determined by spectrophotometry and samples were diluted to a working concentration of 20 ng / µL. A set of 7 polymorphic microsatellite primers - Man6, Man13, Lan10, Lan20, Lan22, Maniac-3, Maniac-13 - (Piertney *et al.* 2002, Duval *et al*. 2005, Brumfield R and Braun M, pers.comm.) were selected based on their levels of polymorphism. Polymerase chain reactions (PCR) were run using fluorescently labeled forward primers (Table 1, Applied Biosystems, Inc., Foster City, CA). PCR products were combined (1 to 4 loci at the time) and run on an ABI 3100 automated capillary sequencer. Fragment sizes were determined using a size standard GENESCAN LIZ (500) and genotypes were assigned using Genemapper 4.01 (Applied Biosystems, Inc.). All homozygous individuals were run at least twice to avoid allelic drop-out problems and dubious genotypes were re-run to avoid spurious results. We determined allele frequencies per locus and ran tests for linkage disequilibrium and Hardy-Weinberg equilibrium using FSTAT v. 2.9.3.2. (Goudet 2001). All loci were in Hardy-Weinberg equilibrium and showed no significant linkage disequilibrium. Additionally, we determined the sex of all green-plumaged individuals using two chromo-helicase-DNA-binding (CHD) genes (P2 P8 primers, Griffiths *et al.* 1998). The PCR conditions used were an initial denaturing step at 92 ºC for 2 min, followed by 35 cycles of 92 ºC (45 s), 52 ºC (45 s) and 72 ºC (1 min) and a final run of 72 ºC for 5 min.

Paternity analysis

We used CERVUS 2.0 to assign parentage using co-dominant molecular markers (e.g. microsatellites, Marshall *et al*. 1998). CERVUS calculates a likelihood score (LOD) for each male being the sire of a particular offspring, based on the offspring, maternal genotypes (if known) and candidate male genotypes. The candidate father with the highest LOD score is assigned as the most likely sire. CERVUS uses simulations to assess the confidence for each paternity assignment. Simulations take into account the number of candidate males, the proportion of the male population that is sampled, the completeness, and the rate of typing error in the genetic data (e.g. null alleles, Marshall *et al*. 1998, Webster *et al.* 2004). The simulation parameters used were the following: 1) number of candidate males: 92; proportion of candidate males sampled: 0.95; proportion of loci typed: 0.976; rate of typing error: 0.01 and 0 (we ran both error types, see Morrissey and Wilson 2005); and strict confidence level of 95%. We examined the frequency of genotyping error between mothers and offspring and found no mismatches. To assign paternity of nestlings, we assumed that breeding females were the biological mothers of the chicks in their own nests and entered their genotypes as the known parent. To assign parentage of juveniles, first we used CERVUS to determine paternity of males, and then we used male assignments to determine maternity (i.e., stepwise parental analysis). The simulation parameters used to assign maternity were the following: number of candidate females: 73; proportion of candidate females sampled: 0.75. For paternity analysis, we used additional information (i.e., whether male sired other young at the nest, whether mismatch was likely caused by a null allele) to confirm that CERVUS assignments were reasonable ("total evidence" approach, Webster *et al*. 2004). We accepted CERVUS assignments if the selected male had zero mismatches with the nestling and we rejected CERVUS assignment if selected male had 1 or more mismatches. We did not follow these rules under two circumstances: 1) If assigned male had one mismatch that was consistent with the presence of a null allele (particularly at locus Lan20, which had a high null allele frequency). In this case, we accepted CERVUS assignment despite the mismatch (one case). 2) If two candidate fathers had the same number of mismatches but the male with lower LOD score had sired the other chick in the nest. In this case we assigned paternity to the lower scoring male (one case).

Heterozygosity estimates

Heterozygosity estimators were calculated for all candidate fathers. We calculated two heterozygosity estimators: (1) Standardized heterozygosity (Hs), which is calculated as the proportion of typed loci for which an individual is heterozygous, divided by the mean heterozygosity of typed loci (Coltman *et al.* 1999); (2) Internal relatedness (IR), a measure that is based on genetic correlations between alleles at each locus and that weights allele sharing by the frequencies of alleles involved (Amos *et al.* 2001). Low values of IR indicate high heterozygosity levels. Qualitative results with both heterozygosity indices were equivalent; thus, in this manuscript we report only the internal relatedness index.

It is unclear how robust neutral molecular estimators of heterozygosity generated by a few genetic markers are with respect to genome-wide heterozygosity (Balloux *et al.* 2004, Slate *et al.* 2004, Smith *et al.* 2005). If heterozygosity at microsatellite loci reflects genome-wide heterozygosity, then molecular estimators of heterozygosity will provide informative results about individual inbreeding levels. However, if this relationship is weak, then molecular estimators of heterozygosity would be an outcome of local effects of microsatellites or loci linked to them instead of inbreeding (Hansson and Westerberg 2002), and we may not be able to detect the effects of heterozygosity (Smith *et al.* 2005). In this study, we assume that our 7 microsatellite markers reflect genome-wide heterozygosity, but we recognize that this may not be the case, and thus our results should be taken with caution.

Genetic similarity

To assess the degree of genetic similarity among pairs, we used microsatellite data to calculate Queller and Goodnight pairwise coefficients of relatedness (r) using Relatedness v. 5.0. This measure estimates relatedness between two individuals on the basis of allele frequency differences from the population mean (for more details see Queller and Goodnight 1989). High values of r indicate high levels of relatedness (e.g., r = 0 unrelated, r = 0.25 half siblings, r = 0.5 full siblings).

Statistical analysis

To test the heterozygosity hypothesis, first we examined the relationships between heterozygosity and behavioral and spatial male traits as potential heterozygosity cues, using generalized linear models (Fox 1997, McCullagh and Neder 1989). In all models, we used Poisson errors and a log-link function because heterozygosity violated linear regression assumptions. Second, we determined if males that sired offspring had higher heterozygosity than expected by chance using Monte Carlo simulations. To do this, we randomly drew 19 males with replacement from the total male population and designated them as sires. We repeated this procedure 1000 times, calculated the average heterozygosity (IR) for each set of 19 males and generated a random distribution using these values. Next, we compared the mean heterozygosity of males observed siring offspring to this random expectation (one-tail probability, Gotelli and Ellison 2004). In addition, we used non-parametric one-tail Mann-Whitney tests to compare the heterozygosity between (1) males that sired and did not sire offspring, and (2) territorial and non-territorial males. Further, to test the relationship between heterozygosity and female visitation, we performed regression analyses.

To test if female mate choice was independent of the genetic similarity of potential partners, we compared relatedness estimates of observed mating dyads with the average relatedness of each female with all potential candidate males using a paired t-test. Consequently, the relatedness of an individual female with her observed mate and the average relatedness between this female and all other potential mates constituted a pair; the number of replicates equals the number of females with known mates. Support for the compatibility hypothesis requires that mating pairs have significantly lower relatedness than non-mating dyads. Moreover, we performed a binomial goodness-of-fit test to examine if females were at least selecting males with lower relatedness than the median relatedness of candidate mates. We performed analyses at the lek level (assuming females assessed only males in the lek where they mated) and at the population level (assuming females assessed all males in the population). Results at both levels were equivalent, so we report results only at the lek level. Analyses were conducted using SPSS v 13.0 and R v 2.4.1.

RESULTS

Can spatial traits act as male heterozygosity cues?

We found a significant relationship between heterozygosity and territory size (GLM, $R^2 = 0.02$, $p < 0.001$) and distance of territory from the center of the lek (GLM, $R^2 = 0.19$, $p < 0.001$, Table 2). The effect of male heterozygosity on territory size was weak (~2% of variance explained) compared to the effect of territory centrality (~20% of variance explained). In addition, territorial males tended to have slightly higher heterozygosity than non-territorial males, but this relationship was not statistically significant (IR: Mann-Whitney U = 753.0, $p = 0.088$, Figure 1). Non-territorial males, however, showed higher variation in heterozygosity values than territorial males. This may be the result of a few non-territorial males establishing territories out of our study area, leading to errors in territory status assignment.

Can behavioral traits act as male heterozygosity cues?

We found no relationship between heterozygosity and courtship display time, number of aggressive interactions during courtship displays (GLM, $R^2 < 0.002$, $p > 0.08$), or number of coordinated interactions among males and territory attendance ($R^2 < 0.028$, $p > 0.007$, not significant after Bonferroni corrections). A weak but significant relationship existed between heterozygosity and vocalization rate (IR: $R^2 = 0.053$, $p < 0.001$, Table 2). Thus, vocalization rate seems to be the most honest behavioral indicator of male heterozygosity (explains 5% of variance) and males with high heterozygosity were found to sing more.

Assigning paternity

We sampled 20 nestlings (11 nests), 3 fledglings, and 34 first-year juvenile males. We successfully assigned paternity of 28 offspring (16 chicks from 9 nests, 3 fledglings and 9 juveniles) to 19 territorial males, out of the 92 potential fathers sampled in the Harpia neighborhood. All but two males that sired offspring were observed defending territories. Clutch size was typically two and we found mixed paternity in only one case. We assumed that breeding females were the biological mothers of the chicks in their own nests (no allele mismatches) and we assigned maternity to only one fledgling. Thus, we had a total of 10 observed female-male mating dyads (both parents known).

Are heterozygous males siring more offspring?

The average heterozygosity of males that sired offspring was not statistically different from the average heterozygosity of males that did not sire offspring (Mean IR ± SE, successful: -0.025 ± 0.023, unsuccessful: 0.026 ± 0.020, Mann-Whitney U = 625.5, p = 0.165). Moreover, Monte Carlo simulations showed that successful males did not have significantly higher heterozygosity than expected by chance ($p = 0.103$, $\alpha = 0.05$, Figure 2). When we examined the relationship between heterozygosity and male mating success in more detail, we found that males that sired offspring had intermediate heterozygosity levels, and that individuals with extremely high or low heterozygosity did not reproduce (Figure 3). Further, we found that successful males had lower variability in heterozygosity levels than unsuccessful males (successful: $\square^2 = 0.010$, n = 19, unsuccessful $\square^2 = 0.031$, n=73, Levene’s test, F = 7.742, p = 0.007), as might be expected if males with intermediate levels of heterozygosity are preferentially selected by females.

Are heterozygous males receiving more female visits?

We observed a total of 98 female visits distributed across 31 territorial males during 1,308 observation hours. The best fit, although not significant, was a quadratic repression between female visitation and heterozygosity (IR: t = 0.83, p = 0.413, $IR^2 = 0.058$, Model: $y = 0.175 + 0.11x – 1.053x^2$, p = 0.16, $R^2 = 0.102$, Figure 4). These results appear to reject the heterozygosity hypothesis, and are consistent with those based on known parentage (males with the highest female visitation had intermediate levels of heterozygosity).

Are females mating with genetically compatible males?

The average relatedness of observed female-male mating dyads was -0.043 ± 0.054 (SE, n = 10) and the average relatedness of females with all other potential mates at the leks where they mated was -0.002 ± 0.016 (SE, n = 88). Two females mated with the most dissimilar males (i.e., least related), one mated with the most similar male (i.e., most closely related) and seven mated with males with intermediate relatedness. Moreover, we found no evidence that females were selecting more dissimilar males than the average candidate male available (paired t-test = -0.76, df = 9, p = 0.234), or that they were selecting males less related than the median relatedness of candidate mates (binomial test, p = 0.623). We were not able to record the identity of most visiting females during behavioral observations; thus, we did not examine the relatedness between visiting females and males.

DISCUSSION

The goal of this study was to test two competing hypotheses for genetic fitness benefits in female mate choice using the White-crowned Manakin as a model system. Specifically, we tested if females select (1) highly heterozygous males or (2) genetically dissimilar males, to increase the genetic diversity of their offspring. The heterozygosity hypothesis predicts that females preferentially mate with highly heterozygous males. On the other hand, the compatibility hypothesis predicts that females mate with genetically dissimilar males. Overall, our results support only partially the heterozygosity hypothesis. We found that spatial (i.e., territory size and centrality) and behavioral traits (i.e., vocalization rate) appear to be honest indicators of male heterozygosity. However, contrary to expected, males of intermediate heterozygosity appeared to be more successful in mating than either the most homozygous or heterozygous males.

Heterozygosity hypothesis

In accordance with the first prediction of the heterozygosity hypothesis, we found a positive relationship between heterozygosity and territory size and centrality. Heterozygosity explained 20% of the variation in territory location and 2% of territory size. Highly heterozygous males had larger and more centrally located territories. To the best of our knowledge, only one previous study has demonstrated a relationship between heterozygosity and territory centrality at leks (Höglund, *et al.* 2002). Our results agree with this study and suggest that territory centrality may act as a cue of male heterozygosity in female mate choice. Moreover, we found weak evidence that territorial males tended to have higher heterozygosity than non-territorial males. A more detailed study is needed to confirm this result, but if real, it suggests that heterozygosity may influence territory acquisition. If territoriality determines male access to females at least to some extent (Höglund and Alatalo1995, Johnson *et al.* 2000), then heterozygosity may impact mate choice by acting as a filter of male reproductive potential prior to female visitation and male assessment at leks.

