The spatial distribution of avian relatives: do obligate army-ant-following birds roost and feed near family members?

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Abstract

The ocellated antbird (Phaenostictus mcleannani) feeds in groups and therefore is an informative species in which to study the biological factors that modulate avian group living. These birds congregate at swarms of army ants to capture fleeing prey, and previous observations suggest that males may be philopatric, feed with close relatives, and defend communal feeding ranges. We assessed whether kin selection could be an important factor maintaining group formation in a population of ocellated antbirds inhabiting continuous forest at La Selva Biological Station, Costa Rica, using radiotelemetry and 15 novel microsatellite markers. We predicted that the roosting areas of closely related adult males should overlap and that adult males feeding simultaneously at the same swarm should be highly related. We banded and genotyped 65 individuals (≥88% of the population) and radiotagged 30 of them. The results generally did not conform to our predictions. Little overlap occurred among the roosting areas of same-sex individuals, and nearest roosting neighbours (either same or opposite sex) were generally unrelated. A small proportion of male dyads suggested short-distance dispersal, but in general the distribution of genotypes within the study area approached randomness. We found little evidence of natal philopatry in either sex. Less than half of the feeding groups sampled included highly related males; most consisted of unrelated individuals. Hence, we found limited potential for kin selection to favour group living and suggest that other factors, particularly direct benefits (e.g. food intake), are probably more important than indirect effects (nepotism).

Keywords: Eciton burchellii, genetic structure, kinship, sociality, territoriality, Thamnophilidae

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Introduction

Animals form groups for many different reasons (Alexander 1974), and these groups can be loosely allocated into one of two categories. Some animal groups can be thought of as ephemeral ‘aggregations’, here defined as loose assemblages of individuals that regularly undergo fission or fusion events. Examples of such aggregations include flocks, schools, or herds that congregate daily to forage (Krause & Ruxton 2002). The benefits of ephemeral aggregations include reduction in predation risk, increased discovery and intake of food, increased access to mates, reduced costs of transportation, and reduced heat loss (Krause & Ruxton 2002). Alternatively, some social groups have more permanent affiliations such as troops, clans, or prides (Mitani et al. 2000; Van Horn et al. 2004). Often, these strong affiliations consist of genetic relatives (families) that form cooperative groups (Emlen 1995; Cockburn 1998; Hatchwell & Komdeur 2000; Clutton-Brock 2002; Ekman 2006). Just as in ephemeral aggregations, family members benefit directly from the association. However, family members can also benefit indirectly by cooperating with relatives, a process often referred to as kin selection (Clutton-Brock 2002; West et al. 2007).

Some cooperative behaviours are costly (West et al. 2007), which suggests that family living should confer advantages beyond those of simple aggregations (Nystrand 2007). Hence, the study of species that aggregate with relatives can be important to our understanding of the evolution of both ephemeral aggregations and families. Furthermore, the analysis of cooperative behaviours other than breeding
can be particularly insightful (Whitehouse & Lubin 2005; Grieser et al. 2006). Here, we assess evidence for kin structuring in obligate army-ant-following birds that feed in groups.

Obligate army-ant-followers are tropical birds that aggregate on swarms of nomadic army ants, particularly Eciton burchellii, to capture arthropods and small vertebrates that flee from the ants (Willis & Oniki 1978; Willson 2004). Diurnal swarms of E. burchellii capture leaf-litter invertebrates by forming a moving army that raids from a nest called a bivouac (Schneirla 1971). At night, the entire ant colony retreats into the bivouac and the colony usually resumes foraging the next day. Colonies of E. burchellii are nomadic, moving the bivouac ~100 m everyday for 2 weeks out of their 5-week brood-production cycle (Willis 1967; Franks & Fletcher 1983). As a consequence of the foraging and nomadic behaviour of the ants, these swarms can be thought of as a patchy resource that ant-following birds must locate daily. Aggregations of ant-following birds disappear when the ants stop foraging in the afternoon and reform the next morning at another swarm. E. burchellii colonies generally occur at low densities (Vidal-Riggs & Chaves-Campos 2008), yet some avian species are obligate ant-followers that always feed at swarms (Willis & Oniki 1978; Swartz 2001; Brumfield et al. 2007).

Our study species is the ocellated antbird (Thamnophilidae: Phaenostictus melamnani). Previous behavioural observations of banded individuals suggest these birds are completely dependent on army ants, form stable mated-pair bonds, and feed at swarms within large and stable home ranges (hereafter called feeding ranges) that extensively overlap with the feeding ranges of several neighbouring mated pairs (Willis 1973). Willis never banded chicks at nests, but his observations of banded juveniles led him to predict that males do not disperse from their natal feeding range and that they eventually mate with females who dispersed from other families. If so, this natal philopatry among males should produce multigenerational patrilineal families where male feeding and roosting/nesting ranges overlap (i.e. clans; Willis 1973). Willis also indicated that although ocellated antbirds congregate daily at swarms, presumptive relatives do not necessarily aggregate at the same swarm simultaneously. Further, he suggested that individuals on their own feeding range are aggressive towards trespassers but not towards presumptive relatives.

