Kin selection may contribute to lek evolution and trait introgression across an avian hybrid zone

MOIRA R. CONCANNON,*† ADAM C. STEIN*‡ and J. ALBERT C. UY*§

*Department of Biology, Syracuse University, Syracuse, NY 13244, USA, †Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, MA 01003, USA, ‡Monteverde Institute, Monteverde, Puntarenas, Costa Rica, §Department of Biology, University of Miami, Coral Gables, FL 33146, USA

Abstract

Understanding the mechanism(s) that favour cooperation among individuals competing for the same resources provides direct insights into the evolution of grouping behaviour. In a hybrid zone between golden-/yellow-collared (*Manacus vitellinus*) and white-collared (*Manacus candei*) manakins, males form aggregations composed of white and yellow males solely to attract females ('mixed leks'). Previous work shows that yellow males in these mixed leks experience a clear mating advantage over white males, resulting in the preferential introgression of yellow plumage allele(s) into the white species. However, the yellow male mating advantage only occurs in mixed leks with high frequencies of yellow males, and only a few of these males probably mate. Hence, it remains unclear why unsuccessful males join leks. Here, we used microsatellite markers to estimate pairwise relatedness among males within and between leks to test whether indirect genetic benefits of helping kin ('kin selection') can promote grouping. We found that yellow males are significantly more related to each other within than between leks, while relatedness among white males did not differ within and between leks. This suggests that yellow males may indirectly enhance their own reproductive success by preferentially lekking with relatives because yellow plumage is under positive frequency-dependent selection (positive FDS). Our results are consistent with the hypothesis that kin selection may promote grouping and facilitate positive FDS for yellow males, mediating the movement of yellow plumage across this hybrid zone.

Keywords: frequency-dependent selection, hybrid zone, kin selection, lek, *Manacus*, trait introgression

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Introduction

Grouping or aggregative behaviour has independently evolved across many taxa and must therefore provide distinct advantages, either through enhanced individual reproduction or through survival (Hamilton 1971; Alexander 1974; West *et al.* 2007). For instance, grouping may decrease individual risk of predation (Foster & Treherne 1981; Landeau & Terborgh 1986) or provide a more stable food supply (Thompson *et al.* 1974; Clark & Mangel 1986). These benefits, however, can be offset by the costs associated with grouping (Krause & Ruxton 2002), such as intragroup competition for resources (Clutton-Brock *et al.* 1982; Pride 2005) and increased transmission of parasites (Freeland 1976; Tella 2002). Therefore, because of the opposing selective forces imposed on individuals within a group, it can be difficult to understand the evolution of this social behaviour.

In species where cooperation leads to increase in per capita prey capture (Creel & Creel 1995) or reduction in individual predation (Cresswell 1994), explaining grouping can be straightforward (Krause & Ruxton 2002). However, in some species, the benefits of grouping are unclear. For instance, in lekking species, males form aggregations ('leks') solely to attract females (Höglund & Alatalo 1995). In some of these lekking

Correspondence: J. A. C. Uy, Fax: 305-284-3039;
E-mail: uy@bio.miami.edu

species, each male defends a permanent cleared space ('court') that is used as a stage to display to females, allowing females to simultaneously evaluate several males displaying at adjacent courts. Larger leks may be more attractive to females, thus enhancing each male's reproductive success (Alatalo *et al.* 1992; Balmford *et al.* 1992; Lank & Smith 1992; Shelly 2001). However, leks are also typically characterized by a high male mating skew, with a few males receiving the majority of copulations and leaving the remaining males with little or no direct reproductive benefits (Mackenzie *et al.* 1995; Shorey 2002; Stein & Uy 2006a).

