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Fine-scale genetic structuring on *Manacus manacus* leks

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Leks have traditionally been considered as arenas where males compete to attract females and secure matings. Thus, direct fitness benefits mediated through competition between males to fertilize females have been considered to be the primary force driving the evolution of lekking behaviour^{1,2}. Inclusive fitness benefits mediated through kin selection³ may also be involved in lek formation and evolution^{4,5}, but to date this theory has been largely ignored. According to kin-selection theory, both reproducing and non-reproducing males may gain indirect inclusive fitness benefits. If females are attracted to larger leks, non-reproducing males add attractiveness to a lek, and therefore, in a genetically structured population, boost the reproductive success of kin. Theory predicts that the attractiveness of leks is plastic, and that males establish themselves on a lek in which the top male, in terms of reproductive success, is a close relative⁶. Here we show that in white-bearded manakins (*Manacus manacus*), for which larger leks are more attractive to females^{7,8} and so secure the maximum number of matings, there is extraordinary fine-scale genetic structure, with leks being composed of clusters of related kin. We propose that males establish themselves where they find relatives to such an extent that they form groups within leks, and that such behaviour is consistent with kin-selection theory to maximize reproductive success of the group.

Manacus manacus males aggregate on display grounds (leks) to attract females for the purpose of mating. Each male defends a small court on the lek where he performs an acrobatic display. Leks are present all year round, annual mortality is low and birds may live up to 15 years^{9,10}. Birds remain at the same court all year and from one year to the next. Among these courts, however, male mating success is highly skewed⁷.

It is assumed that all males on a lek aim to increase their individual fitness by fathering as many offspring as possible. There are, however, potential indirect inclusive fitness effects that could operate, which have largely been neglected as a cue for lek evolution. In many lekking species, females prefer to mate in larger male aggregations than in smaller aggregations or with single males¹, which for a number of species leads to the general positive relationship between lek size and number of mating females. This is

certainly the case in *M. manacus*^{7,8}, for which larger lek sizes appear to attract more female visits (Spearman's coefficient of rank correlation (r_s) = 0.9, $n = 5$, $P = 0.037$; data from leks within our study in 1999 and 2000 and from two leks in ref. 7 where comparable data exist, female visits corrected for observational effort). As such, an individual could enhance his own inclusive fitness by joining a lek in which a relative is dominant and increasing the relative's reproductive success^{4–6}.

We used allele frequencies at four polymorphic microsatellite loci to estimate pairwise relatedness between males on two leks. Relatedness information¹¹ was combined with data on positions of male courts to delimit groups of related individuals. *M. manacus* males positioned themselves on a lek with relatives (Fig. 1). Furthermore, they positioned themselves in the lek to form spatially separated clusters of relatives, each with one or more reproductively successful male(s) (Fig. 2).

M. manacus females choose centrally positioned males in better physiological condition (L.S., unpublished data). Relatives may be more likely to gather around a top male and be accepted if they help in the initial attraction of females to the lek. By clustering near a top male, a family member may also improve his chances of acquiring a central court in the event of the death or injury of his successful relative. As suggested by our data, there may be more than one such family group on a single lek.

Related males may end up on the same lek by chance, as has been suggested in black grouse (*Tetrao tetrix*), for which male kin association on leks may be due to limited natal dispersal of males⁴. By forming related groups within leks, however, it seems that establishment on *M. manacus* leks cannot simply be attributed to limited male natal dispersal where males join the lek closest to their place of birth. Once on the lek, active choice among residential and newly arrived birds must take place¹², as we observed clusters of relatives within leks. Our data show that one group on each lek (group 2 in each case) consisted of individuals with a high average relatedness consistent with first-order relationships, whereas the other two groups consisted of individuals with lower average relatedness (Fig. 1). This suggests that the dynamics of reproductive sharing and competition could vary among groups within leks. Notably, the group showing the lowest average pairwise relatedness (r) (group 1 on lek 2) showed a more equal sharing of matings among the males (Fig. 2).