The behavioural observations described by Willis (1973) suggest that closely related males usually feed together and defend access to food within a communal feeding range. If so, group living could be maintained by indirect components of fitness obtained from these nepotistic interactions (i.e. kin selection). Recent studies suggest that indirect fitness components obtained from cooperation often play a major role in the evolution of family living (Russell & Hatchwell 2001; Baglione et al. 2003; Krakauer 2005; Eberle & Kappeler 2006; Richardson et al. 2007; but see Clutton-Brock 2002). Here, we test the possibility of such indirect fitness effects in ocellated antbirds using radiotelemetry and a large suite of novel microsatellites to evaluate relatedness (i) within groups of birds that fed together at a swarm, and (ii) between nearest roosting neighbours with overlapping feeding areas. In other words, we tested whether ocellated antbird feeding and roosting aggregations were composed of family members. We also evaluated male-biased philopatry by estimating genetic structure for each sex and by comparing the genotypic composition of males and females in the population (Goudet et al. 2002).

Methods

Study site and sample collection

We studied ocellated antbirds at La Selva Biological Station in Costa Rica (10°25'S, 84°01'W). The station encompasses 1611 ha of lowland rainforest partially connected to the 45 000-ha Braulio Carrillo National Park, and it offers convenient conditions for bird mapping, including: 60 km of trails, a grid system covering the entire forest (poles every 50–100 m), and an on-site geographical information system (GIS) laboratory with layers for topography, trail markers, and the orientation grid. Ocellated antbirds are the most common obligate army-ant-following birds at La Selva (Chaves-Campos 2003).

We selected a 392-ha study area within La Selva and conducted five 14-week field seasons between June 2004 and April 2007 to sample the genetic composition of roosting and feeding aggregations. Ocellated antbirds were captured opportunistically using mist-nets set up near bivouacs and swarms at the beginning and at the end of each field season (for a total of 10 sessions). During each session, 3–10 mist-nets were opened every 1–2 days between 05:00 h and 10:00 h. Each session lasted until at least 10 individuals were caught or for 3 weeks, whichever came first. Blood samples (~50 μL) were collected from all individuals by puncture of the brachial vein and mixed with 1000 μL of lysis buffer (Longmire et al. 1988; 1997). All birds were banded with unique colour combinations, and 10–19 individuals carried radio transmitters in each field season to identify their roosting sites and to follow them to swarms. Birds were followed for 9–11 weeks in each field season, and a total of 30 individuals carried transmitters during the study. Radio-tagged birds were deliberately selected to represent five neighbouring pairs per season (different subsets of neighbours in each season) and should therefore represent closely related males and their unrelated mates if these birds congregate in patrilineal clans. The transmitters (Holohil BD-2, 14 weeks of battery life) weighed ~1.8 g (3–4% of bird weight) and were attached using leg harnesses.

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(Rappole & Tipton 1991). Signals were detected using Habit HR 2600 Osprey or AOR 8200 receivers.

Juveniles were excluded from all genetic analyses to prevent inflation of relatedness values due to the presence of predispersing individuals. Juveniles spend about 5 months roosting and feeding with their parents and are easily recognized by their plumage, beak, and facial colouration (Willis 1973).

Molecular sexing and microsatellite isolation

Because ocellated antbirds are sexually monomorphic, we used genetic testing to determine sex. DNA was isolated using the methods described by Rudnick et al. (2005) without the dithiothreitol. The sex of each individual was determined using primers 2550F and 2718R (Fridolfsson & Ellegren 1999). Polymerase chain reaction (PCR) reagents, volumes, and electrophoresis conditions followed those of Rudnick et al. (2005). The thermal profile included an initial denaturation of 94 °C for 2 min, followed by 11 cycles of 94 °C for 30 s, 55 °C for 1 min decreasing 1 °C per cycle, and 72 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 45 °C for 1 min, 72 °C for 2 min, and a final extension of 72 °C for 5 min.

We isolated 13 novel microsatellite sequences from a genomic library using the method described by Williams & DeWoody (2004) with the following 5′ biotinylated oligonucleotides: (GTTT)_7, (GACA)_9, (GCA)_9, and (GGA)_9. Additionally, two other loci were characterized using microsatellite sequences from the spotted antbird (Table 1).

