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# Mate choice for genetic quality: a test of the heterozygosity and compatibility hypotheses in a lek-breeding bird

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# Mate Choice for Genetic Quality: A Test of the Heterozygosity and Compatibility Hypotheses in a Lek-Breeding Bird

Post Print

**ABSTRACT:** Understanding female mate choice in nonresource-based mating systems, where females appear to express strong preferences despite receiving no direct benefits from mate choice, remain a challenge to behavioral ecology. Brown (Brown JL. 1997. A theory of mate choice based on heterozygosity. *Behav Ecol.* 8:60–65) proposed the “good-genes-as-heterozygosity” hypothesis, which sought to establish the genetic nature of male quality. Despite recent attention, few studies have examined how multiple components of genetic quality influence mate choice in a highly promiscuous mating system such as that of the lekking wire-tailed manakin (*Pipra filicauda*, Aves: Pipridae). Based on a 4-year study in the Amazon basin of Ecuador we ask whether female wire-tailed manakins use heterozygosity and/or dissimilarity (relatedness) as mate-choice criteria, and if heterozygosity influences the ontogeny of male social status. Our data show that females select more heterozygous males as their partners. Females do not, however, appear to select mates based on their genetic dissimilarity as mated pairs were more related than expected under random mating. Heterozygosity of territorial males was correlated with 2 male morphological traits (wing and tarsus length), which suggests that females may assess male genetic quality via phenotypic correlates. In addition, our data show that heterozygosity was related to male social rise (territory acquisition) and suggests that heterozygosity may act as a filter of male reproductive potential. Lastly, we find no support for indirect benefits (i.e., increased offspring genetic quality) obtained via female choice for heterozygous males

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Female preferences for exaggerated male traits and the manner by which sexual selection acts on mate choice have been of interest since the time of Darwin and remain a central theme in evolutionary ecology (Andersson 1994; Neff and Pitcher 2005; Andersson and Simmons 2006). It is particularly difficult to explain adaptive mate choice when females receive no direct benefits aside from male gametes (Jennions and Petrie 1997). In nonresource-based mating systems, it has been hypothesized that male ornamental traits are condition-dependent signals that reveal the genetic characteristics of the bearer (see Weatherhead et al. 1999; Aparicio et al. 2001; Hoffman et al. 2007; Hale et al. 2009) and that these phenotypes act as honest indicators by which females assess the quality of potential mates (Andersson 1994). The “good-genes-as-heterozygosity” hypothesis sought to explain the genetic nature of male quality (Brown 1997).

The good-genes-as-heterozygosity hypothesis as proposed by Brown (1997) predicts that females should value heterozygosity in their offspring and under some conditions in their mates. Essential to the selective advantage of heterozygous offspring, however, is the underlying relationship between fitness and heterozygosity. To date, the generality of heterozygosity-fitness correlations are unclear, given that effect sizes vary widely among studies (see Britten 1996; David 1998; Hansson and Westerberg 2002; Coltman and Slate 2003). In studies that show positive effects, heterozygosity has been associated with increased disease resistance (Coltman et al. 1999; Whiteman et al. 2006; Reid et al. 2007), increased survival (Daniels and Walters 2000; Cohas et al. 2009; Mainguy et al. 2009), increased reproductive success (Kruuk et al. 2002; Slate et al. 2004), reduced expression of deleterious recessive alleles, and increased developmental stability (reviewed in Kempenaers 2007). Despite numerous positive studies others have failed to detect significant heterozygosity-fitness correlations (Houle 1989; Savolainen and Hendrick 1995). This has led to the suggestion that detecting heterozygosity-fitness relationships may depend on the extent of variance in inbreeding, which impacts genomewide variation in heterozygosity (Coltman and Slate 2003; Balloux et al. 2004). High variance in inbreeding is thought to be most common in species with highly structured populations of small effective size (e.g., island populations, breeding colonies with high longevity and philopatry, and systems with strong polygyny) (Hansson and Westerberg 2002; Balloux et al. 2004).

