Fine-scale genetic structure and dispersal in cooperatively breeding apostlebirds

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Abstract

In cooperatively breeding species, restricted dispersal of offspring leads to clustering of closely related individuals, increasing the potential both for indirect genetic benefits and inbreeding costs. In apostlebirds (*Struthidea cinerea*), philopatry by both sexes results in the formation of large (up to 17 birds), predominantly sedentary breeding groups that remain stable throughout the year. We examined patterns of relatedness and fine-scale genetic structure within a population of apostlebirds using six polymorphic microsatellite loci. We found evidence of fine-scale genetic structure within the study population that is consistent with behavioural observations of short-distance dispersal, natal philopatry by both sexes and restricted movement of breeding groups between seasons. Global F_{ST} values among breeding groups were significantly positive, and the average level of pairwise relatedness was significantly higher for individuals within groups than between groups. For individuals from different breeding groups, geographical distance was negatively correlated with pairwise relatedness and positively correlated with pairwise F_{ST} . However, when each sex was examined separately, this pattern was significant only among males, suggesting that females may disperse over longer distances. We discuss the potential for kin selection to influence the evolution and maintenance of cooperative breeding in apostlebirds. Our results demonstrate that spatial genetic structural analysis offers a useful alternative to field observations in examining dispersal patterns of cooperative breeders.

Keywords: cooperative breeding, genetic structure, isolation by distance, microsatellites, *Struthidea cinerea*

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Introduction

In most cooperative breeders, social groups are formed primarily through the retention of philopatric offspring, and dispersal is often limited to short distances from the natal territory by one or both sexes (Cockburn 1998; Ekman *et al.* 2004). Restricted or sex-biased dispersal results in clustering of individuals with respect to their relatedness (Sugg *et al.* 1996), and the resulting pattern of gene flow has important implications for the population biology and behavioural ecology of cooperative societies. For instance, genetic structure within a population can facilitate kin selection (Russell & Hatchwell 2001), since indirect genetic benefits will promote the evolution of helping only if

helpers are, on average, more closely related to recipient offspring than they are to other individuals randomly selected from the population (Queller & Goodnight 1989; Queller 1994).

High levels of relatedness among the members of a cooperative group will also increase the likelihood of incestuous matings (Koenig & Haydock 2004). Inbreeding has been linked to a significant loss of fitness in many species (Keller & Waller 2002), including some cooperative breeders (Brown & Brown 1998; Daniels & Walters 2000; Spottiswoode & Møller 2003). Such costs of inbreeding may in turn influence patterns of dispersal in cooperative societies (Greenwood 1980; Pusey & Wolf 1996). In many cooperative bird species, philopatry is biased toward one sex (normally males: Woolfenden & Fitzpatrick 1978; Greenwood 1980; Rabenold 1990) and/or members of one sex (normally females) typically disperse over longer

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distances (Walters *et al.* 2004), resulting in an effective spatial separation of kin.

The social structure of a population is often estimated by observing recruitment and dispersal of individuals in the field. However, recruitment and dispersal can be difficult to measure directly because the temporal and spatial limitations imposed on most studies restrict the detection of rare events, or those that involve large distances (Koenig *et al.* 1996). An alternative approach is to assess dispersal indirectly by examining the genetic structure within and between populations (Koenig *et al.* 1996; Sugg *et al.* 1996). Numerous studies have evaluated genetic structure over a large geographical area, but relatively few have analysed fine-scale genetic structure within avian populations. Most of these have demonstrated the existence of microspatial structuring among birds with lek mating systems (Höglund *et al.* 1999; Shorey *et al.* 2000; Höglund & Shorey 2003; Bouzat & Johnson 2004). Microspatial structuring is also expected to occur in many cooperatively breeding species, where restricted dispersal and high levels of philopatry may decrease genetic variation within breeding groups, relative to genetic differentiation between groups. To date, however, few studies have assessed fine-scale genetic structuring in cooperatively breeding birds (Painter *et al.* 2000; Double *et al.* 2005).

In this study, we examine patterns of fine-scale genetic structuring in the cooperatively breeding apostlebird *Struthidea cinerea* (Corcoraciidae), a medium-sized (33 cm, 130 g), sexually monomorphic, ground-foraging passerine that inhabits dry woodlands in eastern Australia. Apostlebirds are highly social and live in sedentary groups with between three and 20 members that remain together throughout the year (Woxvold 2004). Breeders rely heavily on helpers for successful reproduction, and no unassisted pair has been reported to fledge young (Woxvold & Magrath 2005). Both sexes delay dispersal, and comprise the majority of new recruits into established breeding groups (87.4%), although group membership is occasionally augmented by immigrants of either sex (Woxvold 2004).