M. manacus individuals are unlikely to learn the characteristics of

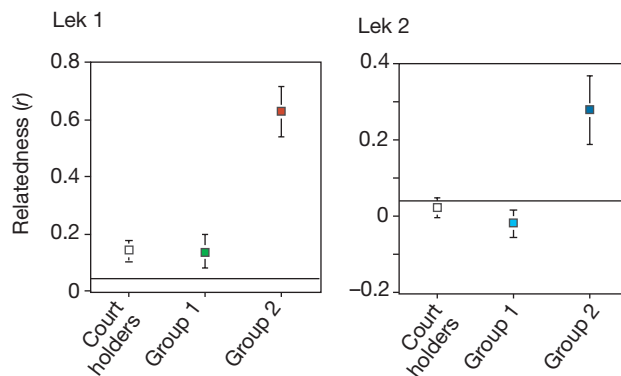


Figure 1 Mean relatedness values of male groupings. The line in each graph indicates the mean relatedness of both leks (bars indicate standard errors). Lek 1: green, group 1; red, group 2. Lek 2: light blue, group 1; dark blue, group 2. Statistical test, analysis of variance (ANOVA) in all cases. Lek 1: population versus court holders, $F_{1,2158} = 23.84$, $P < 0.0001$; population versus group 1, $F_{1,2082} = 3.95$, $P < 0.047$; population versus group 2, $F_{1,2088} = 153.3$, $P < 0.0001$. Lek 2: population versus court holders, $F_{1,2172} = 0.02$, not significant; population versus group 1, $F_{1,2094} = 1.13$, not significant; population versus group 2, $F_{1,2088} = 27.42$, $P < 0.0001$.

their relatives in the nest because clutch size is only one or two eggs and nest predation is high⁹. Thus, any male surviving to become an adult is unlikely to have shared the nest with any other bird in the population. The most plausible mechanism is that both prospecting floaters and established court holders use some kind of phenotype matching¹³ (probably self-referent phenotype matching) when juveniles float around the leks in the local population before settling on a lek.

Our study supports the hypothesis that kin selection is involved in lek evolution and supports a shift away from the model that leks are primarily arenas for intrasexual competition. Cooperation may also influence the evolutionary dynamics of leks, mediated either through kin selection or through by-product mutualism as suggested in long-tailed manakins (*Chiroxiphia linearis*)¹⁴. Moreover, our data suggest that wild populations that have previously been thought of as comprising unrelated individuals can be kin structured at a very fine level, and this structure can have profound effects on reproductive success. □