Several hypotheses have been proposed to explain the influence of genetic quality on mate choice where quality is defined as the contribution of alleles or a genotype to individual fitness (Kempenaers 2007). Models of genetic mate choice which are often not mutually exclusive have largely been divided into the:

- 1) good allele model, in which “specific” alleles or “general” allelic diversity increases fitness independent of genome architecture and
- 2) compatible allele model, in which alleles increase fitness in a specific genetic context or via gene–gene interactions (e.g., epistasis) (Neff and Pitcher 2005; Kempenaers 2007). In the first model, females choose males based on male phenotypic traits or increased vigor, which reflect either specific superior alleles (i.e., traditional good-genes) or general allelic diversity (i.e., heterozygosity). This model implicitly assumes that chosen traits and genetic diversity positively covary (Brown 1997). In the compatible allele model, male attractiveness largely depends on the interaction of male and female genotypes; in some cases (e.g., major histocompatibility complex), this

hypothesis also predicts that females would benefit by choosing genetically dissimilar mates (reviewed in Mays and Hill 2004; Kempenaers 2007).

Here, we focus on the heterozygosity aspect of the good alleles model and the dissimilarity aspect of the compatible alleles model. Importantly, both models could increase offspring genetic diversity, yielding indirect benefits. Yet, their contributions are not mutually exclusive because they contribute to 2 different components of genetic variance (additive and nonadditive) (Colgrave et al. 2002; Neff and Pitcher 2005). Regardless of the model, both hypotheses assume females have either the ability to assess male genetic quality using cues (e.g., morphological, behavioral, and aural) and/or the ability to assess a mate's genotype with respect to their own genotype (e.g., olfactory).

To date, many studies have documented the importance of genetic quality on mate choice (Aparicio et al. 2001; Foerster et al. 2003; Masters et al. 2003; Seddon et al. 2004; Suter et al. 2007; Garcia-Navas et al. 2009); yet, few have addressed the apparent conflict between preferences for heterozygosity and preference for compatibility (but see Colgrave et al. 2002; Hoffman et al. 2007). For example, the 2 models have mutually exclusive predictions about mate-choice concordance among females. Mate choice based on male heterozygosity produces concordant female choice for males with high heterozygosity, leading to unequal distribution of reproductive success and high reproductive skew (Neff and Pitcher 2005). Mate choice for compatibility, however, promotes more evenly distributed reproductive success among males (low skew) because each female's mating preferences will depend on her own genetic makeup. Despite differences between the 2 models of mate choice for genetic quality, recent evidence suggests females might use both cues simultaneously when choosing mates (see Hoffman et al. 2007) or alternate between the 2 cues (see Roberts and Gosling 2003; Puurtinen et al. 2005; Oh and Badyaev 2006). Ultimately, optimal female choice and the strength of preference for genetic quality are likely influenced by the variability of heterozygosity and compatibility among potential mates and the relative costs of obtaining indirect benefits (Colgrave et al. 2002; Roberts et al. 2006; Kempenaers 2007).

Nonresource-based systems such as leks provide an ideal opportunity to test the influence of genetic quality on mate choice because there are fewer confounding variables than in species that defend resources. Highly promiscuous species, with a high degree of variation in mating success and extreme sexual dimorphism, are more likely to have female mate choice driven by overall male heterozygosity (Mays and Hill 2004); yet, no studies to date have examined both components of genetic quality in a lekking species. Here, we test 2 nonmutually exclusive models of mate choice for genetic quality in a lek-breeding bird, the wire-tailed manakin (*Pipra filicauda*). We specifically ask if females choose more heterozygous males and/or more dissimilar males as mates. In addition, we examine the role of heterozygosity in territory acquisition by young males.

## MATERIALS AND METHODS

### Study area

Tiputini Biodiversity Station (TBS) is located on the north bank of the Rio Tiputini in Orellana province, Ecuador (03°38'S, 76°08'W). TBS encompasses approximately 650 hectares of tropical rainforest within the 1.68 million hectare Yasuní Biosphere Reserve, which has extremely high species diversity (Valencia et al. 2004; Blake 2007). TBS contains a complex mosaic of habitats including terra firme, swamp, riparian, and floodplain forests. TBS has more than 25 km of trails and has two 100-hectare plots (ca. 10 × 10 km each) which are gridded with permanent markers (Loiselle et al. 2007).

## Study species

The wire-tailed manakin is an understory frugivorous bird widely distributed throughout the Amazon basin of northern South America (Ridgely and Tudor 1994). Male wire-tailed manakins form exploded leks in both upland terra firme and seasonally flooded forest. Exploded leks differ from classical leks in that male territories are more widely spaced. Leks average one hectare in size (Heindl 2002) and are separated by a distance of 300 m or more (Loiselle et al. 2007). The number of territorial males per lek at TBS ranges from 4 to 12, where each male defends a roughly 40 m diameter area; territory borders are typically 10–15 m apart. The display behavior is complex and males often join in coordinated displays to attract females (Heindl 2002). Female wire-tailed manakins visit the lek only to mate, and they carry out all reproductive activities, including nest building and rearing the offspring, alone (Snow 2004).