Using a set of six polymorphic microsatellite loci, we first ask whether patterns of gene flow in apostlebirds are consistent with observations of a high level of natal philopatry by evaluating the level of genetic structuring within the population and comparing patterns of relatedness among birds within and between breeding groups. To evaluate prior observations of short-distance dispersal by apostlebirds, we then compare the genetic differentiation among groups with their geographical separation; gene flow resulting from restricted dispersal is expected to lead to greater similarity among sedentary groups living closer together ('isolation-by-distance', Wright 1943). Finally, potential differences in dispersal strategies by males and females are examined by assessing patterns of relatedness,

Fig. 1 Map of the study area showing the distribution of breeding groups of apostlebirds (1999, $n = 18$; 2000, $n = 15$) used in genetic structural and isolation-by-distance analyses. Distance between groups was calculated using nest sites. Territorial boundaries were estimated from censuses of group membership taken weekly (1999) or every 10 days (2000).

genetic structuring and isolation by distance (IBD) separately for each sex.

Materials and methods

Study population

The study was conducted on private farmland in southern New South Wales (34°34'S, 145°45'E), where apostlebirds inhabited discrete patches of woodland separated by expanses of grassland. We monitored the breeding activities of resident groups over three seasons (August to January) from 1998 to 2000, although sampling was sufficient to analyse population genetic structure only during 1999 and 2000. Breeding groups included in genetic analyses occupied c. 3100 ha of the study area, within which most individuals were sampled (Fig. 1). The proportion of birds (excluding fledglings) sampled in each season was 83.7% (1999, $n = 147$) and 79.7% (2000, $n = 177$). Group size at the start of each season ranged from 3 to 17 birds, and membership was monitored at least once every 10 days. During each census, the focal

Table 1 Dispersal data for 13 apostlebirds that emigrated to established breeding groups. Dispersal distance was measured as the distance between boundaries of breeding territories. Territorial boundaries were inferred from observations made during regular (≤ 10 days) censuses of each group

		No. males	No. females	Sex unknown
Banded prior to dispersal		5	2	1
Dispersal distance:	< 50 m	2	1	1
	0.75–1.0 km	2	1	
	1.5 km	1		
Banded after dispersal		2	2	
Total		7	4	2

group was followed for 10–15 min after first contact, with positions recorded on an aerial photograph of the study site (1:12 820). Territorial boundaries were inferred from the farthest distances that group members travelled before turning back towards the nest. Distances between the nests and territorial boundaries of separate groups were calculated from the study site aerial photograph.

During our research, most new recruits into established breeding groups were offspring that delayed dispersal (87.4%, $n = 103$; Woxvold 2004). However, group membership was partly augmented by immigration. Among immigrants of known gender, there was no apparent sex-bias in either the likelihood or distance of dispersal, although the sample size was small (Table 1). Of eight immigrants banded prior to dispersal, seven relocated within 1 km of the breeding territory in which they were originally marked, although the origin of unbanded birds ($n = 5$) was unknown (Table 1).

Adults were captured in mist nets (12 m, 63 mm mesh). Each bird was fitted with a metal band (Australian Bird and Bat Banding Scheme) and a unique combination of coloured darvic bands. A blood sample was taken from each adult (25–100 μ L) by brachial venipuncture using a 27G needle. A smaller sample (10–50 μ L) was taken from the medial metatarsal vein of each nestling when first captured. Blood samples were immediately transferred into 750 μ L of either lysis buffer (0.1 M Tris-HCl, pH 8.0; 0.1 M EDTA, pH 7.5; 10 mM NaCl; 0.5% SDS) or 100% ethanol and stored at 4 °C for up to 10 months. DNA was extracted using a salting-out procedure (Bruford *et al.* 1992) and sex was determined using the method described by Fridolfsson & Ellegren (1999).

Isolation of novel polymorphic microsatellite markers

Genomic DNA of apostlebirds was enriched for fragments containing GAAA and GT repeats which were then used to make a plasmid library and screened for repeat-containing colonies using the method of Gardner *et al.* (1999) as modified by Adcock & Mulder (2002). Positive clones were amplified in 50- μ L reactions and the polymerase chain

reaction (PCR) product purified using a QIAquick PCR purification kit (QIAGEN). Amplified DNA 250 ng was sequenced commercially (SUPAMAC).