Behavioral traits are known to act as visual cues of male performance and are sexually selected (e.g. acoustic component: Gibson and Bradbury 1985, Marshal *et al.* 2003, Seddon *et al.* 2004, display rate: Gibson and Bradbury 1985, Höglund and Lundberg 1987, McDonald 1989, Anderson 1989, lek attendance: Hill 1991, Anderson 1989, and male aggression: Trail 1985, Fiske *et al.* 1998 but see Hill 1991). We found that vocalization rate was the only measured behavioral variable significantly related to heterozygosity. Heterozygosity explained approximately 5% of the variance in vocalization rate and males with higher heterozygosity were found to sing more. Thus, our results suggest that females can potentially use vocalization rate in concert with other spatial traits (i.e., territory centrality, territory size) to assess the level of heterozygosity of potential mates.

Regarding the second prediction of the heterozygosity hypothesis, we did not find evidence that males with the highest heterozygosity were more successful at mating. Instead, we found a complex relationship between heterozygosity and male mating success. Most males that sired offspring had intermediate levels of heterozygosity, whereas males with the highest heterozygosity did not appear to reproduce. This pattern could not be explained by limited accessibility of highly heterozygous males, because males with high heterozygosity were present in all leks and, in many cases, they were neighbors of males that sired offspring. Moreover, we found that males with low heterozygosity did not sire offspring.

There is some evidence in the literature that, in some species, females select mates with intermediate levels of heterozygosity to produce offspring with optimal levels of genetic diversity. Aparicio *et al.* (2001) found that male Spotless Starlings (*Sturnus unicolor*) with intermediate levels of heterozygosity were more successful in mating and eliciting mate fidelity. In Bluegill Sunfish (*Lepomis macrochirus*), males with intermediate levels of heterozygosity were also found to have higher reproductive success than males with low or high heterozygosity levels (Neff 2004). Furthermore, Bonneaud (2006) found that female house sparrows (*Passer domesticus*) did not form breeding pairs with males with low allelic diversity or with males that were too dissimilar at MHC loci (class I genes). These authors argue that inbreeding and outbreeding can both compromise fitness. On the one hand, extreme inbreeding may have fitness disadvantages due to the expression of deleterious recessive alleles and lower potential to respond to changing environments (inbreeding depression, Lynch 1991). On the other hand, extreme outbreeding may have fitness disadvantages due to the loss of local genetic adaptations and breaking up of co-adapted gene complexes (outbreeding depression, Lynch 1991). As a result, it has been suggested that females may seek an optimum, by balancing inbreeding and outbreeding costs (optimal outbreeding hypothesis, Bateson 1983). Our results agree with this hypothesis and suggest that female White-crowned Manakins avoid mating with males with low and high heterozygosity levels. We do not know, however, the underlying mechanism by which females recognize males with intermediate levels of heterozygosity during mate selection. We found that heterozygosity was significantly related with centrality of male territories, territory size and vocalization rate, thus females may use these traits as an index of male heterozygosity and select males with intermediate phenotypic expression as mates. A study that addresses the fitness effects of inbreeding, outbreeding and the mechanisms used by females to recognize males with intermediate genetic diversity is necessary to confirm this argument.

Compatibility hypothesis

We did not find evidence that females actively select dissimilar males to increase the genetic diversity of their offspring. A small proportion of females mated with the most dissimilar males; but this proportion of unrelated matings would be expected under random mating. Moreover, we found no difference in the average relatedness between observed and potential mating dyads. The existence of fine resolution mechanisms for genotype recognition in passerine birds is controversial (Mays and Hill 2004). However, even if females have only approximations of their own genotype and those of potential mates, they may increase their offspring heterozygosity by recognizing and avoiding mating with kin. Under this scenario, females would be expected to select unrelated males that by definition will have lower genetic similarity than the median candidate male. Even at this coarser level, we did not find evidence to suggest that females are actively avoiding mating with kin. Females selected males across the entire relatedness continuum and there was no evidence that they were selecting males with lower relatedness than the median relatedness of potential mates. These results suggest that the compatibility hypothesis is an unlikely explanation for female mating preferences in this lekking species.

Additional remarks

We are aware that our results are limited because of small sample size, and may not hold with increased sample effort. We believe this is unlikely because our result from female visits (a potential surrogate of male mating success for which we have a larger sample size) suggests a similar pattern, in which males with intermediate levels of heterozygosity also appear to have higher visitation than males with high or low heterozygosity levels (Figure 4).

Conclusions

We conclude that the compatibility and the heterozygosity hypotheses do not explain female mate choice in the White-crowned Manakin. Nevertheless, heterozygosity may play a role during female mate selection. Males with intermediate heterozygosity were the most successful at mating, suggesting that females may optimize heterozygosity levels of their offspring by negotiating a balance between inbreeding and outbreeding costs.

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Table 1. PCR cocktail recipe, PCR conditions and general characteristics of 7 microsatellite loci used for molecular analyses in the White-crowned Manakin (*Pipra pipra*). All microsatellite loci were developed for other manakins species (Piertney *et al.* 2002; DuVal *et al*. 2005, R. Brumfield and M. Braun, pers. comm.). Allele numbers were based on 142 genotyped individuals. Volumes for the PCR cocktail are in µl.

	Man6	Man13	Maniac-13	Maniac-3	Lan10	Lan20	Lan22
PCR cocktail							
10x NH_4 (Buffer)	1	1	1	1	1	2	1
dNTP mix (1mM)	1	1	1	1	1	2	1
$MgCl_2$ (25 mM)	1.25	1	1	1	0.5	1.5	0.5
Primer R	0.25	0.25	0.25	0.25	0.25	0.5	0.25
Primer F	0.25	0.25	0.25	0.25	0.25	0.5	0.25
DMSO	0.25	0.25	0.25	0.25	0.25	0.5	0.25
Taq DNA Polymerase	0.06	0.06	0.06	0.06	0.06	0.1	0.06
Water	0.96	1.21	1.21	1.21	1.71	2.9	1.71
DNA (20 ng/µl)	1.5	1	1.25	1.5	1	1.5	1
Total volume	6.52	6.02	6.27	6.52	6.02	11.5	6.02

	Man6	Man13	Maniac-13	Maniac-3	Lan10	Lan20	Lan22
<u>PCR program</u>							
Denaturation temperature (˚C)	94	94	94	94	94	94	94
Denaturation time (sec)	30	30	30	30	30	30	30
Annealing temperature (˚C)	56	56	55	54	54	54	54
Annealing time (sec)	30	30	30	30	30	30	30
Extension temperature (˚C)	72	72	72	72	72	72	72
Extension time (sec)	60	60	30	30	30	30	30
Number of Cycles	35	35	30	35	30	30	30
Final extension temperature (˚C)	72	72	72	72	72	72	72
Final extension time (min)	10	10	10	10	10	10	10
<u>Characteristics of microsatellite loci</u>							
Number of alleles	11	26	25	19	7	17	20
Observed heterozygosity, H(O)	0.772	0.901	0.871	0.853	0.547	0.747	0.915
Expected heterozygosity, H(E)	0.783	0.932	0.885	0.877	0.525	0.816	0.920
Null allele frequency	0.0046	0.0153	0.0084	0.0116	-0.0193	0.0452	0.0013

Table 2. Generalized linear models (Poisson family) examining the effects of heterozygosity (measured as Internal Relatedness) on behavioral and spatial traits of territorial males. The effect of heterozygosity on each dependent variable was examined individually (significant relationships after Bonferroni corrections are highlighted in bold, $p<0.00625$). Note that low values of IR indicate high heterozygosity levels and, thus, a negative slope between IR and vocalization rate indicated a positive relationship of this variable with respect to heterozygosity.

Dependent Variable	N	slope	R^2	P-value
Behavioral traits				
Vocalization rate	37	-0.55	0.05	**< 0.001**
Territory attendance	37	-0.03	<0.01	0.0165
Number of coordinated interactions	37	-0.90	0.03	0.0072
Courtship display time	37	0.024	<0.01	0.101
Number of aggressive interactions	37	-0.22	<0.01	0.4410
Spatial traits				
Distance to center of lek	37	1.31	0.19	< **0.001**
Territory size	37	-0.31	0.02	< **0.001**

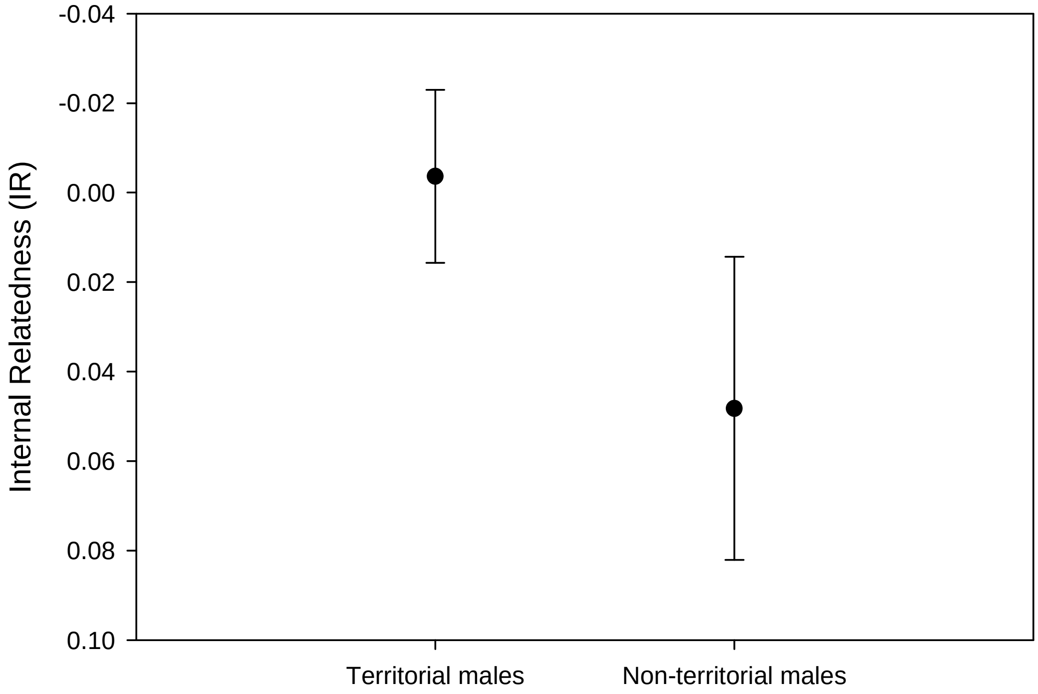

Figure 1. Average Internal relatedness (Mean ± SE) of territorial (n = 63, TM) and non-territorial (n = 29, Non TM) White-crowned Manakin males. Territorial males tend to have slightly higher heterozygosity than non territorial males but the difference was not statistically significant. Low IR values represent high heterozygosity levels, thus to facilitate graph interpretation the x axis (IR) has been inverted.

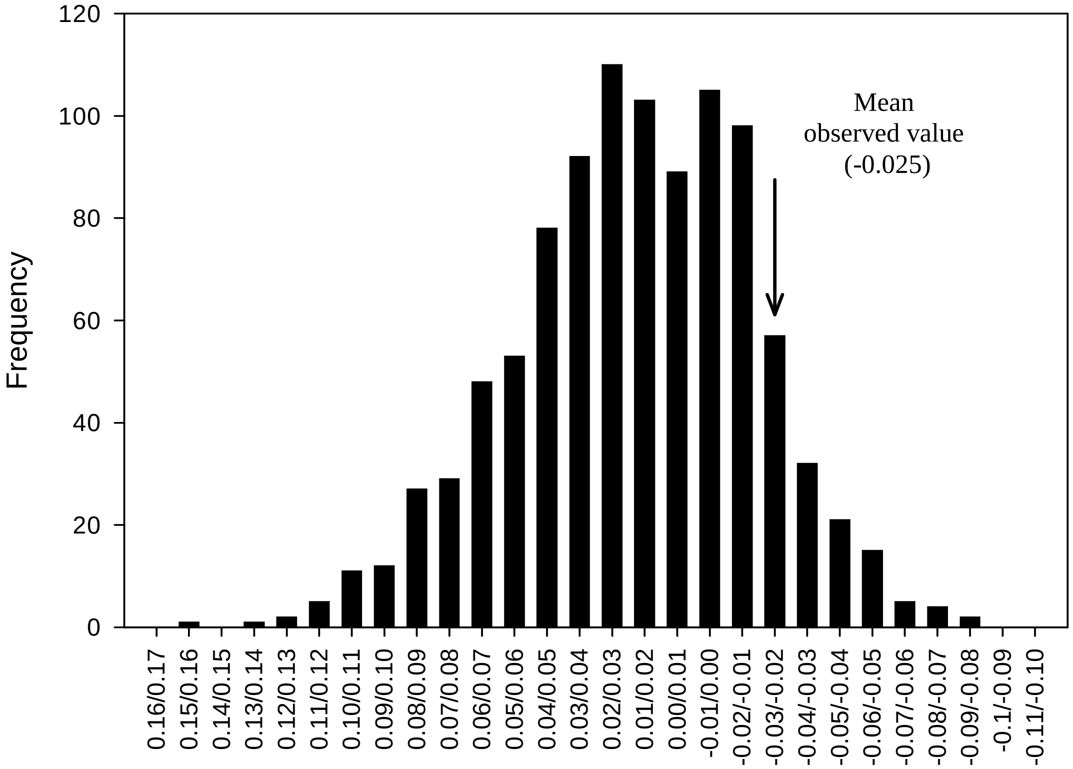

Figure 2. Monte Carlo analysis of males that sired offspring (n =19, observed) and males randomly drawn from the overall population. The histogram illustrates the distribution of the average heterozygosity of 19 randomly drawn males from 1000 randomizations. The average heterozygosity of males that sired offspring (observed) was higher than the random simulated values 897 times (p = 0.103). Note the x axis (IR) is inverted, such that males with higher heterozygosity occur at the right side of the graph.