## Field methods

Leks were located using auditory and visual cues while walking along line transects within the plots from February to April of 2002–2005 and by systematically searching the forest along the trail system from November to April of 2003–2006. Birds were captured using 2 sampling regimes: 1) systematic mist netting activities at 192 permanent net sites in January and March from 2001 to 2006 and 2) directed target netting of individuals at known leks or nests from November 2003 to April 2006. Each bird captured was marked with numbered aluminum and unique colorcombination leg bands. These methods resulted in complete sampling of adult territorial males verified by revisiting male territories within and across 4 breeding seasons. Likewise, these methods enabled the sampling of a large proportion (>75%) of nonterritorial males regularly seen visiting males during coordinated display bouts (see Ryder et al. 2008, 2009). Females were sampled via systematic netting and target netting at active nests and likely represents a random sample of the female population at our study site. A blood sample of 12.5–50  $\mu$ l was taken for paternity analysis. Blood samples were preserved in lysis buffer. Nests were located via systematic searches and by radio tracking females in breeding condition from December to April 2004–2006. Eggs at nests were replaced with plaster replicas and incubated ex-situ to avoid losing the genetic sample to nest predation.

Hatchlings were bled and returned to the original nest to be raised by their mothers (for details see Tori et al. 2006).

Wire-tailed manakins have a complex social system in which males follow age-graded queues and eventually make transitions from being nonterritorial floaters to territory holders (Ryder et al. 2008). We characterized males by plumage and status into one of the following 4 categories: formativeplumage floater, predefinitive-plumage floater, definitiveplumage floater, and definitive-plumage territory holder (for details see Ryder et al. 2008). We monitored changes in male status in each successive year of the study by making repeated visits to each male's territory to resight color-banded males. A male was considered to have socially ascended when he changed from definitive-plumage floater to definitiveplumage territory holder. Definitive plumage was a prerequisite for territorial status ( $n = 65$ ) because predefinitiveplumage birds have never been found to attain territories (Ryder et al. 2008).

## Molecular methods

DNA was isolated using a standard phenol–chloroform extraction method, followed by a cleaning step of dialysis in 1 x TNE<sub>2</sub> buffer (10 mM Tris, 10 mM NaCl, 2 mM EDTA). DNA was amplified using polymerase chain reaction (PCR) with fluorescently labeled primers for 7 microsatellite loci: Man 3, Man 6, Man 7, Man 13, Lan 10, Lan 20, and Man(AC)-13 (Piertney et al. 2002; DuVal and Nutt 2005; Brumfield R, Braun M, personal communication). Post-PCR we multiplexed amplicons to run on an ABI 3100 automated capillary sequencer. Genotypes for paternity and heterozygosity analyses were assigned using Genemapper 4.01 (Applied Biosystems, Inc, Foster City, CA). All homozygous individuals were rerun to confirm genotypes. No loci showed significant linkage disequilibrium and only one of the 7 (Man 7) was not in Hardy–Weinberg equilibrium (FSTAT v. 2.9.3.2, Goudet 2002). We used CERVUS 3.0 (Marshall et al. 1998; Kalinowski et al. 2007) to assign parentage of nestlings and juveniles. For nestlings, we assumed attending females to be the biological mothers. We knew the mother–offspring relationships for 101/125 (81%) of our nestling samples. In addition to nestlings sampled, individuals can be aged as less than a year old using molt limits within their greater coverts (Ryder and Durañes 2005). Thus, we also attempted to assign paternity to all postfledgling individuals born during the 4 years of our study. To assign parentage of nestlings and postfledgling individuals with unknown mothers, we first used CERVUS to determine paternity and then used male assignments as a baseline (known father) to assign maternity. We sampled 250 candidate males (ca. 95% of males sampled), which were nearly completely typed at the 7 loci (96%) and estimated genotyping error rates from mother–offspring comparisons. The combined exclusion power of the loci was 0.99. We assigned parentage using relaxed (80%) and strict (95%) confidence levels as well as a “total evidence” approach (Prodohl et al. 1998; Webster et al. 2004; Ryder et al. 2009).