Primers were designed to flank repeated regions using PRIMER 3 (Rozen & Skaletsky 2000). Primer pairs that yielded specific products of appropriate size following optimization of PCR conditions were subsequently tested for polymorphism. A 5'-M13 tail (5'-CACGACGTTGTAAAACGAC) was added to one primer from each pair for use in the dye-labelling method outlined by Boutin-Ganache *et al.* (2001). Polymorphism was assessed by typing 16 putatively unrelated apostlebirds (separate breeding groups). Each 10- μ L reaction contained 30–100 ng of DNA, 0.15 U of *Taq* polymerase and reaction buffer (Promega: 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100), 3 mM of $MgCl_2$, 200 μ M dNTPs (Promega), an M13 primer (200 nM) 5'-labelled with a Beckman Coulter dye (D2, D3 or D4), and the locus-specific tailed (15 nM) and un-tailed (200 nM) primers. Reactions were run in 0.2-mL microtitre plate wells (Greiner) overlaid with a drop of mineral oil (Sigma) on a Corbett Research PC-960C thermocycler. The thermal cycling program comprised an initial cycle of 90 s at 94 °C, followed by 40 cycles of 20 s at 94 °C, 20 s at the optimal annealing temperature, and 90 s at 73 °C. PCR products (0.25 μ L) were electrophoresed on a Beckman Coulter 8000XL automated sequencer using the CEQ 8000XL fragment analysis kit (Beckman Coulter). Fragment sizes were determined using the Beckman Coulter 8000XL fragment analysis software. The software package CERVUS (Marshall *et al.* 1998) was used to calculate allele frequencies and expected and observed heterozygosities.

Population genetic structure

We used several methods to genetically corroborate our behavioural observations of high levels of philopatry and short-distance dispersal by apostlebirds. First, the extent of genetic structuring within the population was investigated using an analysis of molecular variance (AMOVA) framework in ARLEQUIN (version 2.000; Schneider *et al.* 2000). This method apportions the total genetic variance into hierarchical

Table 2 Characterization of six microsatellite loci found to be polymorphic in the apostlebird *Struthidea cinerea*. The M13 prefix in the primer sequence indicates the site of attachment of a 5'M13 tail (CACGACGTTGTAAAACGAC). The final annealing temperature (T_M) is in °C, and the $MgCl_2$ concentration in mM. The number of alleles (N_A) and observed size range for each in base pairs (bp) was calculated for 254 individuals from 20 breeding groups. Observed (H_O) and expected (H_E) heterozygosities were calculated using 43 birds from 20 groups. Five loci were revealed through cross-amplification with primers developed for *Corcorax melanorhamphos*¹ and *Petroica goodenovii*². The cloned sequence of *Sci1* has been deposited with GenBank under the Accession no. AY642592. *Sci1* was also found to be polymorphic in *C. melanorhamphos*

Locus	Repeat motif	Primer sequence (5'–3')	T_M	($MgCl_2$)	N_A	bp	H_O	H_E
Sci1	(GAAA) ₇ AAAA(GAAA) ₂₆ GA(GAAA) ₂₂ (GA) ₆ (GAAAGAGA) ₂	F: M13-TGAAATAACTTCGATAGTGTCTCAGG R: GAACCGAACAAAACCTTTACTGTG	52	3	27	222–414	0.953	0.921
CmeG5 ¹	—	—	52	3	7	193–221	0.814	0.757
CmeG9 ¹	—	—	50	3	7	257–286	0.721	0.793
CmeH2 ¹	—	—	50	3	5	155–171	0.628	0.669
Pgm2 ²	—	—	52	3	4	223–239	0.558	0.565
Pgm4 ²	—	—	52	3	14	272–424	0.837	0.860

¹Beck *et al.* (2003); ²Dowling *et al.* (2003).

components (within individuals, and within and among breeding groups), and was used to compute Wright's (1951) fixation index F_{ST} for all individuals. The significance of the overall F -statistic was derived from null distributions generated from 5000 random permutations between individuals among breeding groups (= subpopulations). The degree of relatedness (R) between birds within and between breeding groups was then calculated and compared using a regression approach (Queller & Goodnight 1989) implemented by the program RELATEDNESS 5.0 (Goodnight & Queller 1999). Standard errors and 95% confidence intervals for mean R values were obtained by jackknifing across loci. Sex differences in population structure were characterized by calculating relatedness between pairs of the same sex. Finally, in a continuous population, restricted dispersal is expected to result in a pattern of isolation by distance (Wright 1943), whereby genetic differentiation increases linearly with the natural log of the geographical distance between individuals in a two-dimensional plane (Rousset 2000). Mantel tests (10 000 permutations) were used to examine the relationship between geographical distance (ln km) and the genetic distance between pairs of birds in different groups (Fig. 1), as defined by (i) $F_{ST}/(1-F_{ST})$ and (ii) pairwise R , using the program IBD version 1.52 (Isolation By Distance; Bohonak 2002). We tested for a potential sex difference in dispersal strategies by analysing patterns of IBD for males and females separately.