Table 1 Characteristics of 13 polymorphic microsatellite loci isolated from ocellated antbird genomic DNA, plus two loci amplified using unpublished primers (†) developed for spotted antbirds (HyNa). Fifty-seven individuals were genotyped at all loci. Number of alleles, allele range (bp), annealing temperature (T_m), mM of MgCl_2, expected heterozygosity (H_E) and observed heterozygosity (H_O) are provided for each locus.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primers 5′–3′ Repeat</th>
<th>Alleles</th>
<th>bp</th>
<th>T_m</th>
<th>MgCl_2</th>
<th>H_E</th>
<th>H_O</th>
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<tr>
<td>PMC3-27a</td>
<td>ACCTTTCCGCCAGCA</td>
<td>(GCA)_5</td>
<td>3</td>
<td>144–150</td>
<td>59</td>
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<td>AGGATTTGTCTCCCTCCTTTT</td>
<td>(GCA)_4</td>
<td>2</td>
<td>214–217</td>
<td>62</td>
<td>1.5</td>
<td>0.204</td>
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<tr>
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<td>CAAACTGGAGAAGACACCTTGGA</td>
<td>(GCA)_9, AGGA</td>
<td>2</td>
<td>150–152</td>
<td>65</td>
<td>1.5</td>
<td>0.429</td>
</tr>
<tr>
<td>PMC3-42†§</td>
<td>TGCTTGGAGATGAACTGACTCTG</td>
<td>(GCA)_2, RA)</td>
<td>2</td>
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<td>1.5</td>
<td>0.217</td>
</tr>
<tr>
<td>PMC4-11a‡</td>
<td>TTGACCCACCTCTGACTCTG</td>
<td>(GTTT)_10(GTTTT)_4</td>
<td>10</td>
<td>103–123</td>
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<td>PMC4-11b‡</td>
<td>GTCCTAGTTGCACCTCTGCTG</td>
<td>(CRA)_4, (CR)_10</td>
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<td>(AAAT)_2, AAA(AY)_7</td>
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<td>179–183</td>
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<td>(AAAC)_6</td>
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<td>168–179</td>
<td>53</td>
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<td>PMC2-134</td>
<td>TGAACCAATTCGTTAGT</td>
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<td>120–123</td>
<td>60</td>
<td>1.5</td>
<td>0.429</td>
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</tbody>
</table>

*Significant deviations from Hardy–Weinberg proportions at P < 0.0001 as assessed by genepop (Raymond & Rousset 1995); ‡Excluded from relatedness calculations; §Excluded from relationship estimation.
1× Taq polymerase buffer (5 mM KCl, 1 mM Tris-HCL pH 9.0, and 0.01% Triton X-100), 1.5–3.0 mM MgCl₂, 0.2 mM of each dNTP, and 1 U of Taq polymerase. The thermal profile included an initial denaturation of 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, Tm for 1 min (Table 1), 72 °C for 2 min, and a final extension of 72 °C for 5 min. Individuals were genotyped at each locus using an ABI 3730XL automated sequencer and GENEMAPPER version 4.0. More than half of the individuals were genotyped two or more times at every locus as a quality control measure. Microsatellite sequences were deposited in GenBank under Accession nos EU369190 to EU369204.

We evaluated the independence of our markers by calculating gametic phase disequilibria for all 105 possible microsatellite pairs using GENEPOP (Raymond & Rousset 1995) following the recommendations of Narum (2006). Standard assumptions necessary to estimate relatedness using microsatellite markers were tested. Potential deviation from Hardy–Weinberg equilibrium for each locus was assessed using GENEPOP, and the potential presence of null alleles was assessed using CERVUS 3.03 (Kalinowski et al. 2007), MICRO-CHECKER (Van Oosterhout et al. 2004), and by direct inspection of inheritance patterns between known parents and offspring.

**Roosting and feeding groups**

Radiotagged individuals were located throughout each field season to determine the composition of diurnal feeding aggregations and to localize nocturnal roosting sites. This was done by walking all trails within our 392-ha study area at least three times per week, once during the day and once at night during a 24-h interval (~10 km per day). The position of each bird was classified as ‘roosting’ if it was detected after sunset or as ‘feeding’ if the bird was found eating at a swarm (all diurnal radio signals were followed until we saw the birds feeding near ants). Roosting positions were determined by triangulation using bearings from at least two trail markers or grid posts. The precision of the triangulations was determined using the Lenth method (Lenth 1981a,b; Nams & Boutin 1991) implemented in the program LOCATE III (Nams 2006) using 32 randomly selected points for which we had more than two bearings. Feeding positions were directly mapped using landmarks existing both in the field and on the GIS map of La Selva.

Roosting and feeding areas (i.e. ranges) were estimated with the standard kernel method in the program Animal Movement 2.04 (Hooge & Eichenlaub 1997) in ARCVIEW 3.2. We restricted our calculations to individuals with ≥10 different feeding locations following Borger et al. (2006). The mean number of roosting and feeding locations per individual was 25 and 51, respectively. Feeding ranges were represented with 90% kernel isopleths following Borger et al. (2006). Individual ocellated antbirds roosted at different sites every night within a relatively large but consistent area, but most roosting localities for a given individual were restricted to the centre of this area (Fig. 1). To objectively delineate these core areas we used the method of Barg et al. (2005), which defines a core area as the first point where the difference in area between consecutive kernel isopleths (in 10% increments) at least doubles. After determining the roosting and feeding ranges of each radiotagged individual, we identified nearest neighbours by measuring the distance between roost centres in ARCVIEW. Roost centres were calculated using the ARCVIEW extension Weighted Mean of Points version 1.2c (Jenness 2004) using all roosting locations available per individual. We calculated percent overlap between roosting and feeding ranges of nearest neighbours to determine how often they fed and roosted together.

