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Genetic relatedness of communally breeding guira cuckoos

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Abstract. A population of sexually monomorphic, communally breeding guira cuckoos, *Guira guira*, located near Brasilia, Brazil, was studied during three breeding seasons. Previous studies indicated considerable reproductive conflict among adults over contribution to the incubated clutch. In this study parentage and relatedness among nestlings were evaluated by DNA fingerprinting of samples from four nesting groups. The hypotheses tested were: (1) that guira cuckoo breeding groups consisted of monogamous pairs: and (2) that a single female contributed most of the incubated eggs in each group. The data indicate that nestmates were offspring of different adults. Some adults did not breed. The monogamy hypothesis was not supported by the data. Most related nestlings (78%) appeared to be half-, but not full-siblings. Parentage analyses directly suggested polygynandry in one breeding group and polygamy in another. Only 41% of pairs of nestlings selected at random from the same nest were found to share statistically significant numbers of bands. A single female was not responsible for the majority of young in a nest; however, further research is necessary to evaluate the possibility that dominant females contribute more incubated eggs than do subordinates. The data exemplify a breeding system in which nestling rivalries and reproductive conflict among adults would be expected.

Communal breeding, a system in which three or more breeders concurrently raise young in the same nest (Koenig & Pitelka 1981), has been described in a handful of avian species from diverse taxa. Communal breeders include polygynous groups (e.g. magpie geese, *Anseranas semipalmata*, Frith & Davies 1961), polygynandrous groups (e.g. acorn woodpeckers, *Melanerpes formicivorus*, Koenig & Stacey 1990; and pukeko, *Porphyrio porphyrio*, Craig & Jamieson 1990) and groups of monogamous pairs (e.g. groove-billed anis, *Crotophaga sulcirostris*, Vehrencamp 1977, 1978; smooth-billed anis, *Crotophaga ani*, Davis 1940a; Loflin 1983). Some species show all of these possibilities (e.g. dunnocks, *Prunella modularis*, Davies 1990).

Vehrencamp and colleagues (Vehrencamp 1977, 1978; Vehrencamp et al. 1986; Koford et al. 1990) described groove-billed ani breeding groups made up of two or more females and their mates. These monogamous females laid their eggs in a single nest and engaged in several behaviour patterns that increased the number of their own young hatching within a clutch. For instance, prior to egg laying, a female might visit the nest and toss out some eggs laid by other females (Vehrencamp 1977), thus biasing the representation of that female's offspring in the brood. Last-laying females contributed significantly more incubated eggs than earlier layers (Vehrencamp 1977).

Although the success of individual female anis was estimated by determining female specific egg-shell characteristics (Vehrencamp 1977), the mating system has not been examined genetically. Indeed, with the exception of the dunnocks (Burke et al. 1989), studies of communally breeding birds are lacking genetic data addressing relatedness between adults and nestlings.

Guira cuckoos, *Guira guira*, like anis, are members of the Crotophaginae, in the family Cuculidae. Field observations (Macedo 1991) suggest that the communal breeding system of guira cuckoos is similar to that of anis (Davis 1940a, b, 1942; Vehrencamp 1977, 1978; Loflin 1983; Macedo 1991). Like anis, guira group sizes range from two to more than a dozen adults and breeding has been characterized by large communal clutches, high egg

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losses, and variable patterns of participation in caring for the young by individual adults (Macedo 1991). Egg losses are due, in part, to egg tossing, a prominent feature of the reproductive period of guira cuckoos that appears similar to activities of groove-billed and smooth-billed anis (Vehrencamp 1977, 1978; Loflin 1983; Antas & Cavalcanti 1988; Cavalcanti et al. 1991).

Several studies indicate that monogamy is the predominant mating system for anis of the genus *Crotophaga*. Groove-billed anis apparently 'form conspicuous monogamous pair bonds within their groups' (Vehrencamp et al. 1986, page 355), and other studies support these observations (Skutch 1959; Köster 1971; Vehrencamp 1977). There are conflicting reports concerning the mating system of smooth-billed anis varying from 'generally monogamous' (Loflin 1983) to descriptions of monogamy, polyandry and polygyny (Davis 1940a). Regarding a population of guira cuckoos in Argentina, Davis (1940b, page 483) remarked on a 'tendency for the birds to remain in pairs'. Since crotophagines are sexually monomorphic in plumage, behavioural observations of paired birds do not constitute solid evidence regarding mating patterns. It is possible that monogamy has been overestimated as being the predominant mating pattern in this group of birds. Genetic analyses are necessary to verify or refute the behavioural assessments of such mating systems.

Given the apparent similarities between guira cuckoos and groove-billed anis, we expected their mating systems to be similar. Here, we use DNA fingerprinting to examine whether guira cuckoos mate monogamously and whether one pair (presumably the last to lay) contributes significantly more nestlings to the nest. Genetic monogamy predicts that both sexes will parent young with only one mate and that nestlings in the same nest will be either unrelated or full-siblings, but not half-siblings. A breeding bias would be supported if one set of siblings outnumbers other sets in the same nest.