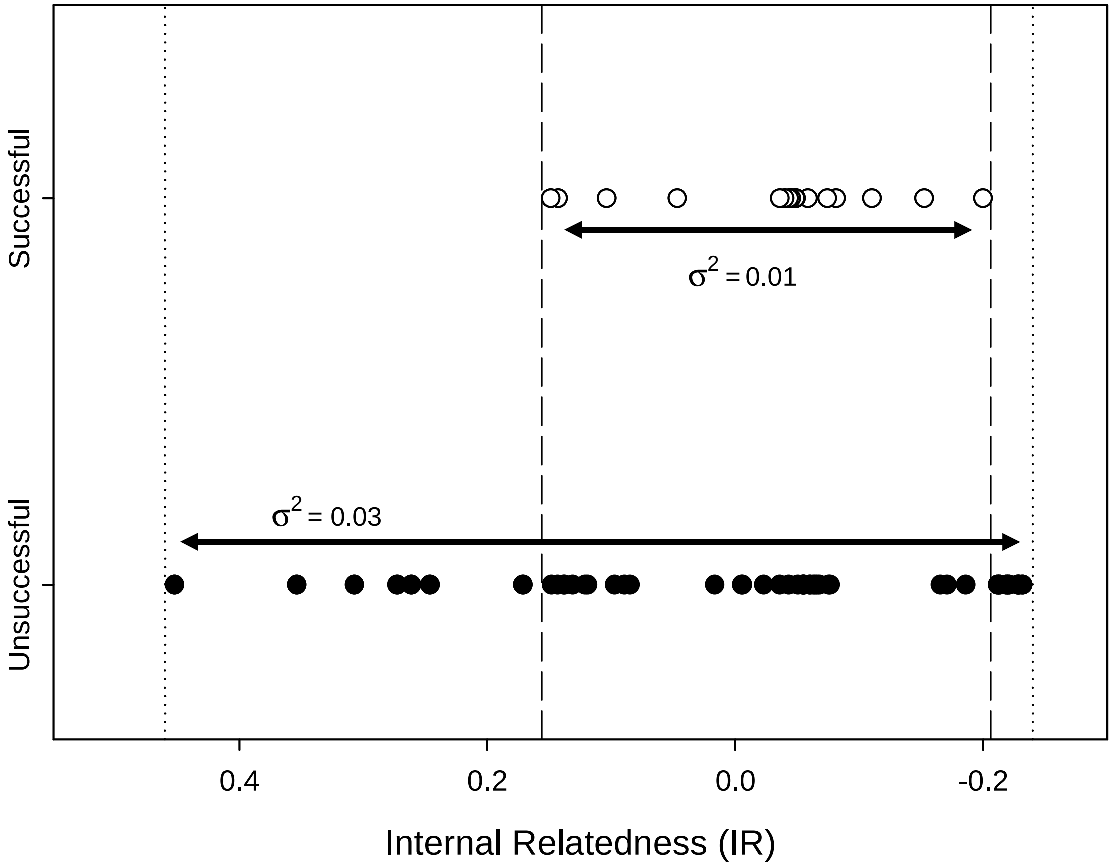

Figure 3. Internal relatedness (IR) for territorial males that did (open circles) or did not sire offspring (black circles). Territorial males with extremely high or low heterozygosity did not sire any offspring. Vertical lines mark internal relatedness range for successful (dashed lines) and unsuccessful males (dotted lines). Successful males had significantly lower variability in heterozygosity than unsuccessful males. Note the x axis (IR) is inverted, such that males with higher heterozygosity occur at the right side of the graph.

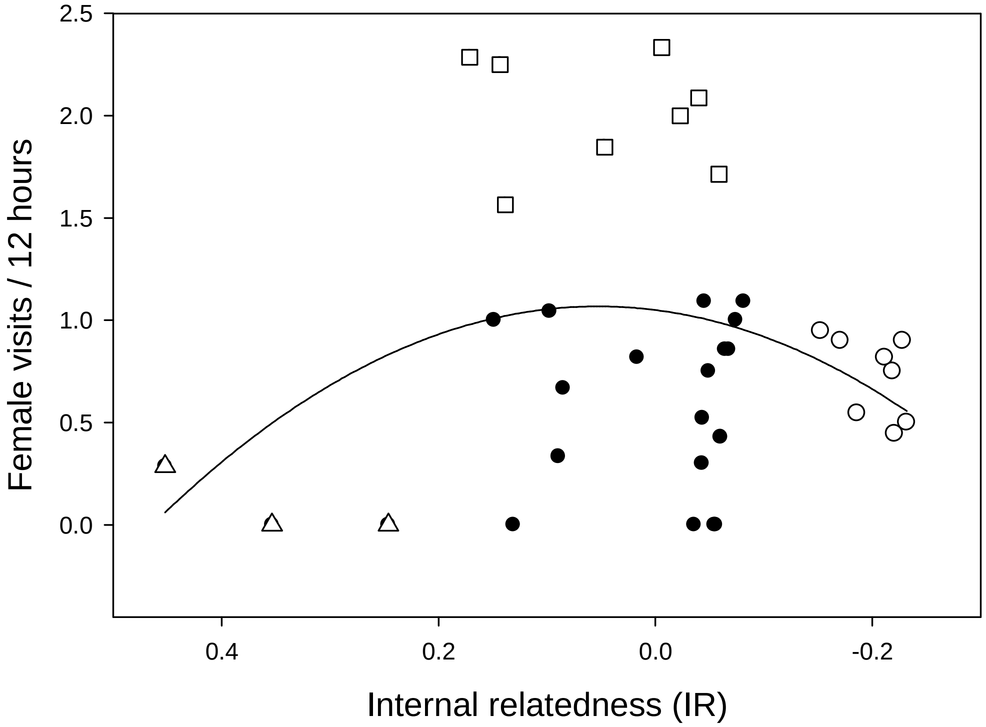

Figure 4. Quadratic regression between internal relatedness index (IR) and standardized female visits of territorial males of 4 White-crowned Manakin leks. Note that: 1) males with high female visitation (open squares) had intermediate heterozygosity levels, 2) males with high heterozygosity (open circles) had intermediate female visitation levels and 3) males with low heterozygosity (open triangles) received almost no female visits.

CHAPTER 2

Do resources affect male reproductive success in exploded lek mating systems? A case study with the White-crowned Manakin (*Pipra pipra*)

INTRODUCTION

Leks are typically defined as assemblages of adult males displaying at a traditional site. Females visit these display arenas to assess potential mates and for the purposes of mating (Bradbury and Gibson 1983, Ligon 1999, Alatalo *et al.* 1991). Accordingly, it is assumed that (1) lek territories rarely contain significant resources, (2) resource availability within leks does not affect the distribution of females, and (3) that female choice of particular males is based on male characters unrelated to immediate gains in fitness (Höglund and Alatalo 1995). The validity of these assumptions, however, is not clear for species with exploded leks, in which males have relatively large territories that may be separated by considerable distances (males are in auditory rather than visual contact). Unlike classical leks, in which males have very small display territories, exploded leks may contain suitable habitats for females, and females can potentially forage and even nest within a male's territory (Höglund and Alatalo 1995, Jiguet *et al.* 2000, Jiguet *et al.* 2002). Thus, male mating success in exploded leks can potentially be affected by the spatial distribution of resources through two mechanisms: (1) influence on female encounter probability (Figure 1a); or (2) influence on female mate choice (Figure 1b and c).

In the first mechanism, territories with high resource availability are expected to be attractive to females, increasing the encounter probability between sexes (Figure 1a, Bradbury and Gibson 1983, Bradbury *et al.* 1986). Males with more access to females will have more opportunities for copulation and, potentially, higher mating success. Indeed the "hotspot" hypothesis of lek evolution suggests that resources are one of the major factors driving female spatial distributions, and that males are attracted to sites with high female density or activity (Loyau *et al.* 2007), leading to the formation of leks (Bradbury and Gibson 1983; Bradbury *et al.* 1986, 1989).

In the second mechanism, resource distribution can affect female mate choice by acting directly as an honest signal of male quality or by influencing other features that females use to assess males (e.g., display performance). On the one hand, if resources (e.g., fruits) are limited (Leigh and Windsor 1982), one would expect intense competition among males to control high-quality territories. Thus, high-quality males will be able to establish their territories in areas where resource availability is high, and females will be able to assess resource availability as an honest signal of male quality (Figure 1b). On the other hand, lekking species are characterized by high energetic costs during reproduction because of intense energy demands of mate attraction displays (Vehrencamp *et al*. 1989, Höglund *et al.* 1992, Kålås *et al.* 1997). Thus, males that have territories with higher resource availability will be able to find and consume resources more efficiently so that they can maximize their levels of attendance and display performance. If females are using these behavioral variables as cues for male quality, resource availability can potentially be translated into significant effects on male mating success (Figure 1c).

In this study, we evaluated the effect of resource availability on male mating success of an exploded lekking species: the White-crowned Manakin (*Pipra pipra*). To do this, we examined the effect of territory resource distribution on male mating success. We predicted that males with more resources within their territories would have higher reproductive success. Next, we examined which of the previously described scenarios operate in our system. If territory resource availability affects male mating success through an effect on female encounter probability, we predicted that the number of female visits would increase with the availability of resources within territories. On the other hand, if resources affect male traits used as quality cues during female mate choice, we predicted that high fruit availability would enhance male characteristics (e.g., display activity, aggression, lek attendance; see Fiske *et al.* 1998). Lastly, if females use fruit availability within territories as an honest signal of male quality, we predicted that resources would have an effect on female mate choice, but resources would not necessarily have an influence on male behavior. As these predictions are not necessarily mutually exclusive (resources can increase female encounter probability and male attractiveness simultaneously), our examination is preliminary and separation of these hypotheses require future manipulation to unambiguously determine the underlying mechanisms. Nonetheless, information generated in this study will be the first step towards a better understanding of the role of resources, and the mechanisms that shape the relationship between resource availability and male mating success in species with exploded leks.

METHODS

Study site

Research was conducted at the Tiputini Biodiversity Station (TBS, ~0º 38' S, 76º 08' W) located on the north bank of the Tiputini River in eastern Ecuador. TBS encompasses 650 hectares of largely undisturbed rainforest, located within the 1.5 million hectare Yasuní Biosphere Reserve. It includes extensive evergreen terra firme forest, and other habitats such as swamps, floodplains and areas of natural regrowth. The canopy is up to 25 m high with emergent trees around 40 meters tall (Valencia *et al.* 2004). TBS lies on relatively flat terrain at an elevation of 190 – 270 m. The average annual rainfall is 2740 mm and occurs mainly between April and August (average monthly rainfall ~ 383 mm); October to February is drier (average monthly rainfall ~140 mm, Karubian *et al.* 2005). We focused our study within and adjacent to a 100 hectare plot.

Study species

The White-crowned Manakin (*Pipra pipra*) is distributed in lower growth of montane forest and terra firme forest from Costa Rica to SW Colombia and E Peru and also occurs in Amazonia, SE Brazil, S Venezuela and the Guianas (Ridgely and Greenfield 2001). They form exploded leks in which males are within hearing distance of each other (Johnsgard 1994, Castro-Astor *et al.* 2007). Each male has a territory (approximately 570 m^2), where they use several perches to perform advertisement calls and displays (Snow 1961, Théry 1992). They spend a large proportion of their time advertising within their territory (up to 88% of the day, Théry 1992). Females alone have the responsibility of building the nest, incubating eggs, and raising the chicks. White-crowned Manakins inhabit the understory, preferring to stay between 3 and 6 meters from the ground.

Manakins are mainly frugivorous (Worthington 1982), but they also consume some insects. They forage by gleaning from a perch or by hovering or sally-gleaning while on a short flight (Tori, pers. obs.). They eat a wide diversity of soft fleshy fruits and arillate seeds; consuming the pulp or aril and discarding the seed via regurgitation or passage through the gut. Loiselle *et al.* (2007), in a short-term study conducted at our site, reported that White-crowned Manakins consumed 44 different fruit species. Fruits from the families Melastomataceae, Rubiaceae and Araceae make up a large proportion of their diet (Krijger *et al.* 1997, Loiselle and Blake 1990, Tori, unpublished data).

Fruit availability

Mapping activities were conducted in February and April of 2002 – 2004, December to April 2004 - 2005, and November to April 2005 - 2006. Data were used to identify the location of focal leks (n = 4) and territories (2005: n = 29, 2006: n = 28). Territories were defined as defended areas where individual males displayed and advertised for females. Leks were defined as discrete spatial clusters of male territories, in which neighboring males were in auditory contact. We established 30-m transects bisecting the major axis of each male's territory in December 2004 (hereafter called 2005 field season) and November-December 2005 (hereafter called 2006 field season). Transects were 5-m wide and 10-m high. We took pictures, scanned or collected voucher specimens of flowering and fruiting plants for later identification to species or morpho-species. Plants were identified using collections at the Missouri Botanical Garden or at Herbario Nacional del Museo Ecuatoriano de Ciencias Naturales in Quito. We restricted our analyses to plants known or suspected to be consumed by manakins based on personal knowledge and prior studies (Snow 1981, Worthington 1982, Wheelwright *et al.* 1984, Worthington 1989, Marini 1992, Loiselle and Blake 1992, 1993, 1999, Loiselle pers. obs).