Results

Detection and characterization of polymorphic loci

Of 436 colonies screened from the GAAA-enriched library, 15 positive clones were identified and sequenced. Nine of these contained more than five repeats and included

flanking sequences suitable for primer design. From the GT library, 11 positives were identified from 136 colonies, with primers designed for six. A single locus (*Sci1*; GenBank Accession no. AY642592) was found to be polymorphic. This was supplemented with five primer pairs developed for microsatellites isolated in two other Australian passerines, the white-winged chough (*Corcorax melanorhamphos*, three loci, Beck *et al.* 2003) and the red-capped robin (*Petroica goodenovii*, two loci, Dowling *et al.* 2003). Table 2 shows the primer sequence for *Sci1*, and the optimal annealing temperature, $MgCl_2$ concentration, number of alleles found and observed and expected heterozygosities.

Sci1 was tested for cross-species amplification in the white-winged chough, the only other member of the family Corcoracidae (Schodde & Mason 1999). Under conditions optimized for use in apostlebirds, *Sci1* amplified fragments of nine different sizes from eight putatively unrelated individuals, suggesting suitability for use in parentage analysis.

Population genetic structure

Overall population F_{ST} was significantly greater than zero (1999: 0.1749, 2000: 0.1901; $P < 0.001$ both years), indicating that a significant amount of the genetic variance within the population existed between social units. Accordingly, average within-group relatedness (R_w) was significantly higher than that for pairs between groups (R_b) (Table 3). Average R_w was much greater than zero, while average R_b was less than zero (Table 3). A similar pattern was observed when the sexes were analysed separately (Table 3). Average pairwise relatedness between males within groups was similar to that of females within groups ($R_{w-male} - R_{w-female}$: 1999 = -0.0133 , $P = 0.696$; 2000 = 0.0154 , $P = 0.634$), consistent

Table 3 Average pairwise relatedness within (R_w) and between (R_b) apostlebird breeding groups during 1999 and 2000. Sample sizes (n) indicate the number of individuals used in each set of comparisons. P values denote the statistical significance of the difference between R_w and R_b . When analysing each sex, samples used in within-group analyses were sometimes smaller than between-group analyses due to the presence of small breeding groups where data for only one member for each sex was available, precluding comparison between individuals. Standard errors and 95% confidence intervals were obtained by jackknifing across loci

	Within-group				Between-group				
	n	R_w	SE	95% CI	n	R_b	SE	95% CI	$R_w - R_b$
<i>All</i>									
1999	114	0.3467	0.0537	0.1379	114	-0.0352	0.0052	0.0135	0.3819*
2000	112	0.3643	0.0535	0.1375	112	-0.0412	0.0054	0.0139	0.4054*
<i>Male</i>									
1999	69	0.3791	0.0631	0.1623	70	-0.0366	0.0163	0.0420	0.4157*
2000	61	0.4061	0.0662	0.1701	61	-0.0515	0.0176	0.0452	0.4576*
<i>Female</i>									
1999	39	0.3923	0.0501	0.1287	44	-0.0200	0.0118	0.0303	0.4123*
2000	48	0.3908	0.0371	0.0954	51	-0.0223	0.0167	0.0429	0.4131*

* $P < 0.0001$.

with observations that both sexes delay dispersal, and that there is no apparent sex bias among immigrants into established groups.

There was a strong pattern of IBD observed in the population as a whole during both 1999 and 2000. Pairwise relatedness decreased significantly with geographical distance (ln-km) between breeding groups during both years (Mantel test: 1999, $P = 0.007$, $n = 153$; 2000, $P = 0.001$, $n = 105$). A similar though weaker correlation was observed between genetic distance as measured by $F_{ST}/1-F_{ST}$ and geographical distance (1999: $P = 0.030$; 2000: $P = 0.051$). When the sexes were analysed separately, males showed a stronger pattern of IBD than females. Among males, geographical separation was significantly and inversely related to pairwise relatedness (1999: $P = 0.008$, $n = 153$, Fig. 2a; 2000: $P < 0.001$, $n = 105$, Fig. 2b) and positively correlated with genetic distance (1999: $P = 0.013$), although this trend was not significant during 2000 ($P = 0.054$). In contrast, neither kinship nor genetic distance between females was significantly related to geographical separation in any year (pairwise R : 1999: $P = 0.134$, $n = 136$, Fig. 2(c); 2000: $P = 0.119$, $n = 91$, Fig. 2(d); $F_{ST}/1-F_{ST}$: 1999: $P = 0.151$, $n = 66$; 2000: $P = 0.051$, $n = 55$).