![Fig. 1 Map of the study area showing the feeding sites (open figures) and roosting sites (closed figures) of a parent-offspring dyad (male 24, triangles; male 43, circles). Roost centres are separated by four roost-area widths (i.e. nearest neighbours are not shown). Localities were collected between June 2004 and April 2007 at La Selva Biological Station, Costa Rica.](image-url)
The accuracy of these range estimates depends on how efficiently we sampled roosting areas. To estimate our sampling efficiency, we compared the observed number of roosting areas to the maximum expected number of roosting areas under saturation. This method should underestimate the proportion of roosting areas we sampled because most Neotropical insectivorous birds do not saturate habitats (Stouffer 2007). To determine the degree of saturation, we calculated the mean proportion of banded adults from a sample of 42 feeding aggregations that were censused (i.e. the identity of each bird was determined via leg bands).

Ocellated antbirds often feed in dense understorey and it is not always possible to directly observe all the individuals that compose a feeding group. Thus, we used a combination of radio-detections of birds at swarms and direct observations of feeding individuals to determine group composition. In some cases (49 out of 219 swarms), observational and radiotelemetry data were supplemented with individuals simultaneously mist-netted at the same swarms. Levels and patterns of relatedness were similar regardless of data type (observations, radiotelemetry, or mist-netting), so we present only the results of the combined feeding data set. Because birds usually switch swarms during the day (Willis 1973; J.C.-C. personal observation), we considered only the first feeding assemblage found per day unless we found two swarms simultaneously; in such rare cases we considered both.

Genetic analysis of roosting neighbours

We used Queller & Goodnight (1989) approach and the program SPAGEDI 1.2e (Hardy & Vekemans 2002) to calculate pairwise relatedness \( r \) between individuals that shared roosting areas. To estimate \( r \), we used unlinked loci without null alleles (Table 1). Because we found that same-sex individuals do not usually share roosting areas (see Results), we calculated pairwise relatedness between nearest neighbours. Significance was determined with a permutation test implemented in program RPTOOLS (Hood 2006) in which a number of randomly selected pairs (equal to the number of nearest-neighbour dyads observed) were chosen, with replacement, to calculate an average estimate of \( r \). The procedure was repeated 1000 times to generate random mean \( r \)-values to compare with the observed mean \( r \)-value among nearest neighbours. The analysis was conducted separately for same-sex dyads and opposite-sex dyads to evaluate our predictions.

We extended our assessment of spatial relatedness between nearest neighbours to the entire study area by performing a genetic structure analysis. This analysis allowed us to evaluate the male-biased local genetic structure predicted by Willis (1973). We used two approaches for this analysis: the spatial autocorrelation method of Smouse & Peakall (1999) implemented in GENALEX 6 (Peakall & Smouse 2006), and the regression of relatedness coefficients on distance (Vekemans & Hardy 2004) as implemented in SPAGEDI. Again, only unlinked loci without null alleles were utilized. Both methods estimate the genetic similarity between dyads whose geographical separation falls within specific distance classes. The method of Smouse & Peakall (1999) uses its own similarity coefficient while the method of Vekemans & Hardy (2004) uses standard relatedness coefficients. We used the coefficient of Ritland (1996) because it is the most powerful under most conditions (Vekemans & Hardy 2004). The first distance class corresponded to the mean distance between roost centres of nearest neighbours (about 250 m, as suggested by our results). Additional distance classes corresponded to individuals whose roost centres were located from two to seven roosting area widths apart (i.e. 500–1750 m). Seven `roosting area widths' was the maximum distance observed between roosting individuals in this study. Pairwise autocorrelation coefficients were averaged for each distance class and statistical departures from randomness were evaluated through random permutation and bootstrapping (1000 iterations in each case). Bootstrap errors tend to be larger than permutation errors and therefore bootstrap tests are more conservative (Peakall et al. 2003), but we present the results of both methods for comparison. The analysis was also separately conducted for males and females to test for potential male philopatry and female-biased dispersal (Willis 1973). Only adult individuals whose genotypes and roost centres were known were used in this analysis. We assumed distances between roost centres were stable over time based on high adult survival rates and high nestling mortality rates (Willis 1973).