Fleshy fruits in the tropics often take several months to ripen (Loiselle 1987), and birds preferentially eat ripe fruits because of their higher nutritional values (Foster 1977, Moermond and Denslow 1983, Moermond *et al.* 1986). Ripe fruits within territories can be difficult to quantify because fruits can ripen asynchronously over a long time period, and fruits are often consumed quickly after ripening. Blake *et al.* (1990) suggested the use of unripe fruit as a measure of future ripe fruit availability. In an effort to maximize the information about fruit availability across the main breeding season (December-March), we measured (1) number of unripe fruits, (2) number of reproductive (i.e., flowering and fruiting) plants, and (3) total wet fruit biomass (g). When fruits were so abundant that direct counts were not possible, we calculated an average number of fruits in each infructescence (using a sub-sample of 10 infructescences) and multiplied it by the total number of infructescences on the plant (Worthington 1982, Ryder *et al.* 2006). Wet-fruit biomass was calculated only for species of Melastomataceae, Rubiaceae and Araceae (~ 77% of species with fruits). To do this, we combined the number of ripe (when present) and unripe fruit in each territory and multiplied the total by the average weight of ripe fruit for each species (based on ~20 fruits per plant species weighed to the nearest 0.1g; Ryder pers. comm. and Tori unpublished data). If we could not collect ripe fruit of a given species (~36% of species), we used the average weight of a closely related species as a surrogate. We are aware that fruits from different species may vary in nutritional content, yet we believe that the large variation in number of fruits, reproductive plants and fruit biomass found among different territories overwhelm any interspecific variation in fruit nutritional content. Thus, we think that the number of reproductive plants, number of unripe fruit and fruit biomass will provide reasonable estimates of resource availability within male territories across the breeding season.

Male reproductive success

We used two estimates of male success: 1) molecular paternity using microsatellites and 2) female visits. Nests were found via systematic searches of the forest, supplemented by radio-tracking females in breeding condition to their nests (for details see Tori *et al.* 2006). For molecular paternity, we sampled DNA from females, nestlings and candidate males and genotyped them for 7 polymorphic microsatellite loci (Man6, Man13, Maniac-3, Maniac-13, Lan10, Lan20 and Lan22; Piertney *et al.* 2002, DuVal and Nutt 2005, Brumfield and Braun pers. comm.). Paternity of nestlings was assigned using the program CERVUS (Marshall *et al*. 1998, for details see chapter 1). Behavioral estimates of male reproductive success were measured by counting the number of female visits recorded during behavioral observations (see below). We standardized the number of female visits by the number of two-hour observation periods conducted for each male.

Male behavioral observations

We conducted focal behavioral observations of 33 territorial males in 4 leks (same males whose territories were sampled for fruit). Observations were conducted during January–March 2005 and December 2005–March 2006, during peak periods of daily activity for White-crowned Manakins (07:00-09:00, 12:00-14:00 and 14:30-16:30 h). Each behavioral observation lasted 2 hours and males were observed for at least 12 hours (Mean ± SE , 2005: 14.80 ± 0.39 hours, 2006: 26.75 ± 0.99 hours). During behavioral observations, we recorded the (1) number of female visits, (2) time males spent displaying (seconds), (3) territory attendance (minutes), and (4) number of aggressive interactions directed towards conspecifics in the focal male's territory. All behavioral variables were standardized by the number of behavioral observations (i.e., per 2 hours) conducted for each male.

Statistical analysis

The number of female visits (2005, 2006 and 2005-2006), number of reproductive plants (2006), fruit biomass (2005 and 2006), and time spent displaying (2005) were transformed (i.e., $\ln(x+1)$ or $\sqrt{(x)}$) to meet parametric assumptions. We were not able to normalize the variables unripe fruit (all years), fruit biomass (2005-2006), territory attendance (2005 and 2006) and number of aggressive interaction (2005 and 2006). In the cases where data did not meet parametric assumptions, we used non-parametric tests.

To examine if there were general differences in resource availability between field seasons, we compared the number of unripe fruits, reproductive plants, and fruit biomass between 2005 and 2006 using Mann-Whitney or t- tests. Further, to see if resource availability within territories was consistent across seasons, we ran correlations of each resource variable between 2005 and 2006.

To determine if males that sired offspring had more resources within their territories than males that did not sire offspring, we used Mann-Whitney or t-tests. Due to small sample sizes for male success based on molecular paternity analysis, we pooled both seasons (i.e., 2005-2006) and grouped males in molecular success categories (i.e., males that sired vs. did not sire offspring). Further, we used the mean across years for number of fruiting plants, unripe fruit, and fruit biomass as explanatory variables. We felt that averaging values across years was justifiable, as resources within a territory were significantly correlated between years (see results). White-crowned Manakins are relatively long-lived birds (Blake and Loiselle 2008) with relative stability across years in male ownership of territories and female home ranges (see results, Blake and Loiselle, unpublished data). To examine if resources had an effect on female encounter probability (Figure 1a), we ran regression analyses for each year independently. We used the number of unripe fruit, reproductive plants, and fruit biomass as explanatory variables and the standardized number of female visits as the response variable. Residuals were normally distributed for all regression analyses. Finally, to examine if fruit resources had an influence on male performance (Figure 1c), we ran correlations between the three resource variables and male behavioral traits. Because assumptions of normality were not met in all cases, we ran correlations instead of regression analyses. We used SPSS v. 13.0 and R v. 2.6.1 to conduct statistical analyses.

RESULTS

Male territory fidelity

In general, territories were stable across years both in location and male ownership. From 2005 to 2006, 23 of 24 males (present in both seasons) occupied the same territory, while one male moved territories within a lek. In addition, two new males occupied territories in 2006 that were held by other males in the previous year, and 6 males that were territorial in 2005 did not come back in 2006.

Fruit resources

We recorded a total of 94 morpho-species of reproductive (i.e., flowering and fruiting) plants in the transects. The families Melastomataceae (35%), Rubiaceae (22%), Araceae

(15%), Solanaceae (5%), Gesneriaceae (4%), Annonaceae (3%), accounted for most of the plant species. Other families, (i.e., Bromeliaceae, Nyctaginaceae, Erythroxylaceae, Euphorbiaceae, Guttiferae, Monimiaceae, Myrtaceae, Arecaceae, Urticaceae and Heliconiaceae) accounted for less than two percent each.

The number of unripe fruits, reproductive plants and fruit biomass within territories varied among males for both seasons (Table 1). Few male territories were relatively fruit-rich, whereas most were relatively fruit-poor. The number of unripe fruits (Mann-Whitney U = 340.5, p = 0.296), reproductive plants (t test: t = 0.273, df = 55, p= 0.786) and fruit biomass (t test: t = 1.945, df = 55, p = 0.060) did not differ between 2005 and 2006. Moreover, the number of fruiting plants (r = 0.45, p = 0.019) and fruit biomass (r = 0.41, p = 0.032) in territories were significantly positively correlated between seasons, whereas numbers of unripe fruit (Spearman r = 0.37, p = 0.057) were marginally correlated between years.

Molecular Paternity

We assigned paternity of 11 nestlings to seven territorial males (from 33 focal males). Despite small sample sizes that likely failed to identify all males that were reproductively successful, males that were identified as siring offspring from paternity analyses had significantly more unripe fruit (Mann-Whitney U = 45, p = 0.018), marginally more fruiting plants (t = 1.606, df = 32, p = 0.059), but similar fruit biomass (Mann-Whitney U = 63, p = 0.09) within their territories compared to males with no molecular evidence of paternity (Figure 2).

Female visitation

We observed a total of 98 female visits distributed across 19 males in 2005 and 22 males in 2006 during 1,308 observation hours. In a few cases, visiting females were observed foraging within male territories, but the number of female visits was not related to the number of unripe fruit, number of reproductive plants or fruit biomass in 2005 or 2006 (Table 1).

Behavioral traits

Males showed considerable variation in behavior, especially in time spent displaying and aggressive interactions (Table 2). Treating each variable independently, time spent displaying was positively correlated with some measure of resource availability in both years (2005: unripe fruit and 2006: number of plants). That is, in 2005, territorial males with more unripe fruit in their territory spent more time displaying, while in 2006, males with greater number of reproductive plants spent more time displaying than males whose territories had fewer reproductive plants (Table 3). However, with a Bonferroni correction (Rice 1989) for multiple tests, only the relationship between time spent displaying and number of reproductive plants was significant at the adjusted p-value (0.0056). Nonetheless, there appears to be a consistent tendency for fruit resources to affect male display in both seasons ($p < 0.05$), suggesting that the relationship might be biologically meaningful (see Moran 2003).

DISCUSSION

Our results suggest that resources play a role in male mating success for the White-crowned Manakins. We found that males that sired offspring tended to have higher fruit availability within their territories than unsuccessful males. Resources, however, did not affect the encounter probability between sexes (i.e., as measured by female visits). On the contrary, our results suggest that males may compete for territories with more fruit to satisfy their own energetic requirements. Males with more fruit resources within their territories tended to spend more time displaying in both seasons. Thus, resource availability within territories may affect male mating success through its influence on male display characteristics.

Territory resource distribution

An essential requisite for fruit resources to affect male reproductive success is that resources vary among male territories. In tropical forests, fruit resources are known to be patchily distributed (Fodgen 1972, Levey 1988, Loiselle and Blake 1993) and, thus, males might be expected to compete for access to high quality territories. In this study, we found that the number of unripe fruits, reproductive plants and fruit biomass varied among territorial males in both seasons. Males were observed to have high territory fidelity and the availability of resources was correlated within territories between years. These results suggest that only some males are able to settle in areas with high resources and that less competitive males may be “forced” to settle in territories with relatively lower abundance of fruits. These differences in territory quality among males provide opportunity for selection and suggest that territory quality could affect male performance, as well as act as an honest signal of male competitive abilities during female mate choice.

Do resources affect reproductive success of territory owners?

We found that males that sired offspring had more unripe fruits and tended to have more reproductive plants in their territories than unsuccessful males. We are aware that our results are limited because of small sample sizes, however even with small sample sizes, we found differences among success groups, suggesting that these relationships may be real. Consequently, this study suggests that resources may be more important than previously believed in exploded lekking species, and that future studies should consider the role of resources in male success.

Are resources affecting the encounter probability between sexes?

We found no direct relationship between female visitation and resource availability within territories (i.e., males in resource-rich territories did not receive proportionally more female visits than males in resource-poor territories) during any season. This suggests that territory quality is not driving female visitation patterns, and that males do not gain reproductive benefits through this mechanism.

Failure to support this hypothesis, however, does not necessarily refute the importance of fruit resources as a way to gain access to females. It has been suggested that leks are placed in areas of high fruit availability (i.e., hotspot hypothesis), where high density of females are expected regardless of the fine-scale distribution of resources among territories. At the scale of the lek, Ryder *et al.* (2006) found that White-crowned Manakins leks at our study site are located in forest areas where fruit resources (i.e., number of fruiting plants and number of fruiting species) are more abundant. A study that examines the relationship between overall fruit availability at leks and the overall number of female visits received by leks will further elucidate if resources affect the encounter probability among sexes at the lek level. At the scale of individual territories on leks, however, we found no evidence that the distribution of resources within territories determines female visitation.

Do resources have an effect on male performance?

We found that males with more resources within their territories displayed for longer periods of time than males with less resources. On the other hand, we did not find any consistent relationship between the availability of resources and male territory attendance or aggression rate. The lack of relationship between fruit resources and territory attendance was surprising, since attendance has an intuitive relationship with food availability within territories (i.e., the more resources within territories, the longer males can stay without searching for food elsewhere). We suspect, however, that males may need to attend their territories for certain amount of time (regardless of the availability of resources) to be able to own / defend a territory and have access to females (territory ownership in manakins appears to be critical for male mating success, chapter 1, Ryder *et al.* 2008). Consistent with this hypothesis, we found that attendance had the smallest coefficient of variation of all the behavioral traits measured and that males attended their territories for long periods of time (Table 2). Moreover, attendance has been identified as a general predictor of male mating success in lekking species (Fiske *et al*. 1998). This may suggest that other costly sexually-selected behaviors (e.g. display behaviors) may be limited by the ability of males to meet their energetic demands while maintaining high levels of attendance.

Lekking courtship behaviors have been reported to be costly. For example, studies of Sage Grouse revealed that the energetic costs of male display are substantial, and that males have an instantaneous rate of energy expenditure during display of more than 13.9 times their basal metabolic rate (Vehrencamp *et al*. 1989). Similarly, in the Great Snipe (*Gallinago media)*, displaying males have been found to lose up to 6.8% of their body mass during each display night and it has been suggested that display rates are constrained by energetic limitations (Höglund *et al.* 1992). We also have evidence that display behavior can be energetically costly for White-crowned Manakins. Average weights of adult males differed between the breeding (December – March, n = 57, 10.55 ± 0.08 g) and non-breeding season (June-August, n = 20, 11.5 ± 0.14 g, t = 6.385, df = 75, p < 0.001, unpublished data). However, we do not have specific data from individual males between seasons to test if those males in resource-rich territories lose less weight than those in resource-poor territories after controlling for display behavior. Nevertheless, our results are consistent with the idea that White-crowned Manakin display behaviors demand substantial energetic costs, and that displays may be subsidized by resources within territories. Resources, however, are only one factor that may influence the expression of male display rate. Other factors such as genes (Kotiaho *et al.* 2001), age and experience (Trainer and McDonald 1995, Trainer *et al.* 2001) are also known to influence the expression of male display characteristics.