## Heterozygosity estimates

Recently, various indices have been introduced to improve measures of multilocus heterozygosity by incorporating allele frequencies (e.g., internal relatedness and homozygosity by locus [HL]). Here, we used the HL index to estimate the heterozygosity of all candidate fathers because unlike internal relatedness it does not underestimate the heterozygosity of individuals with rare alleles (Aparicio et al. 2006). HL weighs the contribution of each locus depending on its allelic variability, so that highly polymorphic loci with evenly distributed alleles receive larger weight. Moreover, HL is better correlated with genome-wide homozygosity and inbreeding coefficients (Aparicio et al. 2006). HL index ranges from 0 (when all loci are heterozygous) to 1 (when all loci are homozygous); thus, lower values represent higher heterozygosity.

### Statistical analysis

All variables and/or the distribution of the residuals were checked for normality and homoscedasticity. Although no transformations were necessary to meet parametric test assumptions for the majority of tests, nonparametric tests (e.g., Kolmogorov–Smirnov) were adopted when the data failed to meet assumptions. To examine if females choose mates with respect to their heterozygosity, we performed 2 sets of analyses. Given that territoriality was a strong prerequisite for male reproduction (i.e., 98% of offspring sired by territorial males) (Ryder et al. 2008, 2009) all analyses of mating success only include territorial individuals. We first examined if there were heterozygosity differences between successful and unsuccessful males. Given that previous work had established a relationship between territorial tenure (i.e., number of breeding seasons a male held his territory) and probability of reproduction (Ryder et al. 2008), we ran an analysis of covariance to compare the heterozygosity of reproductively successful and unsuccessful territorial males with tenure included as a covariate in the model. Sample sizes for each success category within leks and years were small; to increase our power we pooled data from all leks across years and ran an “overall” model (Figure 2 shows the trends by lek). Second, we examined if heterozygosity predicted male total reproductive success (i.e., number of offspring sired) using leastsquares regression. This later analysis enabled us to examine finer-scale variation in heterozygosity among only successful males. Lastly, given that male genetic quality is likely assessed via male phenotype, we examined the relationship between heterozygosity and male morphology (i.e., wing length, tail length, and tarsus), using a stepwise multiple regression.

To examine if female choice for male genetic quality resulted in higher offspring heterozygosity than expected under random mating (indirect fitness benefits), we designed an EXCEL macro to generate random offspring. The macro generated an equivalent number of random offspring to those observed per mating pair ( $n = 52$ ). Keeping the identity of each female constant, we formed random mating pairs by selecting a male from the lek at which females mated (excluding their known mate). Offspring genotypes were generated by randomly selecting alleles from each parent, and HL values were calculated using IRmacroN4 (Amos et al. 2001). The

distribution of heterozygosity values between observed and random offspring was compared using a Kolmogorov–Smirnov test.

To examine if females preferentially chose dissimilar males, we calculated dyadic relatedness using RELATEDNESS version 5.0 (Queller and Goodnight 1989). The relatedness coefficient ( $r$ ) uses a regression measure of individual relatedness by comparing the frequency of alleles at a given locus in a given allelic position to the frequency of the same allele in some set of partners (i.e., putative relatives) and to the frequency of that same allele in the population at large (Queller and Goodnight 1989). Following Tarvin et al. (2005), we used a paired t-test to compare the dyadic relatedness of known male–female partners with the average similarity of the same female and all other males on the lek at which she mated (excluding her known mates). We used a least-squares regression to determine if parental relatedness of known male–female pairs predicted offspring HL.

To examine if heterozygosity plays a role in male territory acquisition, we examined the relationships between heterozygosity and male social ascension to territorial status. Males were distinguished as those which socially ascended by obtaining a territory and those which remained as nonterritorial floaters more than 4 breeding seasons. The change status analysis only considered males which had the opportunity to rise socially (i.e., all preexisting territory holders were excluded). We compared the average heterozygosity between these 2 categories using a t-test. Analyses were conducted using SPSS v 13.0 and JMP v 7.0. Means and standard errors are reported unless otherwise noted.