We refined our conclusions from the spatial autocorrelation analysis by estimating the specific genetic relationships included in the average genetic autocorrelation values. To do so, we used the program ML-RELATE (Kalinowski et al. 2006) to identify putative relationships between dyad members. This method accounts for the presence of null alleles; thus, we used all of our unlinked loci to increase our power to identify the correct relationships (Table 1). We then classified the pairwise relationships according to the distance between the roosting centres of dyad members (separated in 250-m roosting-area width categories). As with relatedness pairwise calculations, relationships were calculated for all possible dyads and the results were classified according to the sex of the dyad members.

Test of sex-biased dispersal

Willis (1973) proposed a scenario of patrilineal family formation caused by permanent male philopatry and female-biased dispersal. The spatial autocorrelation analysis described above allows inference of dispersal patterns between sexes but does not constitute an explicit
test of sex-biased dispersal. We explicitly tested for female-biased dispersal using the mean corrected assignment index described by Goudet et al. (2002) with 10,000 randomizations. A significantly lower index value for one sex indicates that dispersal is biased towards that sex. This method was chosen because it is the most powerful for detecting sex-biased dispersal within a single population (Goudet et al. 2002). Only adult individuals were included in this analysis (24 males and 24 females).

Genetic analysis of feeding associations

Relatedness was averaged across all pairs of individuals sampled in each feeding assemblage. Statistical departures from randomness were determined using bootstrapping; we constructed a random distribution of $r$ and compared it to the observed mean value of $r$ calculated across all feeding groups. The distribution was constructed using poptools by randomly sampling (with replacement) pairs of individuals from the entire population to create random groups of feeding birds. In other words, we assumed no genetic structure within the study area. Sampling with replacement accounted for the fact that each feeding group was not independent. The number of groups and the number of individuals in each random group were as observed. We calculated $r$ for all pairs within each random group, averaged across pairs for each group, and then averaged across groups to generate a mean value of $r$. The procedure was repeated 1000 times for both same-sex dyads and opposite-sex dyads to test for the expected high levels of $r$ among feeding males. We also used the Kalinowski et al. (2006) method to identify putative relationships between dyad members with the goal of determining the proportion of highly related dyads within each feeding aggregation.

Results

Isolation of microsatellites

A total of 192 recombinant clones were sequenced and 86 (45%) contained microsatellites. We designed PCR primers for 55 of those, of which 46 yielded PCR products. Of these, only 13 loci were polymorphic. When combined with two primer sets derived from a spotted antbird library (courtesy of I. Lovette, Cornell University), we had a total of 15 polymorphic loci with which to estimate relatedness. After excluding loci with null alleles and/or those in linkage disequilibrium (loci 2-96, 3-30, 3-42, 4-2, 4-11a, 4-11b), we used a suite of nine unlinked loci for the relatedness analyses (Table 1). These nine autosomal loci had a mean of 7.4 alleles per locus in the 57 ocellated antbirds that were genotyped, and Mendelian patterns of inheritance were confirmed using three families for which both parents and all nestlings were genotyped at all loci. Mean relatedness between presumptive parent-offspring dyads was 0.46 (not different from 0.5; one-sample T-test, $P = 0.73$). Twelve unlinked loci, three of which probably have null alleles, were used in the estimation of dyadic relationships (7.1 alleles per locus; loci 2-96, 3-30, 3-42 excluded; Table 1).

Roosting and feeding ranges

Sixty-five ocellated antbirds (36 females, 29 males) were caught, bled, and banded from the study area. Feeding ranges were determined for 21 adults and roosting ranges were determined for 16 adults; these almost certainly represent the feeding and roosting ranges of 21 and 16 different mated pairs (see below). Feeding ranges were large (mean: 53 ha; 95% CI: 37–79 ha; $n = 21$) and partially overlapped among same-sex neighbours (54% on average, standard deviation = 30%, $n = 20$). Each feeding range could be represented as a rough circle with a mean diameter of 820 m. Roosting areas tended to be located in the centre of the respective feeding range (Fig. 1), were an order of magnitude smaller than feeding ranges (mean: 5.8 ha; 95% CI: 2.8–8.3 ha; $n = 16$; with a mean diameter of 266 m), and partially overlapped among same-sex neighbours (23% on average, standard deviation = 25%, $n = 15$). Both types of ranges generally remained stable through the study period, as suggested by three lines of evidence. First, most radiotagged individuals restricted their feeding and roosting activities to the same ranges across years. Second, recaptures of adults that were not radiotagged always occurred in the same portion of the study area. Finally, nesting was restricted to roosting ranges ($n = 10$ nests monitored).

Both feeding and roosting ranges were apparently shared by mates (see also Willis 1973). We monitored three nesting mated pairs for several field seasons and determined they completely shared their feeding ranges (94% reciprocal pairwise average) and extensively shared their roosting areas (64% reciprocal pairwise average). Thus, each feeding and roosting range seems to be occupied by a single mated pair.