Do resources act as honest signals of male quality?

This hypothesis suggests that fruit resources within territories affect female mate choice by acting as honest signals of male quality. It predicts that females will directly assess resources within territories, so that there is no necessary relationship between resources and male display traits. Appropriate testing of this hypothesis, however, requires field experimentation not included in this study (e.g., experiments with different amounts of resources controlling for male performance). Thus, we can not discard the possibility that resources are simultaneously acting as honest signals of male quality and increasing male display performance during female mate choice (Figure 1 b and c).

Resources can act as honest signals of male quality at two different temporal scales. On one hand, resource assessment within a breeding season could provide females with information about present (i.e., seasonal) male competitive abilities (snapshot). On the other hand, females may assess resources at a longer temporal scale. Manakins species have relatively long life spans (> 10 years, Snow 1962, Snow and Lill 1974, McDonald 1989, 1993) and male territories occur at specific locations that are used year after year. Moreover, hippocampal measurements of several genera of manakins (*Pipra, Manacus* and *Chiroxiphia*) show that they have large hippocampus, suggesting that they may have good spatial memories (D.B. McDonald, pers. comm.). Thus, females may be able to use information about the overall long-term availability of resources within known territories to make mating decisions. In this case, resources would provide more comprehensive information about male competitive abilities and could be used as a criterion to reduce mate searching costs. Studies that collect long-term data on resources and male mating success are required to test this possibility.

Concluding remarks

Territory quality has been shown to affect female mate choice and reproductive success for many species (e.g., Hews 1983, Poulsen *et al*. 1998, Calf *et al*. 2003, Kerbiriou *et al.* 2006, Maguire 2006, Rubenstein 2007). However, in non-resource based mating systems such as leks, it is generally assumed that territories do not contain significant resources and that resources do not affect male mating success. This assumption is not clear for exploded lekking species, in which males are clustered but have relative large territories that can contain resources that may affect male reproductive output. To the best of our knowledge, there have been only two studies that tested these arguments in birds with exploded leks. In the Little Bustard (*Tetrax tetrax*, Jiguet *et al*. 2002), males settled in areas with resources, but resources within territories were not critical for females and females did not use them as a criterion for mate choice (Jiguet *et al.* 2002). Similarly in Houbara Bustards (*Chlamydotis undulata undulata*, Hingrat *et al.* 2007), males did not monopolize critical resources for breeding females, and resources did not affect female mate choice. In contrast with these studies, our results suggest that resources may affect male mating success in White-crowned Manakins. Resource availability varied among male territories and males that sired offspring were found to have higher fruit availability within their territories than unsuccessful males. We did not find any evidence, however, that males controlled female access to resources to obtain matings, or that resources within territories are critical for females (females feed mostly outside male territories). On the contrary, our results suggest that resources may be important to maximize male display. Males with more resources within their territories tended to display for longer periods of time. Thus, resources appear to affect male mating success by influencing male characteristics that females select during mate choice. Indeed, display rate has been identified as an important determinant of male mating success in the White-crowned Manakin (see chapter 3). Further investigations are necessary to unambiguously determine the mechanisms by which resources affect male mating success and to support our correlative suggestions with experimental data.

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Table 1. Descriptive statistics (mean, standard error and range) and simple regression results to test the relationship between the number of unripe fruit, number of reproductive plants and fruit biomass on the number of female visits received by each territorial male in 2005 and 2006. For regression analyses, the variables female visits (2005 and 2006), reproductive plants (2006), and fruit biomass were transformed and all regressions produced normal residuals. We found no relationship between the number of female visits and any resource variable.

Year	Variable	Mean	SE	Range	r^2	t	P-value
2005	Unripe fruit (per 150 m^2)	1716	675.1	0 – 14,698	0.04	1.108	0.278
	Reproductive plants (per 150 m^2)	7	0.6	2 - 14	0.01	-0.553	0.585
	Fruit biomass (g per 150 m^2)	382	174.1	0.90 – 4,369	0.05	1.125	0.270
2006	Unripe fruit (per 150 m^2)	639	195.2	1 – 5,047	0.01	0.595	0.557
	Reproductive plants (per 150 m^2)	8	1.1	1 - 30	0.00	0.403	0.690
	Fruit biomass (g per 150 m^2)	78	20.6	0.65 - 539	0.01	0.645	0.524

Table 2. White-crowned Manakin male behavioral traits (mean, standard error and coefficient of variation) measured during focal behavioral observations at Tiputini Biodiversity Station for 2005 and 2006.

	2005			2006		
Behavioral trait	Mean	SE	CV	Mean	SE	CV
Time spent displaying (sec / 2h)	166	24.3	0.89	205	21.1	0.55
Territory attendance (min / 2h)	96	2.4	0.13	80	5.5	0.37
Number of aggressions (aggressions / 2h)	0.2	0.03	0.93	0.3	0.04	0.70

Table 3. Correlations between resource availability and White-crowned Manakin male behavioral traits in 2005 and 2006. The variables number of reproductive plants (2006), fruit biomass (2005 and 2006), and time spent displaying (2005) were transformed to meet parametric assumptions and Pearson correlations were run. Other variables such as number of unripe fruit (2005 and 2006), territory attendance (2005 and 2006) and number of aggressions (2005 and 2006) could not be normalized, thus we ran non-parametric Spearman correlations.

		2005		2006	
Variable	Behavioral variable	r_s	P-value	r_s	P-value
Unripe fruit	Time spent displaying	0.368	0.050	0.320	0.097
	Territory attendance	0.089	0.646	0.337	0.080
	Number of aggressions	0.220	0.251	0.135	0.495
Reproductive plants	Time spent displaying	-0.111$_a$	0.567	0.561$_a$	0.002
	Territory attendance	-0.140	0.470	0.001	0.996
	Number of aggressions	0.263	0.167	0.035	0.859
Fruit biomass	Time spent displaying	-0.173$_a$	0.371	0.229$_a$	0.242
	Territory attendance	0.049	0.800	0.142	0.473
	Number of aggressions	0.300	0.114	0.108	0.584

$_a$ Pearson correlation coefficients

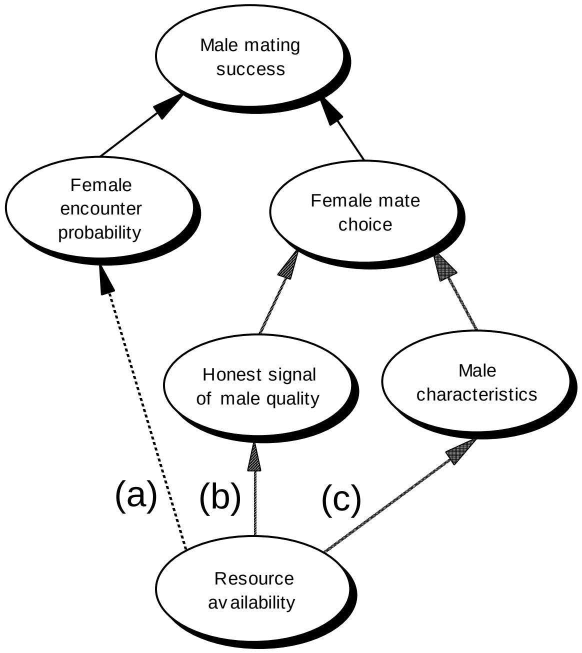

Figure 1. Potential effects of resource availability on male mating success for exploded lek species. (a) Resources can have an effect on male mating success by affecting the encounter rates between sexes. (b) Resources can have an effect on male mating success by acting as honest signals of male quality during mate selection. (c) Resources can have an effect on male mating success by affecting male traits (e.g. display performance) that are used as cues during female mate choice.

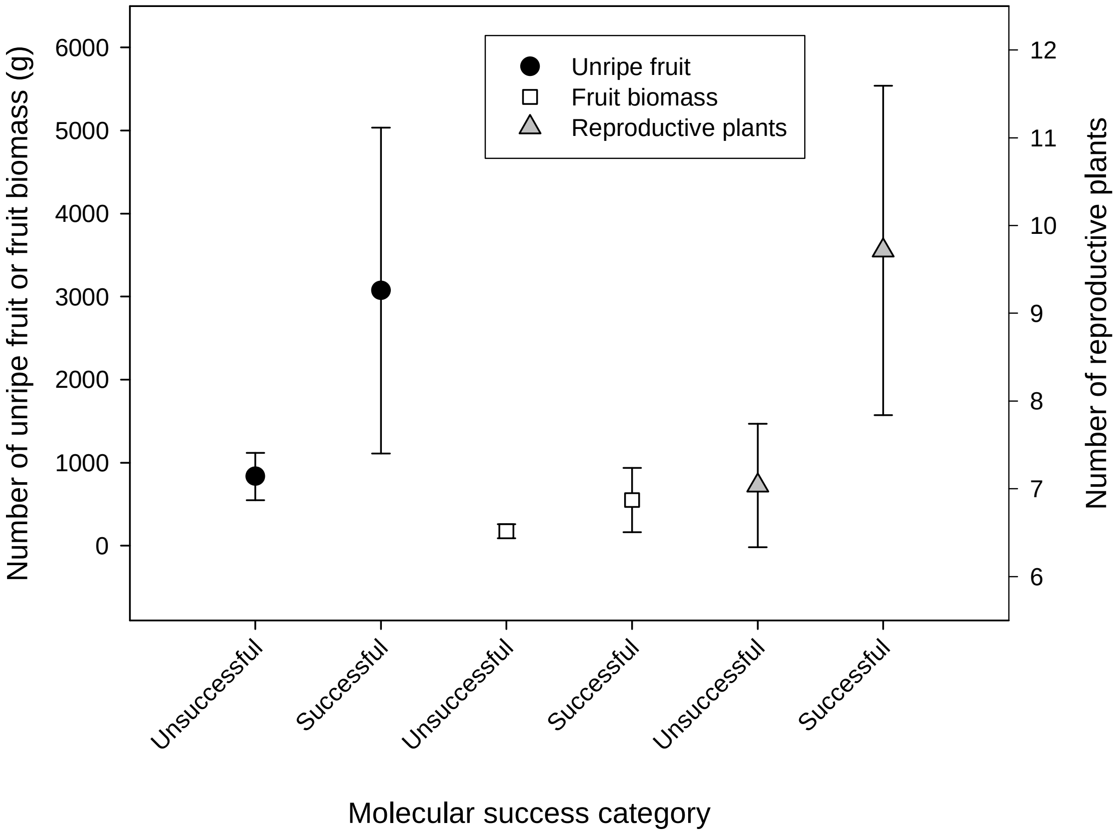

Figure 2. Comparison of resources availability (mean ± standard error) between males that sired (successful) and did not sire offspring (unsuccessful).

THIRD CHAPTER

Sexual selection in the White-crowned Manakin (*Pipra pipra*): Effects of behavioral and spatial characteristics on male mating success

INTRODUCTION

Lek mating systems are characterized by intense sexual selection. Typically there is high variance in male mating success, in which only a few males sire most of the offspring (Höglund and Alatalo 1995, Mackenzie *et al.* 1995). Differences in male mating success result from interactions among males to gain access to females, and from choices females make among the males they have access to. Male-male competition (e.g., Foster 1981, Trail 1990) and female mate choice (e.g., Gibson and Bradbury 1985, Anderson 1989, Pruett-Jones and Pruett Jones 1990, Gratson 1993) have been reported to occur in many lekking species, and it has been suggested that both can act simultaneously (McDonald 1989a, Höglund and Alatalo 1995, Loyau *et al.* 2005).

Male-male competition can influence the outcome of mate choice at three stages of the mating process: 1) detection, 2) evaluation, and 3) choice of mates (Wong and Candolin 2005). First, competition can affect male mating success by influencing which individuals are assessed by females (mate detection). This happens, for example, when male-male competition determines which males get access to territories, or the quality of the territories acquired by males. In many lekking species, the position of the territory on the lek has been reported to have an influence in male mating success. In White-bearded Manakins (*Manacus manacus*, Shorey 2002), Sharp-tailed Grouse (*Tympanuchus phasianellus*, Gratson *et al.* 1991), Great Snipes (*Gallinago media*, Höglund and

Lundberg 1987), Topi antelope (*Damaliscus lunatus*, Bro-Jørgensen 2002, Bro-Jørgensen and Durant 2003), and Marine Iguanas (*Amblyrhynchus cristatus*, Partecke *et al.* 2002), males with more central territories have been found to gain relatively higher mating success. Moreover, territory tenure has been suggested as a prerequisite of male mating success for some lekking species (e.g., Semple *et al.* 2001, Loyau *et al.* 2005, DuVal and Kempenaers 2008, Ryder *et al.* 2008). Thus, it is believed that male-male interactions can affect male success through an effect on whether and where males obtain territories.

Second, competition can affect mate choice by influencing the assessment of prospective mates. In this case, dominant males disrupt female choice by interrupting the courtship of subordinate males (McGhee *et al.* 2007) and, thus place a limit on the female's ability to assess candidate mates (Wong and Candolin 2005). Courtship disruptions have been commonly reported in lekking species (e.g., Foster 1981, Apollonio *et al.* 1992, Partecke *et al.* 2002). For example, Trail (1985) reported that 32% of all Guianan Cock-of-the-Rock (*Rupicola rupicola*) matings were terminated by courtship disruptions, and that females redirected their mating interactions toward disruptive males.