Discussion

Our results reveal the existence of fine-scale spatial genetic structure within a population of apostlebirds. Significant global F_{ST} values, overall patterns of IBD, and higher levels of relatedness among birds within groups than among birds between groups are all consistent with behavioural observations of natal philopatry by both sexes, restricted dispersal over short distances, and the highly sedentary (between-season) nature of most social units (Woxvold

2004). While many birds exhibit restricted dispersal (Greenwood 1980), few studies have analysed fine-scale genetic structure within avian populations (e.g. Shorey *et al.* 2000; Bouzat & Johnson 2004), and this is one of the first studies to demonstrate this pattern in a nonlekking species (Piertney *et al.* 1999; Painter *et al.* 2000; Double *et al.* 2005).

Genetic structuring within cooperative societies is an important prerequisite for the development and maintenance of helping behaviour. Indirect genetic benefits accrued by a helper are the product of (i) its contribution to the number of young raised by the group, and (ii) the coefficient of relatedness between helper and recipient. It has previously been demonstrated that apostlebird helpers enhance the production of nondescendent offspring (Woxvold & Magrath 2005).

However, for kin selection to influence the maintenance of cooperative breeding the degree of relatedness between members of a breeding group must be higher than that of average pairings between groups (Queller & Goodnight 1989; Queller 1994). Our results confirm this pattern (previously based on short-term observations of delayed dispersal in the field) for the present study.

Restricted dispersal and patterns of IBD may also mitigate the reduction of indirect genetic benefits accruing to philopatric helpers in the event of successful matings by an immigrant. Such events are likely to be common given the importance of avoiding incestuous mating (Koenig & Haydock 2004). In our study population, we observed both occasional polygamy (4/15 group-seasons where mating system resolved) and divorce ($n = 1$ group; Woxvold 2005), indicating that nonbreeding group members varied in their degree of relatedness to breeding birds. Moreover, in a detailed analysis of nesting behaviour

Fig. 2 Relatedness between pairs of male (a, b; open triangles) and female (c, d; open circles) apostlebirds in different breeding groups in relation to the logarithm of geographical distance (km) between groups during 1999 (a, c) and 2000 (b, d).

(Woxvold 2005; Woxvold *et al.* in press), most nonbreeding members of these groups continued to provision nestlings. In such circumstances active helpers may enjoy a range of direct genetic benefits (skills acquisition, improved chances of future independent reproduction, etc.), although many will accrue lower indirect returns than those assisting first-order relatives. However, where dispersal normally occurs over short distances and neighbours are more closely related than birds from distant groups, mating by immigrants from nearby groups may not reduce the relatedness between helpers and recipients as much as random dispersal.

Our data revealed a stronger pattern of IBD among males than among females, suggesting that males tend to disperse over shorter distances. It is possible that IBD was not significant among females due to the smaller sample in the population (type II error). Nevertheless, insofar as these results do reflect more restricted dispersal by males, they have important consequences for our understanding of the comparative behavioural ecology of male and female apostlebirds. In the absence of associative cues, birds may have difficulty recognizing unfamiliar kin following dispersal (Koenig & Haydock 2004; Sharp *et al.* 2005), and a sex-bias in dispersal distance will minimize the chances of cohabiting with a relative of the opposite sex. In most cooperative birds, males are the more philopatric sex, and are more likely to acquire breeding status in or near the natal territory (Komdeur & Edelaar 2001; Cockburn *et al.* 2003; Walters *et al.* 2004). Accordingly, females

settle further from their natal territory in most cooperative species for which data are available, a pattern that has been interpreted as a mechanism for avoiding close inbreeding (Walters *et al.* 2004). As yet, there are too few direct observations of dispersal by apostlebirds to indicate which sex is more likely to breed in the natal neighbourhood. However, genetic analysis suggests that females may employ a similar inbreeding avoidance strategy to that observed in other cooperative species.

Our conclusions about philopatry and dispersal from genetic structural analysis of the study population are similar to those obtained from direct observations of individuals of known identity. However, our genetic data also revealed that male and female apostlebirds may adopt subtly different dispersal strategies, a pattern which was not evident from the small sample of known dispersal events. Detecting such subtle differences in the field would require a lengthy study conducted over a large spatial area. Thus, molecular analysis of spatial genetic structure offers a useful and cost-effective alternative to field observations in examining dispersal patterns of cooperative breeders.

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