Given roost range overlap among same-sex individuals, we calculated the distance between the roost centres of nearest neighbours to better estimate the spatial separation between these individuals. The mean distance from the centre of a given roosting area to the centre of the three nearest same-sex neighbour’s roosting areas was 248 m (95% CI: 212–282 m; $n = 29$; mates excluded). The difference between this distance and the mean width of a roosting area (266 m) reflects the degree of overlap between roosting areas, although a small part of the overlap could be due to triangulation error (1.3 ha, on average). Overall, however, these results clearly indicate that same-sex adults do not roost in communal areas and that they do not share roosting.
areas. They also suggest that the distance between roost centres of parents and adult reproductive offspring could be regarded as dispersal distances.

Proportion of roosting areas and birds sampled

The accuracy of distance and range overlap estimates depends on the proportion of roosting areas and birds sampled. We calculated the number of roosting areas that could fit in the study area by using the distance between the centres of nearest same-sex neighbours as the diameter of the area exclusive to each pair (212–282 m as shown above). The extremes of this 95% CI correspond to circular areas from 3.5 to 6.2 ha, which translated to a minimum of 25 and a maximum of 33 roosting areas (i.e. 50–66 adults) that can fit within the study area (we eliminated the area corresponding to secondary forest and open swamps from our calculations because these areas are unsuitable for nesting; J.C-C. personal observation) Thirty-seven resident adults are known to have roosted in our study area and 19 different roosting areas were mapped, which indicates that we sampled 42–75% of all possible roosting areas and 56–74% of all possible resident adults in a saturated population. We calculated the number of banded individuals at swarms within the study area to help determine whether the population actually was saturated. The average proportion of banded adults per feeding aggregation ranged from 88 to 95% (95% CI), suggesting that the population was not saturated and that we banded most of the individuals that feed in the study area. Most of the birds that were banded but not radiotagged were captured several times, but only on the edges of the study area, which suggests they do not roost within the study area. Thus, we likely sampled most of the roosting areas that exist within the study area, and our estimates of range overlap and distance between nearest neighbours should be reasonably accurate.

Table 2 Parent–offspring (PO), full-sib (FS), and half-sib (HS) relationships by distance (in roosting area units; see text) between roosting area centres of adult individuals. Dyads also classified according to the sex of dyad member: male (m) or female (f).

<table>
<thead>
<tr>
<th>Relationship</th>
<th>dyad</th>
<th>250</th>
<th>500</th>
<th>750</th>
<th>1000</th>
<th>1250</th>
<th>1500</th>
<th>1750</th>
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<tbody>
<tr>
<td>PO</td>
<td>m-m</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
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<td>2</td>
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<tr>
<td>FS</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>f-f</td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>HS</td>
<td>m-m</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>m-f</td>
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<td>1</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Combined</td>
<td>7</td>
<td>9</td>
<td>12</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Genetic patterns within potential roosting associations

Mean $r$ between mates (0.04) and between adult nearest neighbours (global $r = 0.0003$) was near zero. Mean $r$ between all male–male, female–female, and opposite-sex nearest neighbour dyads was close to zero and not significantly different from chance (Fig. 2; $P > 0.11$ in all cases; 29 birds). We calculated correlation coefficients between roosting area overlap (reciprocal average percentage) and pairwise $r$ between same-sex nearest neighbours and we found no significant patterns (results not shown).

The autocorrelation analysis (Smouse & Peakall 1999) revealed marginal genetic structure only for males that were not nearest neighbours ($n = 29$ birds). A peak of relatedness was revealed for males whose roosting centres were located two roosting areas apart from each other, but this peak was weak in magnitude and marginal in significance (significant for the permutation test only; Fig. 3a). We did not detect significant peaks for females (Fig. 3b), which suggest that females disperse farther than males. The method of Vekemans & Hardy (2004) produced virtually the same results as the Smouse & Peakall (1999) method (data not shown).

These results do not necessarily imply that all birds living in close proximity are unrelated. The distribution of relatives by distance suggests that some highly related male dyads, including father–son pairs, roost 500–1000 m from each other (Table 2). In fact, the peak of spatial autocorrelation (500 m) was caused by a relatively large proportion of highly related male dyads (four out of 15 dyads; see Table 2 and Fig. 3a). The proportion of related dyads was much lower at other distance classes (Table 2 and Fig. 3a), resulting in average autocorrelation coefficients near zero. The number of highly related female–female dyads was lower than the number of highly related male–male dyads (Table 2). Overall, the two analyses suggest that most individuals roosting in
close proximity to one another are unrelated, as are nearest neighbours. Therefore, we find little evidence for strong natal philopatry among males on roosting areas.

**Sex-biased dispersal**

The mean corrected assignment index was significantly lower for females than males (−0.76 vs. 0.76, respectively, \( P = 0.036 \)). Thus, dispersal is female-biased.