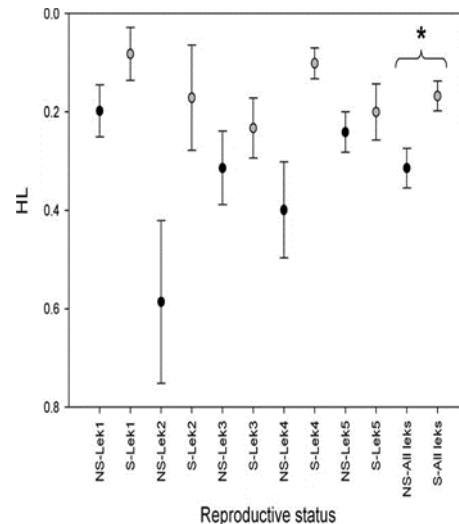


Figure 1

At the individual lek level, the average heterozygosity (mean  $\pm$  standard error) of successful male (S) wire-tailed manakins tended to be higher than the average heterozygosity of nonsuccessful males (NS), yet small sample sizes precluded a statistical comparison (Lek1 nNS = 6, nS = 5; Lek2 nNS = 6, nS = 3; Lek3 nNS = 2, nS = 3; Lek4 nNS = 7, nS = 5; Lek5 nNS = 4, nS = 10). Across all leks, successful male wire-tailed manakins ( $n = 26$ ) had significantly higher average heterozygosity than nonsuccessful males ( $n = 25$ ) denoted with an asterisk ( $P = 0.0008$ ). Note that low values of HL indicate high heterozygosity levels, so the y axis has been inverted.



## RESULTS

### Heterozygosity, reproductive success, and male morphology

Individual heterozygosity in our population of wiretailed manakins ranged from 0 to 0.83 (mean HL, males:  $\bar{X} = 0.22 \pm 0.02$ ; females:  $\bar{X} = 0.30 \pm 0.04$ ). Average heterozygosity of territorial males did not differ among leks ( $F_{4,46} = 1.13$ ,  $P = 0.353$ ) suggesting that genetic diversity of leks were roughly equal. Heterozygosity was related to male reproductive status (i.e., successful vs. unsuccessful males), where territorial males that sired offspring had significantly higher heterozygosity ( $\bar{X} = 0.168 \pm 0.031$ ) than unsuccessful territorial males ( $\bar{X} = 0.314 \pm 0.040$ ) ( $F_{7,43} = 7.78$ ,  $P = 0.0008$ , Figure 1). Heterozygosity, however, did not vary with length of male tenure ( $F_{7,43} = 0.22$ ,  $P = 0.885$ ), and there was no interaction between tenure length and reproductive status ( $F_{7,43} = 1.19$ ,  $P = 0.326$ ). When we examined each lek individually, we found that all leks followed the predicted pattern (Figure 1); however, because of small sample sizes we were unable to test for statistical differences at the lek level. Additionally, heterozygosity predicted a male's total reproductive success (i.e., number of offspring sired) only after removal of a single outlier (i.e., more than 2.5 standard deviations from the mean,  $r^2 = 0.13$ ,  $F_{1,49} = 7.04$ ,  $P = 0.010$ ). Lastly, heterozygosity of territorial males was related to male morphology ( $r^2 = 0.13$ ,  $F_{2,49} = 3.564$ ,  $P = 0.036$ ). Specifically, males with the highest heterozygosity had larger wings ( $\beta = 20.05$ ,  $P = 0.052$ ) and smaller tarsi ( $\beta = 0.12$ ,  $P = 0.03$ ).

### Dissimilarity and indirect benefits

The distribution of relatedness values of breeding pairs ranged from 20.3 to 0.5 (Figure 2). Female similarity to her mate, as measured by dyadic relatedness, was significantly different from her average similarity to the other males at the lek at which she mated ( $t_{29} = 22.00$ ,  $P = 0.05$ ). This relationship, however, was in the opposite direction than predicted by mate choice for dissimilarity (female mate:  $\bar{X} = 0.07 \pm 0.04$ ; female potential mates:  $\bar{X} = 20.01 \pm 0.02$ ; Figure 2); that is, females were significantly more related to their partners than to other potential mates at the leks in which they mated. As expected,

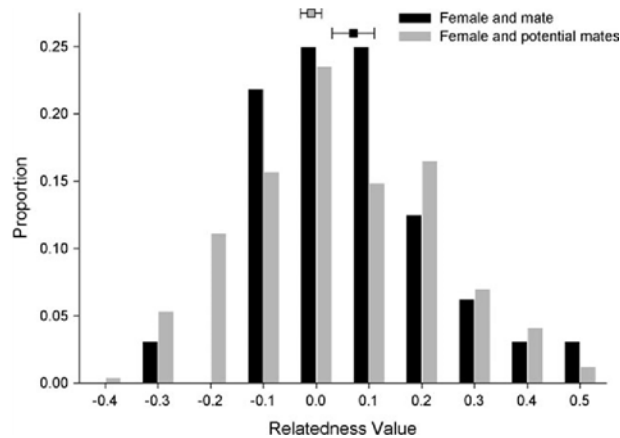


Figure 2

A histogram showing the distributions and average (Mean  $\pm$  standard error) relatedness values for female wire-tailed manakins and their mates (black bars and point) and females and all other males from the lek at which they mated (gray bars and point). Note that approximately half of mated pairs are related at or above the level of first cousins (e.g.,  $r \geq 0.1$ ).