Lastly, competition can affect male mating success by influencing the ability of females to choose mates, such as in *Chiroxiphia* manakins. In these species, adult males typically form teams to perform cooperative displays to attract females. There is a strong dominance hierarchy among males (established and maintained by male-male social interactions, McDonald 2007) and only the dominant male ("alpha") has access to copulations. So, pre-established dominance among males limit the opportunity of females to select specific mates within dancing teams (McDonald 1989a, DuVal 2007a,b).

Although male-male competition is important for some lekking species, the common finding that male mating success is related to a variety of male behaviors and morphologies (see Fiske *et al.* 1998) has led to the predominant view that differences in male mating success are primarily influenced by female preferences (Höglund and Alatalo 1995). In lekking species, males are thought to provide females with no resources except their gametes. Thus, females are believed to base mate selection mostly on male characteristics and to follow strategies that maximize their own reproductive interests. There are three non-exclusive ways by which females can gain benefits from mate choice: (1) females can receive direct benefits by choosing males that will enhance the number of offspring produced (e.g., choose males with better sperm quality, Locatello *et al.* 2006), or they can receive indirect fitness benefits by (2) enhancing offspring attractiveness (Fisherian process, e.g., Jones *et al.* 1998) or (3) obtaining viability-enhancing genes ("good genes") for their offspring (Höglund and Alatalo 1995, Reynolds & Gross 1992, von Schantz *et al.* 1997, Richardson *et al.* 2005, Byers and Waits 2006, but see Kotiaho and Puurtinen 2007). In all cases, females assess and compare males using indicators (cues) that may be expressed as morphological or behavioral traits.

Certain behavioral traits may serve as quality indicators because they either demand high levels of energy expenditure, high levels of experience, or because they interfere with other vital activities such as foraging. Theories of sexual selection have converged on the idea that "honest" signals of quality should be costly to produce and maintain (Zahavi 1975, Andersson 1994). In this sense, if only high quality males can excel in such traits, then, they can provide females with accurate information about the signaler's relative condition (Rowe and Houle 1996, Jennions *et al.* 2001), experience (e.g., Trainer *et al.* 2002, Forsman and Hagman 2006), genetic quality (see references above), freedom from disease (e.g., Dale *et al.* 2001) and competitive ability (Zahavi 1975). Thus, females may be selected to pay attention to these signals, and male performance may have a strong influence on male mating success. Moreover, females may use a combination of cues for mate selection, rather than relying on simply one cue (Iwasa and Pomiankowski 1994, Candolin 2003). Having a superior performance in a larger set of "selected" cues (Møller and Pomiankowski 1993) may produce a greater challenge (Rowe and Houle 1996); consequently, males that achieve overall better performance may be more successful than those that excel in only one behavior.

The objective of this study was to identify behavioral and spatial variables that affect male mating success (measured as female visitation and copulation success) for the White-crowned Manakin (*Pipra pipra*). In particular, we were interested in examining the relative contribution of male-male competition and female mate choice on mating success of territory owners. White-crowned Manakins are a good system to study female mate choice and male mating success because: (1) leks are stable across time and territorial males can be followed throughout and across seasons; (2) leks are typically located in open (not dense) habitats where males are easily observed; (3) song repertoire is simple and consist of only one advertisement call and one whistle call; and (4) males have a relatively simple courtship display (for more information see study species). We hypothesize that male traits, such as number of courtship disruptions, male-male coordinate interactions (connectivity is presumed to increase social status in other species of manakins, Ryder *et al.* 2008), and spatial characteristics of males' territories influence mating success as a result of male-male competition. Further, we predict that males that engage in more male-male coordinated interactions, have fewer courtship disruptions, and defend larger and more central territories, would have higher mating success. Second, we hypothesize that male traits such as territory attendance, vocalization rate and display intensity (measured as number of displays and time spent displaying), act as honest signals of male quality during female mate choice and influence male mating success. We predict that males with higher attendance, vocalization rate and display intensity have higher mating success. Using selection gradient analysis (Lande and Arnold 1983), we identify traits that appear to influence male mating success and, thus, provide insight on the relative importance of male-male competition and female mate choice in influencing reproductive variance in White-crowned Manakin males.

METHODS

Study area

The study was carried out in Tiputini Biodiversity Station (TBS), Orellana province, eastern Ecuador (~0º 38' S, 76º 08' W). TBS includes 650 hectares of continuous lowland rainforest and is located within the 1.5 million hectare Yasuní Biosphere Reserve. TBS has approximately 30 km of trails and two 100 hectare plots. We focused our study within and adjacent to one of these 100 hectare plots (i.e., Harpia plot, for further description see Loiselle *et al.* 2007).

Study species

The White-crowned Manakin (*Pipra pipra*) is a common species at TBS and inhabits terra firme forest. They form exploded leks in which males are situated within hearing distance of each other (Snow 1961). They are sexually dimorphic. Males are black with white crowns and napes, and females are olive-green with blue-grey heads (del Hoyo *et al.* 2004). Males maintain year-round display territories and spend up to 88% of the day within their territories (Théry 1992). They have one advertisement call and one whistle call. Advertisement calls are used for territorial defense and female attraction (Castro-Astor *et al.* 2007). Whistle calls are typically used during male-male “agonistic” interactions (male-male ritualized displays) at the boundaries of territories and during female courtship displays. Males perform courtship displays individually and frequently vocalize (whistle calls and soft version of advertisement call) while dancing. The courtship display consists of forward and back flights among several horizontal display perches and slow butterfly-flights (deep and slow wing beats) around females following them from perch to perch (Snow 1961). Males frequently practice courtship displays in the absence of females (also observed in other manakin species, e.g., Shorey 2002, DuVal 2007b).

Data collection

We conducted focal observations of 37 territorial males at four leks from January - March 2005 and from December 2005 - March 2006, corresponding to the main breeding season of the region. All territorial males on focal leks were captured using mist nests and were marked with uniquely numbered aluminum and unique combination of color leg bands (for details see chapter 1). The sample unit was a 2-hour focal observation period. Observations were made daily during the peak of activity for White-crowned Manakins: 7:30-9:30 h, 12:00-14:00 h, and 14:30-16:30 h. Each male was observed for a minimum of 12 hours; mean observation time was 15 hours per male during 2005, and 27 hours per male during 2006. The order of observations was proportionally distributed (date and time of day) across males and when possible, simultaneous observations took place at two or more leks. During behavioral observations we recorded:

1) Territory attendance: average number of minutes a male was seen in his territory during the 2 hour observation period (unit: minutes / 2 hours).
2) Vocalization rate: average number of advertisement calls during the 2 hour observation period (unit: number of advertisement calls / 2 hours).
3) Number of displays: average number of courtship displays a male performed during the 2 hour observation period (unit: number of displays / 2 hours).
4) Time spent displaying: average time (seconds) a male spent performing courtship displays during the 2 hour observation period (unit: display time / 2 hours).
5) Number of male-male coordinate interactions: average number of male-male ritualized agonistic encounters in the absence of females during the 2-hour observation period (unit: number of coordinated interactions / 2 hours). Male-male coordinated interactions have different display elements than displays performed individually for females and occur during boundary encounters between territorial males. These interactions may act as a mechanism to establish and maintain dominance hierarchies among males (Tori, unpublished data).
6) Number of aggressive interactions during courtship display: average number of courtship disruptions in the 2 hour observation period standardized by the number of displays performed by each male (unit: number of aggressive interactions during courtship display/ number of displays/ 2 hours).
7) Female visitation rate: average number of female visits to a male during the 2 hour observation period (unit: number of female visits / 2 hours).

8) Copulations: copulations attained by territorial males during the observation. Males tend to copulate multiple times with females during the same visit. As these copulations were not independent from each other, we considered all copulations within a single visit as one. Copulations were not frequently observed, so we coded this variable in a binary fashion (1 = males that copulated at least once, 0 = males that were not observed to copulate during behavioral observations).

Because female visitation may affect display intensity (Gibson and Bradbury 1985), we also calculated the number of displays and the time spent displaying by males in the absence of females (i.e., practice displays, see discussion). During behavioral observations we marked perches that males used to perform advertisement calls. We mapped and geo-referenced these perches and used the information to calculate:

8) Territory area: area that territorial males use regularly to advertise and display (unit: m^2). Territory sizes were calculated building a minimum convex polygon around advertisement call perches using the Animal Movement Extension in ArcView v.3 (Hooge and Eichenlaub 1997, for further details see chapter 1).

9) Territory centrality: distance in meters from the center of a male's territory to the geometric center of the lek. To calculate this variable, we used the centroid polygon script in ARCGIS v. 9.1 (ESRI 2005).

Statistical analysis

As female visitation to male territories did not differ across the four leks (2005: Kruskal-Wallis X_i^2 = 2.180, df = 3, p = 0.538; 2006: Kruskal-Wallis X_i^2 = 1.349, df = 3, p = 0.717), data were pooled giving a sample size of 30 territorial males for 2005 and 31 territorial males for 2006. In most analyses, we examined data from the two seasons separately to avoid losing resolution due to potential temporal changes in male status or in female mate choice criteria. However, for analyses using copulation status (whether a male was seen copulating or not), we pooled the two seasons to increase sample sizes. The number of observed copulations (n = 12) was much lower than the number of female visits (n = 98). Thus, to see if female visitation could act as a surrogate of male mating success, we used a logistic regression to determine the predictive relationship between female visits and copulation status, and a Mann-Whitney test to examine differences in female visitation between males with and without copulations. For these analyses, males that had at least one copulation were coded as 1, males not observed to copulate were coded as 0 and only males with at least one female visit were included in the analyses. The number of female visits was found to be a good predictor of copulation status (ß = 10.1 ± 4.34, Wald's $\chi^2 = 5.435$, $p = 0.02$, in 74% of the cases copulation status was predicted correctly by female visitation). Further, we found that the average number of female visits to males that copulated at least once (Mean ± SE: 0.25±0.03 per 2 h observation period) was significantly higher than the average number of female visits to males that were not observed copulating (Mean ± SE: 0.15 ± 0.02, Mann-Whitney U = 30.5, $p < 0.003$). Moreover, this relationship holds when we compared female visitation and paternity using microsatellites (i.e., males that sired offspring had higher number of female visits than males that did not sire offspring; for molecular paternity see chapter 1). So, female visitation appears to be a reliable indicator of male reproductive output and hereafter we use female visits as a surrogate of male mating success.

The relationship between male behavioral traits and spatial characteristics of male territories (hereafter called spatial traits) with male mating success were analyzed separately. Many of the behavioral traits were correlated (correlation coefficient range: 0.4–0.8, $p<0.05$), thus we used principal component analysis (data unrotated) to reduce the number of variables. We use six behavioral variables (territory attendance, vocalization rate, time spent displaying, number of displays, number of aggressive interactions during courtship displays, number of male-male coordinated interactions) and extracted the principal components (PC) that had eigenvalues greater than one. Next, we ran directional selection gradient analyses (Lande and Arnold 1983) between female visitation rate and the composite behavioral variables (i.e., principal components) for each year. Selection gradients measure the partial effect of each trait on fitness controlling for correlations between the focal trait and other traits included in the model (multiple regression analysis). Consequently, selection gradients provide information about male traits that females may use during mate choice (Anderson 1989). For these analyses, PC components were standardized by their standard deviations, and female visitation was standardized by the population mean (relative male mating success). Additionally, we conducted selection gradient analyses to examine the relative contribution of individual traits (as PC are often hard to interpret) on male mating success. As sample sizes were not large enough for multivariate analyses with all cues simultaneously ($n = 30$ in 2005; $n = 31$ in 2006), we used only three variables suggested as important by the PC directional selection gradient analyses: (1) time spent displaying, (2) territory attendance, and (3) vocalization rate. High interdependence of explanatory variables may preclude finding biologically meaningful drivers of male mating success in the selection gradient analysis (Mitchell-Olds and Shaw 1987). Thus, the latter analyses were just exploratory and aimed to examine only the relative importance of each trait with respect to male mating success. Lastly, we used directional selection gradient analyses to test the relationship between male mating success (measured as female visitation) and male spatial traits (territory size and position). Spatial traits were also standardized by their standard deviation. Statistical analyses were conducted using SPSS v 13.0.

A caveat of selection gradient analyses is that direct selection on traits can only be unequivocally tested when all relevant traits are included in the model (Lande and Arnold 1983), since some traits may emerge as important only because of their correlation with other traits that were not quantified. We did not measure all behavioral traits; for example, specific components of male display (e.g., butterfly displays) or quantitative measures of advertisement song and whistles were not measured. Thus, we can not discard the possibility that females use these traits as honest signals of male quality. Our results, however, may serve to design future experiments to test the effects of traits suggested to be important as female cues in a more direct manner.

RESULTS

Variation in male mating success

A total of 98 female visits and 12 copulations were recorded at the four leks. Males varied in their relative female visitation in both seasons (Figure 1). During 2005 and 2006, 19 of 30 (63%) and 22 of 31 (71%) males sampled received at least one female visit, respectively. The remaining 11 (2005) and 9 (2006) territorial males received no female visits. The distribution of copulations (pooled data) was also skewed: three males received two copulations, six males received one copulation and 16 males were not observed to copulate with females.