**Genetic patterns within feeding associations**

Adult ocellated antbirds were detected, observed, and/or caught at 203 *Eciton burchellii* swarms; they were never found feeding with other species of ants or without ants. The mean \( r \) across all 57 ocellated antbirds (global \( r \)) was nearly zero, but feeding groups consisted of relatives more often than expected (Fig. 4; \( P = 0.004, \ n = 203 \) swarms). Specifically, mean \( r \) among female–female dyads was not different from chance (\( P = 0.45, \ n = 81; \) Fig. 4), whereas \( r \) for both male–male and male–female dyads was higher than expected by chance (\( P = 0.01, \ n = 104 \) for male–male dyads; \( P = 0.001, \ n = 182 \) for male–female dyads). The **ml-relate** analysis revealed the presence of at least one pair of presumptive close relatives (i.e. half-sibs, full-sibs or parent–offspring pairs; 148 dyads met this condition) in only 43% of the feeding aggregations. Of the close relatives feeding together, most were estimated by **ml-relate** as filial male–male, filial male–female, brother–sister, or half-sib male–female. Presumably the filial assignments reflect father–son, father–daughter, and mother–son dyads, although **ml-relate** does not provide this level of resolution. There is some degree of uncertainty associated with precise genealogical assignments (Kalinowski et al. 2006), but our analyses clearly indicate that the vast majority (84%) of dyads found at aggregations were unrelated (Fig. 5). Thus, close relatives occasionally congregate in feeding groups but most groups do not include close relatives.
Discussion

Behavioural structuring of genetic relatedness

Our results show that the population of ocellated antbirds at La Selva is not structured in patrilineal clans that share communal areas as suggested by Willis (1973). Rather, the population is structured into roosting areas that are occupied by individual mated pairs. These roosting areas do not overlap substantially, and nearest neighbours are generally unrelated. Hence, there is no evidence to support the idea that relatives roost together as families. Both sexes disperse from their natal roosting area, but females tend to disperse farther than males, as revealed by the sex-biased dispersal analysis, genetic correlation analysis and by the spatial distribution of highly related female–female dyads. The low levels of mean genetic autocorrelation (Fig. 3) suggest that many individuals, principally females, immigrated into our study area and subsequently established roosting areas randomly (i.e. without regard to the relatedness of neighbours). Under this scenario, most offspring probably need to disperse only one or a few roosting areas from their natal home to find unrelated adults that could serve as potential mates (Table 2). In general, autosomal genes are effectively dispersed (mostly by females) and there is little genetic structure within the population.

Although related birds do not share roosts, our evidence suggests that close relatives occasionally (~40% of the time) feed together, presumably as a product of limited male dispersal. We found that ocellated antbird males who fed together were more related to one another than expected by chance. Feeding males were also more related to feeding females than expected. This pattern could have been caused by first-year birds in adult plumage that had not yet dispersed and were foraging with one or both parents. It could also be due to full- or half-siblings feeding together, either as adult or first-year birds. Simultaneous tracking of a few parent–offspring dyads confirmed that at least some predispersal birds remained with their parents for at least 1 month after they moulted into adult plumage (data not shown). We could not test whether males remain on natal territories longer than females; but if the males do remain longer, it could explain the relatively high levels of relatedness between adult males and between adult males and females within feeding aggregations (Fig. 4). Few parent–offspring dyads were nearest neighbours (seven out of 45 highly related dyads; Table 2), and thus, the proportion of predispersal birds potentially classified as adults was low. The erroneous inclusion of predispersers as adults could have inflated average pairwise relatedness within feeding aggregations, but even so, fewer than half (~40%) of the feeding aggregations were composed of close relatives. Thus, our data are largely inconsistent with the idea that group feeding is mainly maintained by kin selection.

The disparities between our results from La Selva and Willis (1973) study on Barro Colorado Island (BCI) could have been caused by the degree of isolation of BCI. BCI is a 1500-ha artificial island created by the damming of the Chagres River to form the Panama Canal around 1914, and the large body of water surrounding this island has proven to be an effective barrier for dispersal of ocellated antbirds (Willis 1974; Karr 1982, 1990; Robinson 1999). On the contrary, La Selva is part of a large continuous habitat with no obvious constraints on emigration and immigration. Thus, our results may represent more typical behaviour. Alternatively, the spatial distribution of relatives may not have been substantially different between BCI and La Selva. At La Selva, we found that some highly related individuals that were not neighbours overlapped considerably with respect to their feeding ranges but not their roosting ranges (Fig. 1). Roost area overlap seems to be the most reliable way to determine whether individuals occupy the same home range or not, but because of the lack of radiotelemetry technology Willis (1973) may have incorrectly interpreted extensive overlap in feeding ranges of fathers and offspring that were not nearest neighbours as permanent philopatry. Another possible explanation is that ecological conditions that lead to male natal philopatry were stronger at BCI.