Several hypotheses may explain lekking. First, the female preference model posits that females prefer to mate with males that are clustered together, whether as protection from predation or to facilitate evaluation of the best mate (Bradbury 1981). Second, the hotshot hypothesis proposes that lekking is a by-product of inferior males establishing territories near successful males in an attempt to gain copulations by associating with attractive males (Beehler & Foster 1988). A third alternative, not driven by female choice, is the hotspot model, which proposes that males cluster where females are abundant (e.g. foraging sites, Bradbury & Gibson 1983). Most recently, kin selection has been evoked as a possible explanation for lekking if males preferentially group with and enhance the mating success of relatives (Kokko & Lindstrom 1996). Support for kin selection driving lek evolution has been equivocal, with kin structure found within some leks (Petrie *et al.* 1999; Shorey *et al.* 2000; Segelbacher *et al.* 2007) but not in others (Gibson *et al.* 2005; Loiselle *et al.* 2007; McDonald 2009). With inconsistent support across taxa, it is clear that no single hypothesis can account for lek evolution (Loiselle *et al.* 2007; Knopp *et al.* 2008; Bush *et al.* 2010).

Manakins (family Pipridae) are known for their extravagant displays and elaborate plumage and have been a model system for understanding lek evolution (Johnsgard 1994; Prum 1994). In bearded manakins (genus *Manacus*), males form leks and defend permanent courts where they display to females by dancing with their beards and collars extended. *Manacus candei* has a white beard and collar and ranges from Mexico to Panama. *Manacus vitellinus* has a yellow or golden beard and collar and ranges from Panama to Colombia. The two species meet and form a hybrid zone in northwestern Panama (Parsons *et al.* 1993). Individuals within the hybrid zone with intermediate yellow beards and collars (i.e. less chromatic yellow instead of golden) were initially described as a distinct species, *M. cerritus* (Ridgely & Gwynne 1989). Genetic studies, however, confirmed that this taxon is a hybrid of *M. candei* and *M. vitellinus* (Parsons *et al.* 1993; Brumfield *et al.* 2001).

Genetic and morphometric analyses show that steep and concordant clines are formed along the Rio Robalo, showing a clear distinction between *M. candei* and *M. vitellinus* populations (Parsons *et al.* 1993; Brumfield *et al.* 2001). However, a plumage colour cline is displaced from the genetic and morphometric clines by about 150 km north into the white-collared population along Rio Changuinola, suggesting that alleles for yellow plumage have introgressed preferentially into the white species (Brumfield *et al.* 2001). In essence, birds in the region between the plumage and genetic/morphometric clines have yellow beards and collars but are otherwise genetically and morphometrically *M. candei* (Parsons *et al.* 1993; Brumfield *et al.* 2001). The presence of the steep plumage cline near Rio Changuinola, however, suggests that there must be a mechanism preventing or at least slowing the movement of yellow plumage allele(s) into the white species.

Males along the plumage cline on the banks of Rio Changuinola form leks where both yellow and white males directly compete for access to females ('mixed leks'; see Fig. 1) (McDonald *et al.* 2001; Stein & Uy 2006b). These mixed leks provide unique, natural
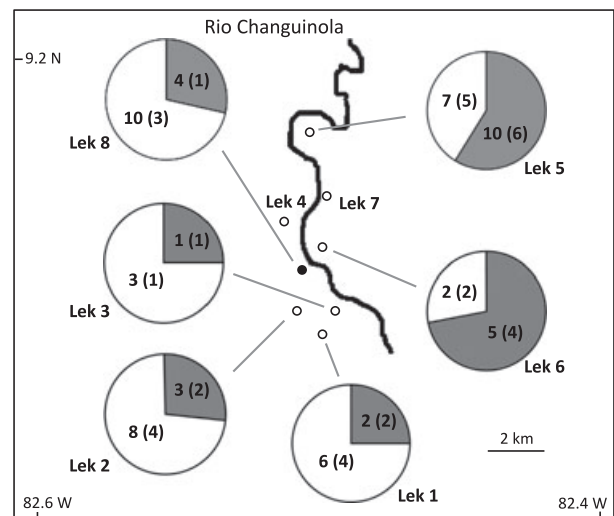
Fig. 1 Location and male colour ratios of mixed leks used in our study along the Rio Changuinola (see also Stein & Uy 2006b). White and grey sections in the pie charts represent proportion of white and yellow males, respectively. Numbers within the pie charts indicate number of white or yellow males within the mixed lek. Numbers in parentheses indicate the number of white or yellow males sampled for blood. Open circles represent leks monitored in 2005 (Stein & Uy 2006b), and the black circle represents the lek sampled in 2006 (this study). Note, leks 4 and 7 were monitored in 2005, but birds were not banded and sampled for blood. Lek 5 frequency reflects both court owners ($N = 14$, with 6 white and 8 yellow males; see Stein & Uy 2006b) and noncourt owning but resident males ($N = 3$, with 1 white and 2 yellow males).