inbreeding reduced offspring genetic diversity. Unrelated pairs had more heterozygous offspring ( $\bar{X} = 0.18 \pm 0.02$ ) than related pairs ( $\bar{X} = 0.35 \pm 0.04$ ), such that the degree of parental relatedness predicted offspring HL ( $r^2 = 0.22$ ,  $F_{1,52} = 14.39$ ,  $P = 0.0001$ ). Consequently, females did not gain indirect benefits (i.e., increased offspring heterozygosity) as evidenced by nonsignificant differences between the distributions of observed offspring heterozygosity ( $\bar{X} = 0.26 \pm 0.03$ ) and random offspring heterozygosity ( $\bar{X} = 0.24 \pm 0.02$ ; Kolmogorov–Smirnov  $Z = 0.58$ ,  $P = 0.879$ ).

## Heterozygosity and territory acquisition

Male heterozygosity was also related to male social rise from floater to territory holder. Males which ascended in social status by acquiring a territory had significantly higher heterozygosity ( $\bar{X} = 0.17 \pm 0.02$ ) than males that remained as nonterritorial floaters ( $\bar{X} = 0.28 \pm 0.04$ ;  $t_{54} = 22.33$ ,  $P = 0.023$ ). We consider this to be a conservative test because floater males which did not rise still had the potential to change status in the future.

## DISCUSSION

The importance of genetic benefits for the evolution of mate choice has been a focal point of interest in the fields of behavioral and evolutionary ecology over the last decade (see reviews by Mays and Hill 2004; Neff and Pitcher 2005; Roberts et al. 2006; Kempenaers 2007). In general, females are thought to select for male genetic quality because it indirectly benefits offspring viability and future reproductive success. Here, we have examined the good-genes-as-heterozygosity hypothesis in a

promiscuous lekking species, the wire-tailed manakin with a specific focus on heterozygosity and dissimilarity. We found that female wire-tailed manakins preferentially mate with males with high genetic diversity as measured by heterozygosity. More specifically, a male's heterozygosity predicted his reproductive status (successful vs. unsuccessful) and his total reproductive success (i.e., number of offspring sired). Heterozygosity was positively correlated to wing size and negatively related to tarsus length, such that highly heterozygous males had larger wings than expected by their structural body size. In addition, our data show that females did not select dissimilar (unrelated) males as partners. In fact, counter to the traditional view of compatibility, the average dyadic relatedness of pairs was significantly higher than the relatedness of breeding females and other potential mates. Our data also show that, on average, females' choices for highly heterozygous males did not increase offspring heterozygosity (i.e., indirect benefits); yet, offspring genetic diversity varied markedly with the degree of parental relatedness. Lastly, we found that males which inherited territories were significantly more heterozygous than males which remained as floaters, suggesting that heterozygosity plays a role in male social ascension.

### Heterozygosity, good genes, and male phenotype

Female choice of male morphological (i.e., ornaments) or behavioral traits is one potential mechanism for targeting male genetic quality. The traditional good-genes hypothesis posits that some males have superior alleles or certain combinations of alleles that are revealed by condition-dependent traits (Hamilton and Zuk 1982). The heterozygosity hypothesis, however, suggest that females select for male heterozygosity because it is linked to male quality (reviewed in Brown 1997; Kempenaers 2007). Given that male condition-dependent traits are often reliable indicators of male quality and may be correlated with either general heterozygosity (Aparicio et al. 2001; Foerster et al. 2003) or specific superior alleles (Mays and Hill 2004), disentangling the 2 hypotheses remains extremely difficult. Ultimately, under both hypotheses female preferences are likely driven by the phenotypic attributes of male genetic quality and assume that traits under selection are heritable and increase offspring fitness. The heterozygosity hypothesis further assumes estimates of genetic diversity using microsatellite markers (noncoding DNA) are good surrogates for trait-specific coding genes or are indicators of genetic quality (e.g., inbreeding levels) (Reid et al. 2005; but see Balloux et al. 2004; Slate et al. 2004). Here, we have shown that females select partners that are more genetically diverse as measured by heterozygosity. It remains unclear, however, if females are selecting for specific genetic attributes (i.e., superior alleles/good genes) or overall general genetic diversity (i.e., heterozygosity).