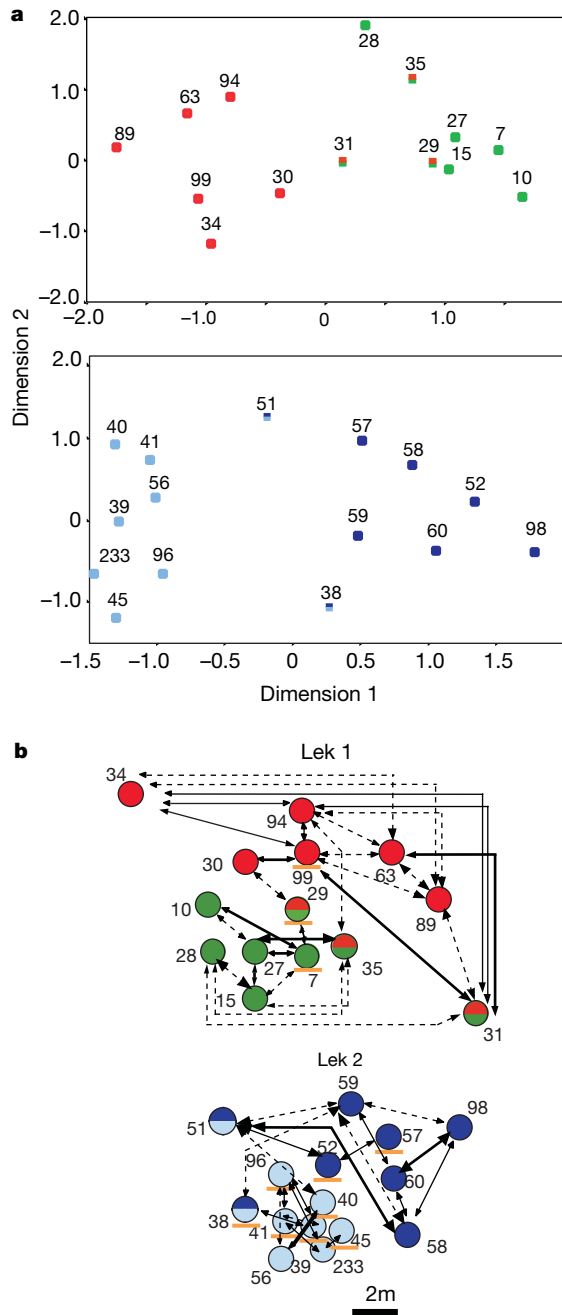


Figure 2 Levels of relatedness and spatial positioning on two *M. manacus* leks. **a**, Multidimensional scaling of the r values using a Euclidean distance model for leks 1 and 2. **b**, Maps showing the positions of individual court holders on each lek. Arrows connect related males: bold arrows, significant at $\alpha = 0.05$; thin arrows, $\alpha = 0.25$; dashed arrows, $r > 0.05$. Groups are coloured as in Fig. 1, males successful in obtaining copulations are underlined. We tested the spatial distribution of court holders by assigning them to one of either group found at each lek. Disregarding males spanning two groups, the observed spatial distribution of males was highly nonrandom (Fisher's exact test: lek 1, $P = 0.002$; lek 2, $P = 0.00058$). Males with two colours were related to both groups.

Methods

M. manacus leks were studied in the Paria area of Trinidad, West Indies (10° 45' N, 61° 15' W). We caught the males ($n = 250$) on 10 leks during April 1998, using mist nets. Roughly 4 μ l of blood was taken from the wing vein and stored in EDTA buffer. Each bird was ringed with a unique colour combination and subsequently released to facilitate behavioural observation. We observed two leks (lek 1, $n = 34$; lek 2, $n = 27$) over an eight-week period to establish whether a male was a court holder, and if so his position in the lek and mating success. Fourteen and fifteen males defended constant territories in a lek, and were thus defined as court holders. The remaining individuals were occasional visitors or unestablished juveniles.

We extracted DNA from blood samples using standard proteinase K/phenol–chloroform procedures. Four microsatellite loci (Man3, Man7, Man6 and Man8) specifically cloned in *M. manacus* were genotyped, resolving 14, 9, 11 and 11 alleles for each locus, respectively. For details on primer sequences and laboratory procedures, see Supplementary Information. Briefly, alleles were amplified using polymerase chain reaction (PCR), separated using polyacrylamide electrophoresis and visualized using silver staining.

We obtained unambiguous genotypes for every bird at every locus. No locus was found to deviate from Hardy–Weinberg expectations. The allele frequencies were then used in a regression estimator¹¹ to estimate pairwise relatedness (r) among all individuals on the lek. We obtained 95% and 75% confidence intervals for each estimate by bootstrapping the loci 1,000 times for each pair of individuals (program obtainable by request from J.S.). We used multidimensional scaling (MDS) to provide an unbiased representation of kin groupings on leks. MDS transforms pairwise relatedness generated from the microsatellite data to distance between points in two-dimensional space. We then compared kin groupings with the permanent territory positions observed. Males were considered related if the estimated r was above 0.05, as the expected r based on all possible dyads for both leks was 0.04 ± 0.004 (s.e.). Individual pairwise relatedness values ranged from -0.188 to $+0.939$ (lek 1) and -0.219 to $+0.914$ (lek 2). Owing to the limited number of microsatellite loci studied only some dyads were significant at $\alpha = 0.05$ (α , type I error probability) (Fig. 2).

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Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

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