experiments to explore the mechanisms underlying the dynamics of yellow plumage introgression, as well as to explore the evolution of lekking in general. Previous behavioural studies indicate that yellow males experience a clear mating advantage over white males but only in leks with high frequencies of yellow males independent of lek size (Stein & Uy 2006b). That is, female preference for yellow males increases linearly in response to increase in yellow male frequency (Stein & Uy 2006b). The mating success of white males, in contrast, is consistently low and thus independent of the frequency of white or yellow males. These results suggest that positive frequency-dependent selection (positive FDS), in which the yellow phenotype has a mating advantage when common, mediates the introgression of the yellow plumage allele(s) into the white species. However, in *Manacus* leks, only a few males gain matings (Chapman 1935; Lill 1974; Shorey 2002; Stein & Uy 2006a,b), making it unclear why unsuccessful males would lek with successful males, especially for attractive yellow males, whose mating success is mediated by positive FDS.

Here, we explore the hypothesis that kin selection can facilitate lekking if unsuccessful males within leks receive indirect fitness benefits by enhancing the attractiveness of related males. We test a key prediction of the kin selection hypothesis as it applies to the mixed leks in the *Manacus* hybrid zone: yellow males should preferentially lek with kin because a higher frequency of yellow males within a lek results in increased mating success for related yellow males.

Materials and methods

Field sampling

The white- and golden-/yellow-collared manakins come into secondary contact and form a hybrid zone with mixed leks in Bocas del Toro, Panama (Parsons *et al.* 1993). Each male defends a permanent court about 1 m in diameter on the forest floor surrounded by small saplings and cleared of all leaf litter and debris. Because male courts are located 2–10 m apart within a lek, females may view several displaying males before choosing to mate with a particular male (Chapman 1935; Stein & Uy 2006a). Male manakins display to females by hopping on their courts and between surrounding saplings, while clicking their wings to produce a popping sound ('snap-grunt display', Chapman 1935). We studied males from six mixed leks, which varied in the composition of yellow and white males (Fig. 1). We caught individuals using mist nets and collected blood from an unbiased subsample of adult, lekking males in these six mixed leks from 17 March to 13 June 2005 and from 29 March to 7 June 2006 (Fig. 1). At the time of capture, body mass (g) and morphometric data, including tarsus, head, wing, and tail lengths (mm), and bill dimensions (mm), were collected. Males were fitted with a unique combination of colour leg bands and a numbered metallic band to facilitate behavioural observations and confirmation of court ownership (see Fig. S1 for court arrangement within leks, Supporting information). Although a complete sampling of all males in the mixed leks is ideal, logistical constraints of working in a remote region like Bocas del Toro prevented us from banding and collecting blood samples from every male in each lek. Blood was preserved in Longmire lysis buffer (Longmire *et al.* 1997), and genomic DNA was extracted using the DNeasy Blood and Tissue Kit following manufacturer protocol (QIAGEN, Valencia, CA, USA).

Five individuals in the hybrid zone were white with minor yellow streaks (one in lek 3, one in lek 4, two in lek 5 and one in lek 6). These potential 'intermediate' phenotypes were essentially white in beard and collar, and assigning them as 'intermediate' did not affect our estimates of mean relatedness grouped by plumage colour (Fig. S2, Supporting information). For these reasons, we decided to assign these males as white-collared in our analyses.