Regardless, our results contribute to the evidence that heterozygosity is indeed related to male traits that could impact fitness. In our population, for example, highly heterozygous males had larger wings and smaller tarsi. Wing attributes may be particularly important for wire-tailed manakins given their complex acrobatic aerial displays (i.e., butterfly and sloop-inflight) (Schwartz and Snow 1978). In particular, long wings reduce wing loading and have been favored in those species that exhibit

hovering or fast agile displays (Théry 1997). Importantly, butterfly flights are only performed for females and swoop-in-flight displays culminate in copulation events making them ideal targets for female selection. Two other display elements, side-side and twist displays, could be impacted by tarsus length yet exactly how shorter tarsi might affect the biomechanics of these display elements remain unclear. Regardless, phenotypes that impact display quality are one potential mechanism for how female might select mates with higher heterozygosity.

### Compatibility, dissimilarity, and inbreeding

Here, we show that females mated with males that were more closely related than the average candidate male at the lek. This finding may seem incongruous given the fairly well established negative fitness consequences of inbreeding depression (Keller and Waller 2002). In our population, roughly half the mated pairs were related, whereas the remaining pairs were unrelated. Given that the inbreeding coefficient is the percent chance of 2 alleles being identical by descent, then mating among only relatives results in a high coefficient with low variance, whereas mating among only nonrelatives results in a low coefficient with similarly low variance. As such, our intermediate mix of related and unrelated pairs should produce an intermediate coefficient estimate with high variance. Furthermore, extreme polygyny as would be expected for lekking taxa leads to small effective population sizes and, ultimately, high variance in the inbreeding coefficient (Hansson and Westerberg 2002; Balloux et al. 2004).

Manakin populations have several demographic characteristics that may increase the likelihood of inbreeding. For example, Ryder et al. (2009) found significant reproductive skew at 4 of the 5 leks studied here, with as few as 3 males siring 80% of all offspring. Moreover, recent work on blue manakins (*Chiroxiphia caudata*) suggests that fine-scale genetic structuring is likely a direct consequence of strong philopatry (i.e., limited dispersal), reproductive skew, and potential inbreeding (Francisco et al. 2007). Although dispersal data are limited for female wiretailed manakins, we have observed 2 events in which young males eventually recruited into that same lek with their father (Ryder TB, personal observation). If females also have low dispersal in a system with strong reproductive skew, then this would increase the average relatedness of females to territorial males and hence the potential for inbreeding.

Given the nearly roughly equal split of related and unrelated partners it remains unclear if females are actively selecting related males. Mate choice for related individuals would require the ability to recognize kin or assess relatedness using either olfactory or phenotypic cues. Recent work on bluecrowned manakins suggests that female inability to choose compatible males (i.e., unrelated individuals) may be due to a female's inability to assess relatedness (Durañes et al. 2009; see also Keller and Arcese 1998; Kruuk et al. 2002). Moreover, strong olfaction is lacking in most birds making it unlikely that females use olfactory cues to recognize kin and assess mate compatibility; thus, mate choice in birds is more likely to be based on obvious phenotypic traits (e.g., ornamentation; Mays and Hill 2004). Overall, our findings suggest that female choice is based on phenotypic traits

irrespective of mate relatedness and that inbreeding is most likely an inevitable consequence of demography rather than active female choice (but see below).

Regardless of the mechanism promoting inbreeding, it is nonetheless occurring in our population. Although inbreeding avoidance is considered adaptive under most circumstances, there may be instances when inbreeding need not be avoided (Kokko and Ots 2006). Recent theory suggests that inbreeding must result in at least a 33% reduction in fitness before mating with full or half sibs is actively avoided (Lehmann and Perrin 2003; Kokko and Ots 2006). If the costs of inbreeding are limited, then mating with relatives may in fact yield inclusive fitness benefits because you and your mate share genes (i.e., “altruistic inbreeding,” Lehmann and Perrin 2003). This fitness benefit may be increased if females mate with an otherwise unsuccessful male relative (Kempenaers B, personal communication). Given that many males are unsuccessful when there is strong reproductive skew, mating with relatives might accrue greater fitness benefits in lekking species (Kempenaers 2007). Moreover, if inbreeding has occurred for several generations, then the population genetic architecture might be such that deleterious alleles have been selected against and thus purged from the gene pool (Crnokrak and Barrett 2002). Thus, inbreeding may be expected to persist if the benefits of inclusive fitness outweigh the potential costs of inbreeding depression.