Effect of kin selection on group living

Willis (1973) hypothesized that group formation in ocellated antbirds was driven largely by kin selection. Our data suggest that while opportunities for kin selection exist, they are limited. We confirmed Willis’ prediction that ocellated antbirds often forage with relatives, but our results clearly show that his hypothetical scenario of adult male relatives roosting near one another and cooperating to defend a communal feeding range is unlikely at La Selva. The dispersal of both sexes from the natal roosting area suggests it is more advantageous for males to establish
and defend an exclusive roosting area than to remain in the natal roosting area. Furthermore, the absence of shared roosting areas between mated pairs indicates that the costs of grouping into communal home ranges outweighs potential indirect as well as direct components of fitness that could be gained by cooperatively defending communal home ranges. Although mated pairs seem to establish exclusive roosting areas, the areas are effectively exclusive only at night. During the day, the feeding ranges of neighbouring pairs overlap extensively, with the consequence that several pairs congregate at the same swarms to feed in groups where some individuals can be highly related.

Ocellated antbirds normally feed with several conspecifics (Chaves-Campos 2003, 2005; mean of ~5 individuals per group in this study; range 2–20) who are often related to each other, thus providing favourable conditions for kin selection. Kin cooperation can occur in the form of nepotism, such as biased tolerance towards family members. Previous work on another obligate army-ant-following species, the bicoloured antbird (Gymnopithys leucaspis), suggests they may tolerate kin. There, mated pairs feeding near their own nesting areas gain access to the most productive parts of the ant swarm by displacing conspecifics from other nesting areas (i.e. site-related dominance; Willis 1967). The same behaviour occurs in ocellated antbirds (Willis 1973). Under this scenario, group formation could be favoured by indirect components of fitness if dominant birds identify kin and bias tolerance towards them (Lukas et al. 2005). However, even if kin recognition and biased tolerance exist in the ocellated antbird, fewer than half of the foraging aggregations include relatives. Thus, we do not expect the influence of kin selection on the maintenance of adult group formation to be strong.

Delayed dispersal is an alternative mechanism that could favour family living via kin selection if predispersing males are tolerated by their parents (Ekman 2006; Griesser et al. 2006). Both parents and offspring could benefit directly from short-term delayed dispersal if offspring established reciprocal tolerance with neighbours. In that case, parents and neighbours might benefit ultimately by having another nonaggressive neighbour that could help them defend the neighbourhood from aggressive strangers (Ridley et al. 2005).

In principle, kin selection could also play a role in the evolution of group formation through the recruitment of relatives to swarms. Because the ant bivouacs move (usually at night), diurnal obligate ant-followers face the daunting task of re-locating swarms every morning. Obligate ant-followers could use the vocalizations of other obligate ant-followers as cues to help locate swarms (Willis 1967, 1972, 1973; Chaves-Campos 2003). If ocellated antbirds cooperate to find swarms, then any bird discovering a swarm could conceivably signal (call) to conspecifics and directly benefit if they reciprocated in the future. Signalers could also benefit indirectly if close relatives were recruited. However, even under this hypothetical scenario, the effect of kin selection would not be strong because less than half of feeding aggregations contain close relatives (as in other species that usually forage in groups; Van Horn et al. 2004; Lukas et al. 2005; Langergraber et al. 2007; but see Morin et al. 1994; Wahaj et al. 2004).

In general, our data suggest that indirect components of fitness are unlikely to be the only factor maintaining group feeding among adult ocellated antbirds. Our results are in agreement with other studies that suggest that kin selection is not necessarily the most important factor in the evolution of vertebrate group formation (Clutton-Brock 2002; Ekman 2006; Bergmüller et al. 2007). Cooperation between individuals, regardless of their relatedness, has been invoked as a major factor driving sociality (Packer et al. 1990; Emlen 1995; Grinnell et al. 1995; Mitani et al. 2000; Werdenich & Huber 2002). Future research on group formation in obligate ant-followers can evaluate whether cooperation has evolved given the frequency with which these birds interact at swarms, particularly nearest neighbours. Other general benefits directly relevant to obligate ant followers include foraging tactics and individual energetic benefits (Giraldeau & Beauchamp 1999; Krause & Ruxton 2002), as well as the interaction of relatedness, food quality, food patchiness and food renewal on foraging decisions (Beauchamp & Fernandez-Juricic 2005; Nystrand 2007). The effect of food patchiness and renewal deserve careful attention because army ant colonies are inherently patchy over space and time (Franks & Bossert 1983; Franks & Fletcher 1983), such that specialization on ants could itself promote group feeding (Waser 1981; Johnson et al. 2002; Safran et al. 2007; but see Revilla 2003a, b).

Conclusions

Our data suggest there is little opportunity for kin selection to drive group feeding in ocellated antbirds. In consequence, direct benefits (such as increased food intake) may have contributed more to the maintenance of group feeding than indirect effects (e.g. nepotism). Our data contribute to a growing body of evidence that suggests indirect components of fitness are unlikely to be the most important factor in the evolution of vertebrate group and family living (Clutton-Brock 2002; Ekman 2006; Bergmüller et al. 2007).

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