Aside from preferentially forming leks with kin, relatives may also preferentially cluster within leks (Shorey 2002). To test for a relationship between fine-scale spatial structuring and relatedness within leks, courts in each lek were mapped to estimate the distance between courts (as in Stein & Uy 2006a). Within each lek, we established a reference point and measured from the centre of each court to this point. After plotting the courts on a Cartesian plane (Fig. S1, Supporting information), we calculated pairwise Euclidean distances between courts within each lek.

Estimating relatedness

To estimate the pairwise relatedness of males from these mixed leks, we genotyped each male using 10 fluorescently labelled microsatellite primers: Man 1, Man 3, Man 4, Man 6, Man 7 and Man 8 developed for *Manacus* (Piertney *et al.* 2002) and Chiro 1, Chiro 2, Chiro 5 and Lan 22 developed for *Chiroxiphia* (DuVal & Nutt 2005; DuVal *et al.* 2007) manakins. All birds were genotyped at the Core Laboratories Center at Cornell University (Ithaca, NY, USA) using the Applied BioSystems 3730xl DNA Analyzer (Foster City, CA, USA). GeneMapper v3.0 (Applied BioSystems) was then used to visualize fragment lengths and call alleles for each bird. Estimates of relatedness require that the markers used are not in linkage disequilibrium and are in Hardy–

Weinberg equilibrium. Using the program GENEPOP 3.4 (Rousset 2008), we found that two loci (Man 7 and Chiro 5) were in linkage disequilibrium with other markers and so were excluded from our analyses. We found that one primer (Man 3) was not in HW equilibrium, but estimates of relatedness were similar with and without this locus (Fig. S3, Supporting information), and so it was included in the main analyses to maximize the number of markers used to estimate relatedness. Pairwise relatedness was calculated using the Queller & Goodnight (1989) estimation as implemented in the program SPAGeDi (Hardy & Vekemans 2002). The method calculates relatedness as r, with the expected value of 0 for unrelated individuals, 0.25 for half siblings and 0.5 for full siblings or parent–offspring relationships. Note, however, that the variance around these expected r values is influenced by the variability of the markers (i.e. number of alleles for each marker) used to estimate relatedness (Alderson *et al.* 1999; Francisco *et al.* 2004). Using the jackknife method in SPAGe-Di, standard error for r was estimated by iteratively calculating relatedness in the absence of one of the microsatellite markers in each calculation. Other indices of relatedness (e.g. Loiselle *et al.* 1995; Wang 2002) provide similar estimates (Fig. S4, Supporting information). We acknowledge that estimates of pairwise relatedness may be biased with a small sample size (e.g. few males within leks, Ritland 1996; Wang 2002); however, because we estimate population gene frequencies from a larger sample (e.g. across leks), the potential biases associated with a limited sample size are ameliorated (Ritland 1996).

Statistical analyses

We tested two key predictions of the kin selection hypothesis. First, we determined whether individuals were more related within than between leks. Because the mating success of some yellow males is enhanced in the presence of other yellow males (Stein & Uy 2006b), we also compared relatedness of males grouped by plumage colour within and between leks (i.e. relatedness of yellow males to other yellow males, of white males to other white males and of yellow males to white males). We ran a randomization generalized linear model (GLM) ANOVA with relatedness as the dependent variable, and 'lek comparison' (relatedness within vs. between leks) and 'colour comparison' (relatedness of yellow males to yellow males, of white to white and of yellow to white) as fixed factors. We also tested for an interaction between lek comparison and colour comparison. Corrected post hoc pairwise comparisons (Tukey–Kramer) were subsequently carried out to determine whether relatedness within leks is different from

relatedness across leks among the three colour groups. Because our data violated assumptions of parametric tests (e.g. estimates of pairwise relatedness were not independent), we used randomization tests to calculate probability values for hypotheses testing (Manly 1991; Cassell 2002).