Behavioral data

Male variation in all behavioral traits was evident (Table 1). When we combined behavioral variables using Principal Component Analysis, we obtained two principal components in 2005 and three in 2006 that had eigenvalues greater than one (Table 2). For 2005, number of displays, time spent displaying, territory attendance and vocalization rate, all scored high on PC1 (48.5% of variance explained). Therefore, we interpret PC1 as a measure of male attendance and advertisement effort. Here, vocalizations are viewed as long range attractants to females and displays as short range attractants. PC2 (20.7% of variance) loaded mainly and positively with coordinated and aggressive interactions among males. Thus, PC2 can be viewed as an index of male-male interactions and dominance. Interpretation of PC axes in 2006 differed slightly. For the first principal component, number of displays, time spent displaying and vocalization rate were positively correlated and had the highest loadings (PC1 explained 40.2% of variance). Thus, PC1 for 2006 can be interpreted as having components of both long and short-range male advertisement. Territory attendance was the main contributor of PC2. PC3 is largely a measure of male dominance status, as aggressive interactions during displays was the most important variable contributing to this axis (Table 2).

In 2005, PC1 (attendance/advertisement index) contributed significantly to the selection gradient model explaining male mating success ($\beta' = 0.72 \pm 0.141$, $p < 0.0001$, Table 3); this model explained 49% of the variance and was highly significant ($p < 0.001$, Table 3). In 2006, PC1 (~ advertisement index) and PC2 (~ attendance index) had significant directional selection gradient coefficients; the overall model explained 30.4% of the variance in relative male mating success ($p = 0.019$; Table 3). Results from 2005 and 2006 were consistent, and identified similar male traits as key characters under selection (Figure 2). These results suggest that sexual selection favors males with higher advertisement (in the form of calls and courtship displays) and territory attendance across seasons.

Using individual behavioral variables directly in the selection gradient models, we found that in 2005, time spent displaying had the highest response ($\beta' = 0 .661$) to selection, followed by vocalization rate ($\beta' = 0.124$), and territory attendance ($\beta' = 0.032$, model: $R^2 = 0.512$, $n = 30$, $p < 0.001$). Conversely, in 2006 territory attendance ($\beta'=0.349$) had the highest response followed by time spent displaying ($\beta' = 0.229$) and vocalization rate ($\beta' = 0.093$, model: $R^2 = 0.339$, $n = 31$, $p = 0.010$, Table 4).

Spatial data

Territories were generally stable across years, as most males were "faithful" to their territories and only a few new territories were formed on the periphery of the leks in 2006 (Tori, unpublished data). Mean territory area and distance to the center of the lek were 486 ± 40 m^2 (± standard error) and 71 ± 6 m in 2005; and 654 ± 40 m^2 and 74 ± 7 m in 2006 (respectively). Territory area and centrality did not contribute significantly to selection gradient models either in 2005 (model: $p = 0.291$, $R^2 = 0.09$, $n = 30$; Area: $\beta' = 0.13$, $t = 0.67$, $p = 0.509$; Centrality: $\beta' = 0.26$, $t = 1.37$, $p = 0.183$), or 2006 (model: $p = 0.641$, $R^2 < 0.01$, $n = 31$; Area: $\beta' = -0.08$, $t = -0.51$, $p = 0.613$; Centrality: $\beta' = -0.14$, $t = -0.89$, $p = 0.384$). Consequently, territory characteristics do not appear to have an influence on male mating success in any year.

DISCUSSION

Sexual selection in lekking species has received considerable attention during the last decades. To date, however, there is still considerable discussion over the relative importance of male-male competition and female mate choice on male mating success for many lekking species (Höglund and Alatalo 1995). Male competitive abilities and female mate choice can influence male reproductive success simultaneously (Stapley 2008), and their relative importance (within and across species) are the outcome of differences in selection pressures (e.g., sex ratios, densities, predation, food resources) and the spatial and social organization of leks. In manakin species, both of these mechanisms have been suggested to play an important role in male mating success (e.g., Beehler and Foster 1988, McDonald 1989a, 1989b, Shorey 2002,). Our results are consistent with the hypothesis that female mate choice is the main mechanism that affects male mating success in territorial White-crowned Manakins. Our data suggest that female visitation is a reliable indicator of male mating success, and that it is strongly associated with measures of activity and display behavior of males, but not associated to behavioral traits associated with male dominance (e.g., courtship disruption or agonistic interactions). In concordance with this finding, we found that spatial characteristics (presumed to be the result of male-male competition), were not related to male mating success. Thus, our results provide evidence that female mate choice plays an important role for the White-crowned Manakin, and that male behavioral characteristics may be selected during mate choice.

Behavioral correlates of male mating success

Our results support the hypothesis that sexual selection consistently acts to increase male advertisement behaviors and attendance, and that (1) vocalization rate, (2) territory attendance, (3) number of displays and (4) time spent displaying may be important cues used by females during mate choice. Selection gradients for the principal components show that inter-specific signals used to attract females (e.g., displays) had greater effect on male mating success than proportional changes in traits used during male-male competition (e.g., male aggression). In 2005, selection gradients indicated that an increase of one standard deviation of the "attendance–advertisement composite variable" (PC1) would generate 71% increase in relative male mating success, compared to only a 5% increase with changes of one standard deviation in male-male interactions traits (PC2). Similarly, in 2006 an increase of one standard deviation of the "advertisement composite variable" (PC1) and "attendance composite variable" (PC2) predict an increase of 37% and 29% in relative male mating success, respectively; while a similar standard deviation change in the PC related to aggression would only increase relative male mating success by 0.5%. Thus, our results concur with previous studies that found that behavioral traits, such as vocalizations (e.g., Gibson and Bradbury 1985, McDonald 1989a, Gerhardt *et al.* 2000), display activity (e.g., Gibson and Bradbury 1985, Höglund and Lundberg 1987, Anderson 1989, McDonald 1989a, Pruett-Jones and Pruett-Jones 1990, Gratson 1993, Whittier *et al.* 1994) and lek attendance (e.g., Cherry 1993, Fiske *et al.* 1994, Friedl and Klump 2005), are likely important determinants of male mating success.

A caveat of this analysis, however, is that we can not distinguish the individual contribution of each behavioral trait on female mate choice. Variables found to be important for male mating success were positively correlated, and composite variables may mask the relative importance of one or a few traits prominent on the PC. So, the question remains whether females use attendance, vocalization and display individually or in combination as an index of male quality during female mate choice. Experimental studies that control for certain male characteristics while testing for others are necessary to answer this question. In this study we did not perform these experiments.

In an effort to obtain a preliminary sense of the relative contribution of each of these traits, we ran selection gradient analyses between each of these variables and relative mating success. We found that the influence of male traits on male mating success varied across years. In 2005, the trait that had the highest effect on male mating success was display rate, while in 2006 the most important trait identified was territory attendance. These results suggest that the strength and direction of sexual selection pressures may differ across years, leading to temporal variability in the weight given to cues used for female mate choice.

Temporal variability in mate choice decisions has been reported for other species (e.g., Lifjeld and Slagvold 1988, Reid and Weatherhead 1990, Gibson and Bradbury 1991, Fiske *et al.* 1994, Forsgren 1997, Friedl 2006, Chaine and Lyon 2008). Different environmental contexts can affect condition-dependent traits (Hill 1995), and the strength and direction of female mate choice preferences (Jennions and Petrie 1997, Hingle *et al.* 2001, Qvarnström 2001, Candolin 2003, Kodric-Brown and Nicoletto 2005, Fisher and Rosenthal 2006, Friedl 2006). On one hand, environmental conditions (e.g., availability of food resources, weather) can affect the expression of sexually selected traits (Siefferman and Hill 2005). On the other hand, environmental conditions can influence female mate decisions by affecting female benefits (Jia and Greenfield 1997, Qvarnstrom 2001, Welch 2003, Chaine and Lyon 2008), search costs and the accuracy of signal detection and discrimination by females (Jennions and Petrie 1997). Further, temporal variability in sexual selection regimens may have important implications for the maintenance of phenotypic variation in male traits in lekking species, and may be a solution for the lek paradox (Taylor and Williams 1982, Kirkpatrick and Ryan 1991, Fiske *et al.* 1994).

In systems in which female mate choice takes place, females are believed to rely on trait variation to discriminate the relative quality among males. It has been suggested that multiple cues may facilitate mate choice by allowing females to base mate decisions on the trait (or traits) that show the largest variation among males (Reid and Weatherhead 1990). Giving higher weight to cues with the largest variation may reduce mate choice costs, as differences among males may be more difficult to detect when variability is low (Jennions and Petrie 1997). Moreover, traits with higher variation may be more reliable indicators of male quality because only individuals in good condition may be able to excel in the expression of the trait (costs of traits are higher for males in poor condition, Kotiaho *et al.* 2001). If females give higher weight to cues that show the largest variation, and the expression of cues is affected by environmental conditions, then the relative importance of mating criteria could vary over time. Support for this argument has been found in a few species (Fiske *et al.* 1994, Reid and Weatherhead 1990, Forsgren 1997). For example, female Ipswich Sparrows (*Passerculus sandwichensis*) preferentially use male cues with higher variability during mate choice (Reid and Weatherhead 1990). In warm years, when males sang more and there was low variability in song rate, females preferentially mated with males with larger territories. Conversely, in cooler years, when there was a larger variability in song rate, females preferentially mated with males with higher song rates. Further, in Great Snipe, the relative importance of cues selected by females varied across years, and females appeared to prefer traits with high variance among males as mate choice cues (Fiske *et al.* 1994). In concordance with these studies, we found that mate-choice criteria matched the relative variability of male traits in each year. In 2005, the year in which display had the larger contribution in mate choice, males showed higher variability in the time they spent displaying (CV 2005 = 0.82) than in 2006 (CV 2006 = 0.55). Conversely, in 2006, when territory attendance had higher influence on male mating success, males showed higher variability in the time spent in their territories than in 2005 (CV 2005 = 0.19, CV 2006 = 0.35). We do not believe these results are a statistical artifact generated by the difficulty of finding an association between male mating success and variables with little variability. We found that traits with the highest variation among males (male-male interaction traits) had little effect on male mating success. Thus, these results suggest that cue variability may be important during female mate choice. It is unclear, however, whether variability is important because it contributes to the reliability of the signal or because more variable traits are easier to assess for females. Clearly more research is needed to confirm this pattern and to understand the mechanisms that link trait variability and female mate choice in species that use multiple criteria during mate selection.

Territory attendance

Attendance has also been reported as an important correlate in male mating success in other lekking species (e.g., Gibson and Bradbury 1985, Höglund and Lundberg 1987, Anderson 1989, Appollonio *et al.* 1989, Hill 1991, Cherry 1993, Fiske *et al.* 1994). The importance of attendance in male mating success, however, is hard to interpret, because there is more than one reason why males that stay longer in their territories can achieve greater success. First, attendance has an intuitive relationship with mating success, because as males spend more time at their territories, they increase their chances to participate in mating activities (passive female choice, Mackenzie *et al.* 1995). Second, successful males may be more willing to invest energy in reproduction and stay longer in their territories than unsuccessful males (attendance is not a cause but a consequence of success, Fiske *et al.* 1994). In this study, we have circumstantial evidence that suggests that this is not the case. We found that two males (GRPU 2005 and BWR 2006) with high female visitation rate at the beginning of the breeding season abandoned their territories early, suggesting that high lek attendance is not a consequence of high mating outcome (GRPU returned to his territory in 2006). Finally, females may use territory attendance as an honest signal of male quality (Friedl and Klump 2005). Attendance can interfere with foraging activities, and, thus it may reflect the relative condition and stamina of males (ability to meet energetic demands imposed by displaying without feeding, Pruett-Jones 1988, Vehrencamp *et al.* 1989). Using attendance as an honest indicator of male quality, however, implies that females would need to visit males more than once and that they will use memory of previous visits for their mating decisions (Hill 1991). Field experiments (not included in this study) are required to unambiguously test the mechanisms by which attendance affects male mating success in this system.

Advertisement displays

Male courtship displays are specifically designed to attract female's attention and gain access to copulations, and have also been recognized as important mate choice cues for many other lekking species. For example, in Great Snipe (Höglund and Lundberg 1987), Sage Grouse (*Centrocercus urophasianus*, Gibson and Bradbury 1985, 1991), Woodhouse Toad (*Bufo woodhousei*, Sullivan 1987), Fruit Fly (*Drosophila melanogaster*, Talyn and Dowse 2004), and Mediterranean Fruit Fly (*Ceratitis capitata*, Whittier *et al.* 1994) display rates play an important role in male mating success. Moreover display duration has been suggested as an important mate choice cue in Sharp-tailed Grouse (Gratson 1993), Long-tailed Manakin (*Chiroxiphia linearis*, duration of butterfly display, McDonald 1989a) and Gray Tree Frog (*Hyla versicolor,* call duration Klump and Gerhardt 1987). Because displays are complex and involve high levels of energy expenditure (Taigen and Wells 1985, Vehrencamp *et al.* 1989, Höglund *et al.* 1992, Kålås *et al.* 1997) and experience (Trainer *et al.* 2002), females may be able to assess display performance as an honest signal of male quality. Alternatively, preferences could also originate simply because males that display more are more conspicuous. In accordance with both of these hypotheses, we found a positive relationship between male display and male mating success for the White-crowned Manakin. Males that display for longer periods of time tended to have higher mating success than less active males. It has been suggested, however, that correlations between mating success and display can be the result of males responding to the proximity of females (reverse causation, Gibson and

Bradbury 1985). To account for this argument, we re-ran the analyses using only the information from displays in the absence of females (i.e., practice displays). We obtained qualitatively similar results (PC selection gradient analyses, 2005 model: $R^2 = 0.388$, $p = 0.001$, 2006 model: $R^2 = 0.269$, $p = 0.035$). This provides strong evidence against such reverse causation. Male courtship displays in White-crowned Manakins, however, are mostly quiet (males only whistle and sometimes perform a soft version of the advertisement call). So, it is highly unlikely that females use courtship displays as a long-distance mate attraction cue. Males may be using advertisement calls as a long-range signal to attract females to their territories. Once females have arrived in the territories, females may use other cues such as display performance or attendance, to decide which males to mate with. This division of sexual selection signals into long and short distance cues has also been suggested for other manakin species (McDonald 1989a).