The traditional view of mate compatibility is that females select sires that are genetically dissimilar in order to maximize offspring genetic diversity (Mays and Hill 2004). However, this view of compatibility (i.e., mate choice for dissimilarity), although common in the literature, would not necessarily be favored if compatibility depended on gene–gene interactions. For compatibility, what matters is the optimal combination of male and female genotype. As such, if locally adapted gene complexes existed and the fitness costs of inbreeding were limited, female choice of more similar (i.e., related) males might provide the most compatible genes for some females. Unfortunately, our data do not currently enable us to differentiate between the role of compatible alleles and good alleles as a mechanism underlying female mate choice in manakins.

### Indirect benefits and offspring genetic quality

Essential to models of adaptive mate choice for genetic quality are the potential fitness benefits accrued via female selectivity. In nonresource-based systems, indirect (e.g., genetic) benefits are assumed by default, and it has been suggested that pairing with high heterozygosity partners could result in higher offspring heterozygosity (Jennions and Petrie 2000). Although heterozygosity is thought to have low heritability (Mays and Hill 2004), a correlation between offspring and parental heterozygosity could arise because highly heterozygous individuals carry alleles that are, on average, different from alleles drawn randomly from the population (e.g., Mitton et al. 1993; Kempenaers 2007). Here, we failed to find evidence that mating with males of higher heterozygosity resulted in more genetically diverse offspring. Moreover, this current lack of evidence for indirect benefits suggests that other mechanisms drive the operation of female choice on mating arenas.

## Genetic variability and social status

Both male–male competition and female choice can affect male mating success in lekking taxa (Pruett-Jones SG and Pruett-Jones MA 1990; Höglund and Alatalo 1995). Male–male competition can affect male mating success on leks in 2 ways: 1) by influencing which individuals are assessed by females (e.g., territory acquisition) or 2) by influencing the ability of females to choose mates (e.g., copulation disruption and or control of copulation via dominance). Heterozygosity is suggested to promote developmental stability, thereby influencing male condition and vigor (Brown 1997). Despite the importance of this link, there have been only a few studies that have attempted to relate individual genetic variation to social status (but see Höglund et al. 2002; Tiira et al. 2006). If males with high heterozygosity are in better body condition and more vigorous, we might expect these males to have higher survival and, potentially, higher social standing. Male–male competition could preclude female choice if males of high genetic quality assert their dominance and control access to reproduction.

Male wire-tailed manakins follow an age-graded queue in which young males develop social partnerships via coordinated male–male displays between territorial individuals (Heindl 2002; Ryder 2008). Moreover, the existence of partnerships and their number determine the likelihood of territory acquisition (see Ryder et al. 2008). Given the longterm nature of male affiliations, dominance among males is well established, making copulation interruption and aggressive control of access to females (e.g., overt aggression between males) rare (Ryder TB, personal observation). Thus, although male–male competition impacts territorial inheritance, male reproductive success appears to be largely driven by territory level female choice. Here, we have shown that individuals with higher heterozygosity are more likely to ascend in social status (i.e., inherit a territory) than individuals of the same social standing with a less diverse genetic makeup. Our results suggest that heterozygosity may act as a filter of male reproductive potential prior to female visitation and male assessment at leks.

Here, we found that highly heterozygous males were more likely to attain territories and had higher reproductive success than males with low heterozygosity. Our findings with respect to mate dissimilarity, however, generate an interesting paradox with respect to population maintenance of genetic diversity in wire-tailed manakins. If inbreeding and the resulting reduction of population genetic diversity are inevitable consequences of manakin demography and reproductive ecology, then selection for heterozygosity (i.e., territory acquisition and male reproductive success) could theoretically mitigate the impacts of inbreeding depression. Moreover, variation in the inbreeding coefficient resulting from strong reproductive skew, low dispersal and a mix of related and unrelated mating pairs may have increased our ability to detect a significant positive heterozygosity–fitness correlation. Clearly, further investigation of the underlying genetic mechanisms and heritability of male phenotypic traits used as cues by females is required to understand the evolutionary processes shaping mate choice for males of high genetic diversity. Overall, our results present interesting questions on how population-specific demography can interact with

sexual selection to shape population genetic structure and potentially maintain genetic diversity.

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