Second, we tested the prediction of kin selection that related males should cluster preferentially within leks. To do so, we ran a randomization correlation analysis (Manly 1991) to test for an association between relatedness and distance between courts within leks. To perform a single test for our entire data set, we pooled court distances across leks after standardizing court distances by lek (i.e. z-scores). Three males (two yellow and one white) in lek five were not court owners, but reliably displayed on the periphery of courts within the lek throughout the breeding season, perhaps waiting for an opportunity to establish a permanent court. (see also Snow 1962 for similar behaviour in *M. manacus*). These three males were included in the estimates of pairwise relatedness, but were excluded from analyses involving distance between courts. All tests of hypotheses were two tailed. Randomization GLM ANOVA and correlation analyses were implemented in SAS ver. 8.1 (SAS Institute, Cary, NC, USA) using a modified SAS wrapper (Cassell 2002).

Results

Relatedness within vs. between leks

Pairwise relatedness of males was significantly greater within than between leks, but the relatedness values
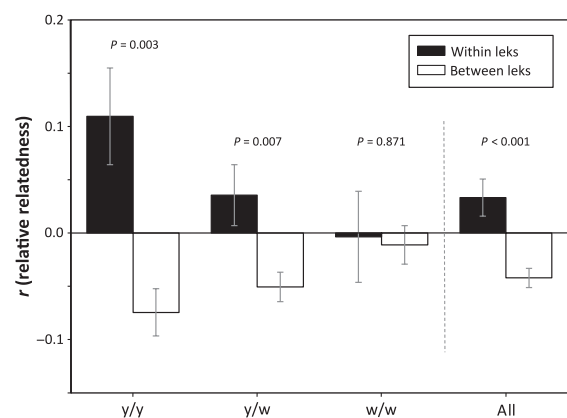
Fig. 2 Mean pairwise relative relatedness (±SE) of males within and between leks grouped by plumage colour (y/y = pairwise relatedness of yellow males, y/w = pairwise relatedness of yellow and white males, w/w = pairwise relatedness of white males, all = pairwise relatedness of all males regardless of colour).

Table 1 Randomization generalized linear model ANOVA of relatedness within vs. between leks.

Factor	df	Type III SS	F	P
Lek comparison	1	0.639	13.50	<0.001
Colour comparison	2	0.037	0.390	0.679
Lek × colour comparison	2	0.314	3.31	0.037
Residuals	589	27.887		

were relatively small and near zero (Fig. 2). A more detailed analysis of relatedness based on male colour, however, revealed a significant interaction between male colour and relatedness within and between leks (Table 1). We found that yellow males were significantly more related to other yellow males within leks (comparable to cousinship) than between leks (Fig. 2). In contrast, white males were no more related to each other within than between leks (Fig. 2). Comparing the relatedness of yellow and white males within leks, we found that they were likewise significantly more related within than between leks, but the relatedness values were near zero (Fig. 2). With respect to overall relatedness regardless of lek membership, mean relatedness of yellow males to other yellow males is comparable to the mean relatedness of white males to other white males (yellow males: -0.036 ± 0.018, $N = 120$ pairwise comparisons; white males: -0.012 ± 0.015, $N = 171$ pairwise comparisons; Fig. S5, Supporting information).

Fine-scale spatial and genetic structuring within leks

A randomization correlation test did not find a significant association between relatedness and intercourt distance within leks when comparing yellow males to yellow males ($r = 0.029$, $N = 14$ pairwise comparisons, $P = 0.922$), white males to white males ($r = -0.078$, $N = 22$ pairwise comparisons, $P = 0.730$) or white males to yellow males ($r = -0.159$, $N = 43$ pairwise comparisons, $P = 0.309$).