Spatial correlates of male mating success

In many lekking species, females have been reported to preferentially mate with males with more central territories. Although the mechanisms underlying this relationship are still not clear, it has been proposed (1) that territory characteristics can be the outcome of male-male interactions (e.g., aggression), and that females use these cues during mate choice to obtain males that are good competitors (high quality males, Höglund and Alatalo 1995, Isvaran and Jhala 2000, Höglund *et al.* 2002); or (2) that females prefer to mate with "hotshot" males that end up in central positions because unattractive males settle around their territories to intercept females (Beehler and Foster 1988, Höglund and Robertson 1990). In this study, we did not find any evidence that spatial territory cues (i.e., territory size or location) affected male mating success. The lack of relationship between these cues and male mating success does not necessarily eliminate the possibility that spatial components influence male success. It has been suggested that male-male interactions may play a critical role for territory acquisition (see chapter 1, Ryder *et al*. 2008) and social status (McDonald 2007) in manakins. Moreover, territoriality may be a prerequisite for male reproductive success (DuVal and Kempenaers 2008, Ryder *et al*. 2008), thus territory status can act as a filter that dictates which males are assessed by females, potentially having an influence in male reproductive output (Wong and Candolin 2005). In this study, we only examined correlates of male mating success among territory holders. Studies that examine the relationship of male-male interactions and territory acquisition during territory establishment may be important to elucidate if male-male competition affects male reproductive potential prior to female visitation at leks.

Conclusion

We found that male mating success in the White-crowned Manakin appear to be mediated by a combination of male behavioral characters. Our data suggest that female mate choice (or at least visitation rate) is more related to individual male differences in display performance, vocalization rate and attendance, than to spatial or male-male interaction traits. The relative importance of these traits however changes over time, and females appear to emphasize the use of mate choice cues that are more variable among males.

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Table 1. White-crowned Manakin behavioral traits (mean, standard deviation and coefficient of variation) measured during male focal behavioral observations for 2005 (n = 30 males) and 2006 (n = 31 males) seasons. All variables were standardized by the number of 2-hour behavioral observations performed.

	2005			2006		
Behavioral trait	Mean	SD	CV	Mean	SD	CV
Territory attendance (min / 2 hrs)	93.3	17.7	0.19	79.1	27.9	0.35
Vocalization rate (calls / 2 hrs)	276.6	114.9	0.42	291.9	123.9	0.42
Time spent displaying (sec / 2 hrs)	161.7	132.9	0.82	199.7	108.9	0.55
Number of displays (Disp. / 2 hrs)	1.0	0.7	0.70	1.7	0.8	0.46
Number of aggressive interactions during display (Agg. / 2 hrs)	0.1	0.2	1.63	0.1	0.1	1.17
Number of male-male coordinate interactions (Coord. / 2 hrs)	0.3	0.4	1.32	0.3	0.2	0.67

Table 2. Component loadings of six behavioral variables as quantified by Principal Component Analysis for 2005 (n = 30) and 2006 (n = 31).

	2005		2006		
Trait	PC1	PC2	PC1	PC2	PC3
Territory attendance	0.873	-0.211	0.429	0.757	-0.325
Vocalization rate	0.809	-0.128	0.767	0.516	-0.107
Time spent displaying	0.842	0.025	0.820	-0.330	0.122
Number of aggressive interactions during display	0.185	0.741	0.082	0.251	0.897
Number of displays	0.856	0.026	0.888	-0.261	0.191
Number of male-male coordinated interactions	0.134	0.795	-0.416	0.573	0.290
Eigenvalue	2.911	1.244	2.413	1.408	1.056
% of variance explained	48.520	20.733	40.219	23.463	17.602

Table 3. Directional selection gradients of composite behavioral variables for White-crowned Manakin in 2005 (n = 30 males, model: $R^2 = 0.491$, $p < 0.0001$) and 2006 (n = 31 males, model: $R^2 = 0.304$, $p = 0.019$). In 2005, PC1 and PC2 represented an index of attendance-advertisement effort and male interactions-dominance (respectively). In 2006, PC1 represented an index of advertisement effort, PC2 reflected territory attendance and PC3 reflected male dominance. Selection gradients are given in units of phenotypic standard deviations.

	2005				2006		
Trait	β’	t	P-value	Trait	β’	t	P-value
PC 1	0.72	5.09	<0.001	PC 1	0.37	2.7	0.012
PC 2	0.05	0.33	0.746	PC 2	0.29	2.13	0.043
				PC 3	-0.01	-0.04	0.972

Table 4. Directional selection gradients for three behavioral variables: territory attendance, vocalization rate and time spent displaying in 2005 (n = 30 males, model: $R^2 = 0.512$, $p < 0.0001$) and 2006 (n = 31 males, model: $R^2 = 0.339$, $p = 0.010$).

	2005			2006		
Trait	β'	t	P-value	β'	t	P-value
Attendance	0.03	0.16	0.874	0.35	2.07	0.048
Vocalization rate	0.12	0.69	0.494	0.09	0.54	0.595
Time spent displaying	0.66	4.02	<0.001	0.23	1.66	0.108

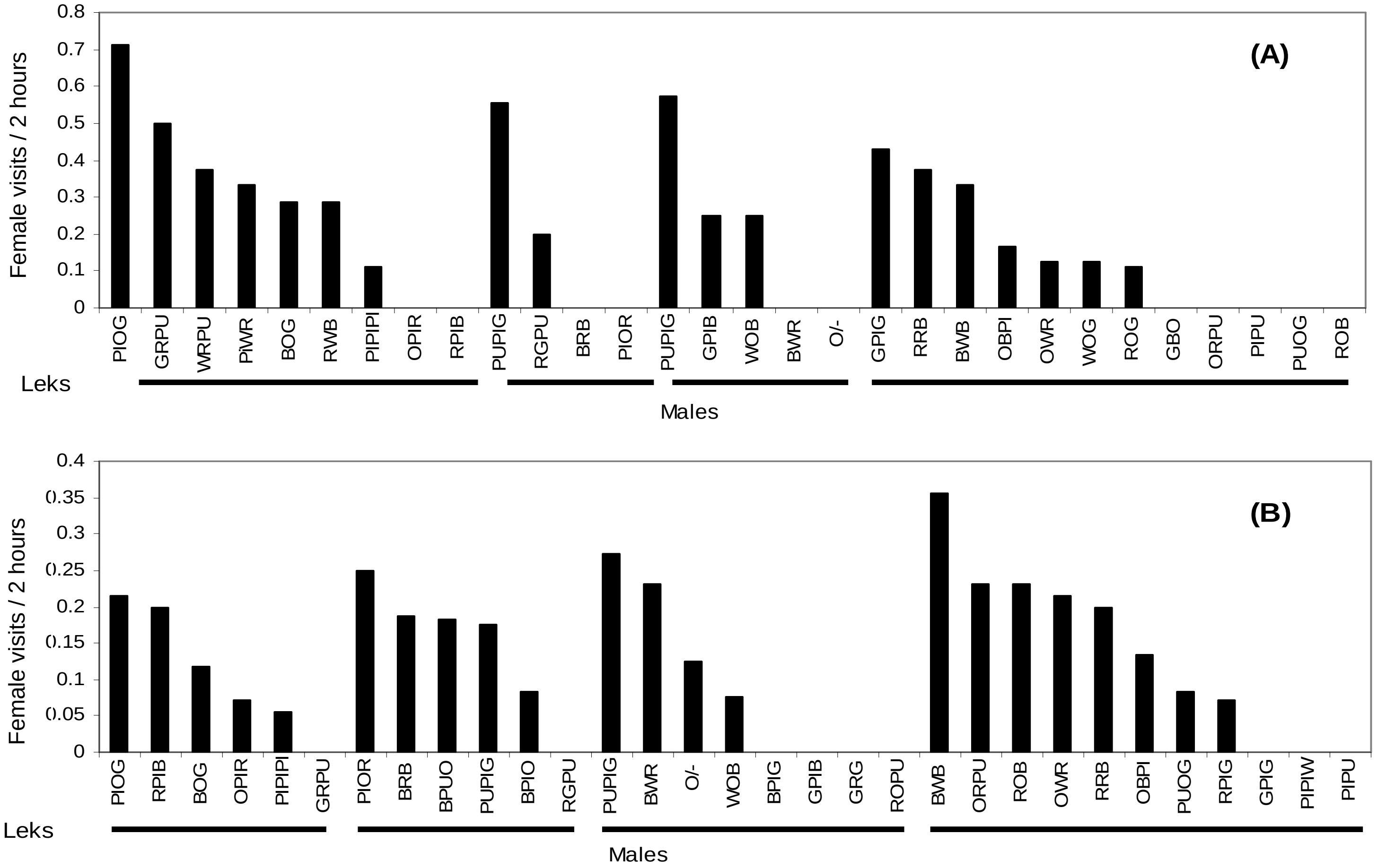

Figure 1. Distribution of female visits (surrogate of male mating success) among White-crowned Manakin territorial males in 2005 (A) and 2006 (B) for 4 focal leks at Tiputini Biodiversity Station. Males are coded following their color band combination and lek membership is indicated by black bars under male codes.

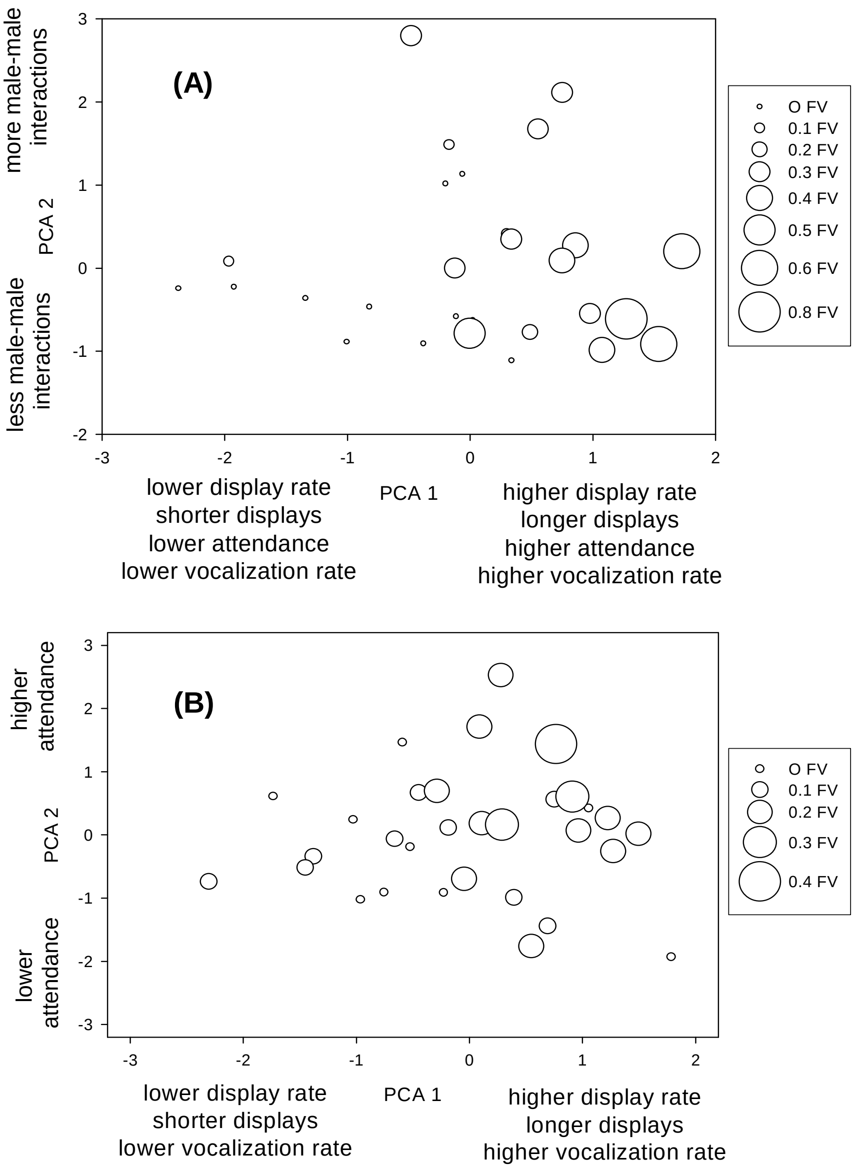

Figure 2. Relationship between male mating success (measured as number of female visits) and principal components 1 and 2 of behavioral traits in 2005 (A, n = 30 males) and 2006 (B, n = 31 males). The sizes of the dots are proportional to the average number of female visits (FV) received by males per 2 hours observation (see figure legend). In both years males that displayed more often, for longer periods of time, have higher vocalization rate and higher territory attendance received more female visits. Note the different contribution of territory attendance to PC1 and PC2 in each year.