Discussion

Kin selection may facilitate positive frequency-dependent selection for yellow males

The kin selection hypothesis posits that individuals should behave to increase the reproductive fitness of kin (Hamilton 1964). In the *Manacus* mixed leks, yellow males experienced a mating advantage in the presence of other males with the same plumage colour, while white males did not (Stein & Uy 2006b). Therefore, yellow and not white males are predicted to cluster with kin. Indeed, we found that yellow males were more related within leks, whereas white males were not (Fig. 2), suggesting that only yellow males preferentially cluster with kin. Several studies, including one in manakins (Shorey *et al.* 2000), similarly found that males preferentially lek with kin (Petrie *et al.* 1999; Regnaut *et al.* 2006). However, these studies did not identify the benefit(s) of lekking with related males (Petrie *et al.* 1999; Regnaut *et al.* 2006) or only suggested benefits to the entire group including nonkin (e.g. female preference for larger leks, as in Shorey *et al.* 2000). Our work differs from these because we not only show clustering of kin, but also link this to a possible benefit to kin—yellow males experience a mating advantage in mixed leks with more yellow males (Stein & Uy 2006b). This is an important distinction because the mere presence of kin groups within leks does not necessarily mean that kin selection is operating. For instance, relatedness within leks may be high if there is low male natal dispersal in the population (Regnaut *et al.* 2006). Studies testing the role of kin selection in lekking should therefore establish not only the presence of kin groups but also the likely fitness benefits males gain by lekking with relatives. Notably, mating success may not be a complete measure of reproductive success, as it only explicitly considers precopulatory and not postcopulatory sexual selection. However, observed matings have been shown to be reliable indicators of paternity in several lekking species (Alatalo *et al.* 1996; Reynolds *et al.* 2007; Semple *et al.* 2001; but see Lank *et al.* 2002). While direct assessment of paternity is ultimately ideal, mating success can serve as a useful proxy of fitness in manakins given this observation.

Assuming that mating success indeed relates directly to reproductive success, female preference for yellow males has probably led to the unidirectional introgression of yellow plumage across this hybrid zone and into the white-collared population (Parsons *et al.* 1993; Brumfield *et al.* 2001; Stein & Uy 2006b). The yellow male mating advantage, however, only occurs in leks with high frequencies of yellow males, indicating that yellow plumage is under positive FDS (Stein & Uy 2006b). In most instances of positive FDS, individuals of a similar phenotype benefit equally or similarly, which facilitates the increase in frequency of the advantageous phenotype (e.g. Allen 1988; Borer *et al.* 2010). In lekking species, however, only a few males typically receive matings (e.g. Mackenzie *et al.* 1995; Friedl & Klump 2005; Stein & Uy 2006a; Young *et al.* 2009), making it difficult for positive FDS alone to favour a shared phenotype. In the case of the yellow birds in the *Manacus* hybrid zone, it was unclear why yellow males would group if the mating advantage is only experienced by a

few males and only after yellow males reach a high frequency in mixed leks (Stein & Uy 2006b). Our results provide a possible solution to this paradox: indirect benefits of helping kin may promote grouping behaviour in yellow males despite the possible absence of direct benefits from matings. Once enough yellow males are present in a mixed lek, positive FDS acts synergistically with kin selection. That is, kin selection facilitates positive FDS by initially favouring grouping in yellow males. In this way, the clustering of kin enhances the reproductive success of related yellow males. This, in turn, facilitates the spread of the yellow plumage across the hybrid zone.

Alternatively, yellow males may group in leks with high frequencies of yellow males, not to benefit indirectly by enhancing the reproductive success of kin, but to increase the likelihood of receiving matings, or to later inherit attractive sites/courts (McDonald 2009). However, this scenario is unlikely to be operating in *Manacus* leks because typically few males receive matings in a given breeding season (Shorey 2002; Stein & Uy 2006a). Likewise, this hypothesis does not predict kin structuring, as selection only favours grouping with other yellow males and not necessarily with yellow kin. Additional work monitoring the mating success of all males across mixed leks of different colour composition will provide a more direct test of this hypothesis.

Within leks, the courts of some males are clustered together, whereas others are farther apart, providing the potential for preferential interactions between kin within leks. We did not find an association between relatedness and distance between courts, suggesting the absence of fine-scale genetic structuring within leks. These results contrast those of Shorey *et al.* (2000), who found fine-scale kin structuring within leks in another *Manacus* species (*M. manacus*). One possible explanation for the lack of fine-scale clustering of kin within these mixed leks is that the dispersed arrangement of relatives within leks may mediate the costs associated with lekking. Males whose courts are adjacent are likely to be in more intense direct competition when females visit a lek because females compare adjacent males before choosing a mate (Stein & Uy 2006a). That is, kin selection and positive FDS may favour the grouping of yellow relatives to attract females to leks, but the intense competition between adjacent courts may select against close clustering of relatives within leks. Alternatively, spatial structuring within leks may be a result of unrelated individuals, who are not receiving indirect fitness benefits from lekking with relatives, establishing courts near successful males. In this way, their proximity to favoured males allows them more opportunities to court or attempt sneak copulations with visiting females (Beehler & Foster 1988). As aggression typically increases as neighbouring males become closer within a lek (Trail & Koutnik 1986; Shorey 2002), this may not always be a successful strategy for white males. Still, under this hypothesis, unsuccessful males would attempt to establish courts near attractive males, but no fine-scale genetic structuring within leks is necessarily expected.

While a model of kin selection facilitating positive FDS may explain the evolution of grouping in yellow males, our results suggest that kin selection is not operating in white males. This pattern may be due to the lack of opportunities for white males to group with other white males. That is, there might be fewer relatives in the area for white males, thus limiting their ability to preferentially lek with kin. The distribution of relatedness for white males and yellow males, however, is similar (Fig. S5, Supporting information), suggesting that white males have the potential to preferentially lek with kin. Alternatively, because white males do not experience a mating advantage regardless of lek composition (Stein & Uy 2006b), white males cannot gain indirect benefits from lekking with kin. However, white males do lek, requiring an alternative explanation to account for lekking in these males. Considering that a lekking mating system is the ancestral state for the manakin family, with only one (*Antilophia galeata*) of 40 species losing this social behaviour (Prum 1994), it is possible that phylogenetic constraints or strong stabilizing selection on this mating system may prevent the evolution of alternative mating strategies. Several potential hypotheses may account for the origin of lekking in manakins, including female preference for male aggregations, unattractive males clustering around attractive males or males congregating in areas with high densities of females (reviewed in Höglund & Alatalo 1995). Regardless of original mechanisms that favoured lekking in manakins, the lack of evidence for kin selection acting on white males, as well as in other manakin species (DuVal 2007; Loiselle *et al.* 2007; McDonald 2009), suggests that kin selection was not a major mechanism in the origin of lekking in this group. However, because positive FDS favours yellow males but not all yellow males benefit equally from grouping, kin selection may instead act to further promote or maintain lekking only for yellow males. While the results of our study pertain to mixed leks at this hybrid zone and may not be broadly applicable to the evolution of lekking in general, the inconsistent results across studies implicating kin selection in lekking in some instances but not in others suggest that the selective pressures acting on leks are dynamic and that kin selection may have a limited role in the origin of lekking in manakins.

Possible factors that slow the introgression of yellow plumage

Alleles with even a slight selective advantage are predicted to sweep through a hybrid zone relatively quickly (Barton & Hewitt 1985). The yellow plumage alleles should, therefore, rapidly move into the white species because yellow males have a distinct mating advantage over their white counterparts (Stein & Uy 2006b). Comparison of male morphometrics indicates that the yellow and white morphs in mixed leks do not differ significantly in tarsus, wing, head or tail length, bill dimensions or body mass (Stein & Uy 2006b). This observation, coupled with the concordant and steep genetic and morphometric clines, indicates that males in mixed leks are genetically and morphometrically the white species (*M. candei*) but introgression of the yellow collar and beard has left some males superficially resembling the golden/yellow species (*M. vitellinus*) (Brumfield *et al.* 2001; Stein & Uy 2006b). Strong sexual selection by female choice probably facilitates the preferential introgression of allele(s) for a yellow beard and collar into the white population (Stein & Uy 2006b), which should lead to the fixation of favoured alleles (Smithson & Macnair 1997; Gigord *et al.* 2001). As white males are still common in mixed leks and the plumage cline is steep, there must be a mechanism slowing the movement of the yellow allele(s) further into the pure white population.

The Changuinola River may present a physical barrier between the purely white population and the northern edge of the hybrid zone that limits the movement of yellow plumage (Brumfield *et al.* 2001). However, mixed leks are found on both sides of the river, indicating that males can and will fly over rivers to establish courts in leks. Although mere speculation, we offer four potential explanations to account for the slowed introgression of yellow allele(s) further into the white population. First, the interaction between positive FDS and a high male mating skew may create a scenario in which trait introgression occurs only under restrictive conditions—it only occurs when a high frequency of related yellow males are in each lek. As only two of the mixed leks we sampled were dominated by yellow males, it is possible that reaching the high frequency of yellow males necessary for positive FDS to act slows the introgression of yellow allele(s). Second, previous work suggests that variable ambient light and visual background across these species' ranges result in yellow males being more conspicuous within and southeast (allopatric golden population) of the hybrid zone and white males being more conspicuous northwest (allopatric white population) of the hybrid zone (Uy & Stein 2007). Yellow males may therefore be less attractive beyond the hybrid zone, thereby slowing the introgression of yellow plumage into the white population (Uy & Stein 2007). Third, partial genetic incompatibilities between the two manakin species in the hybrid zone may result in negative pleiotropic effects associated with yellow plumage that ultimately decreases hybrid fitness (e.g. lower survival rate). Finally, studying a natural population for one or two field seasons yields just a snapshot of its evolutionary history. The yellow plumage allele(s) may in fact be sweeping through the population, but not at a rate appreciable by a few generations.

Conclusions

Several studies suggest that kin selection may contribute to lekking in manakins (Shorey *et al.* 2000) and other species (Höglund *et al.* 1999; Petrie *et al.* 1999; Krakauer 2005; Regnaut *et al.* 2006; Reynolds *et al.* 2009). Others, in contrast, failed to find evidence that kin selection alone can promote lekking in manakins (McDonald & Potts 1994; DuVal 2007; Loiselle *et al.* 2007; McDonald 2009) and other species (Madden *et al.* 2004; Gibson *et al.* 2005; Knopp *et al.* 2008; Lebigre *et al.* 2008). These inconsistent results beg the question of why kin selection may be important for lekking in some instances but not in others. In this *Manacus* hybrid zone, low levels of relatedness among white males but higher levels of relatedness among yellow males within leks suggests that kin selection alone cannot explain lekking in these mixed leks and perhaps manakins in general. In the hybrid zone, however, the operation of positive FDS on yellow plumage requires an additional mechanism to promote grouping because not all yellow males benefit equally from lekking. Indirect benefits through kin selection may therefore favour grouping of yellow males, which then facilitates positive FDS on yellow traits and the movement of this advantageous phenotype across the hybrid zone. Such dynamics may offer insights into why evidence of kin selection acting on lekking species has been inconsistent. In instances where alternative mechanisms are sufficient to drive lekking (e.g. female preference for male aggregations, Young *et al.* 2009), kin selection may play a limited role. Our results suggest that future studies should consider a more complex model with multiple independent mechanisms acting in unison to create positive group dynamics, which together enhance the individual's benefits of grouping in leks and perhaps mitigate the costs of this social behaviour.

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Data accessibility

Sample locations and microsatellite data: DRYAD entry doi: 10.5061/dryad.df7kh26m.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Maps showing the position of courts and colour of court owners in each lek.

Fig. S2 Mean pairwise relatedness (±SE) of males within and between leks grouped by plumage colour and including the five intermediate males.

Fig. S3 Randomization correlation between estimates of relatedness when Man3 was included and excluded in the analyses ($r = 0.97$, $N = 630$, $P < 0.001$).

Fig. S4 Randomization correlation between estimates of relatedness using the Queller & Goodnight (1989) method, and the Loiselle *et al.* (1995) and Wang (2002) methods.

Fig. S5 Distribution of pairwise relatedness values for yellow to yellow males (within leks = yellow diamonds; between leks = open diamonds), and white to white males (within leks = grey triangles; between leks = open triangles).

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