We use multi-locus DNA fingerprinting as a tool to infer parent–offspring and sibling or half-sibling relationships. Such an approach is relatively simple and conclusive (showing that all bands present in a nestling can be accounted for in one or other parent and declaring an exclusion when this is not so) when DNA samples from both parents are available (Burke & Brufford 1987; Wetton et al. 1987; Burke et al. 1989; Gibbs et al. 1990; Rabenold et al. 1990). In this study we examine relatedness in families to evaluate the utility of DNA fingerprinting under less than ideal conditions (i.e. not all group members sampled). Because of incomplete sampling, we were not able to base our analyses on exclusions. Instead, we base the analyses on the proclivity of relatives to share greater numbers of fingerprint bands than non-relatives do. Other studies have used band-sharing frequency confidence limits of known genetic relationships for determination of *r*-coefficients (e.g. Westneat 1990; Piper & Parker Rabenold 1992). Such data are not available for guira cuckoos. Instead, we used a 2×2 chi-squared contingency table with adjusted expected frequencies (based on band sharing between unrelated adults) to test for the significance of band-sharing coefficients. High levels of band sharing were accepted as an indication of a genetic relationship if found to be statistically significantly higher than expected by chance in the study population.

METHODS

The Study Species

The guira cuckoo is 36 cm in length and is a sexually monomorphic bird that is conspicuous and common throughout the savanna habitats of South America (Sick 1984). Guira cuckoos forage on the ground for insects and small vertebrates (Macedo 1991). In the study area, all nests were built near the tops of a single tree species, *Auraucaria angustifolia* (a thorny tree introduced from southern Brazil). Nests were either reoccupied by the birds and renovated through the addition of a few twigs, or constructed anew near the tree top. Several group members contributed to incubation, nest attendance and chick feeding. Competitive interactions among group members included egg and chick removals (see Macedo 1991).

Field Methods

We studied group structure and reproductive behaviour for a population of guira cuckoos in central Brazil, near Brasilia (15°47′S, 47°56′W) from July 1987 to January 1988, August to November 1988, and July to October 1990 (Macedo 1991). Breeding activity was most intense during the rainy season (September–April; Macedo 1991). The

DNA fingerprinting data reported here are from the 1988 field season. The study area of approximately 3000 ha included the breeding territories of 28 groups in 1988. We used nest identity codes to designate a field site location, the identity of a group, and the breeding attempt number. For instance, in the nest code A3.1, the 'A' specifies a locality, '3' is the group in question and '1' indicates the group's first breeding attempt of the season. Individuals are designated by a sample number preceded by an 'A' for adult samples or a 'N' for nestling samples (e.g. A15, N17).

We captured and colour banded 91 adult guira cuckoos over the entire study, using caged, hand-reared lure birds (two adult guira cuckoos) surrounded by hardware cloth with monofilament nooses. We conservatively estimated that the adult population under study consisted of at least 173 birds in 1987, 125 in 1988 and 130 in 1990. Guira cuckoos are sexually monomorphic, and we did not perform laparoscopies to determine the genders of sampled birds. In 1988, blood samples (200 to 500 µl) were collected with sterile hypodermic syringes from brachial veins of 41 adults and 32 nestlings from 12 groups, combined with 100 µl of TNE_2 (0·01 M Tris-HCl, 0·01 M NaCl, 0·002 M EDTA, pH 8·0) and stored at −20°C.

We examined active nests by climbing the nest tree daily during egg laying, and less frequently during the incubation period. These census data included a number of breeding parameters (e.g. communal clutch size, number of group members in attendance during each visit, etc.). After the eggs hatched, we checked the nestlings on days 1, 2 and 3, after which we banded the young and collected blood samples from them (see Macedo 1991 for details).

Molecular Methods

We extracted genomic DNA from whole blood stored frozen in TNE_2 (50–200 µl of blood in 100 µl TNE_2). We mixed the equivalent of 50–100 µl of whole blood in 3·4 ml of 'lysis buffer' (4 M Urea; 0·2 M NaCl; 0·1 M Tris-HCl, pH 8·0; 0·5% *n*-lauroylsarcosine; 0·01 M CDTA), and then digested the mixture with 500 µl proteinase K (60 units) at 55°C overnight. We then extracted samples (in an automatic nucleic acid extractor; Applied Biosystems, Foster City, CA) twice with phenol/chloroform (70:30) and once with chloroform, and then precipitated each sample in ethanol (70%) and sodium acetate (0·3 M). We dissolved the DNA in 0·5–1·0 ml of TNE_2 and quantified it by spectrophotometry and agarose gel electrophoresis.

Multi-locus DNA Fingerprinting

We digested DNA samples with *Alu*I or *Hae*III, precipitated them in 70% ethanol and dissolved them in 15–20 µl of TNE_2. Then we combined 3–4 µg of each DNA sample with 3 ng of a mixture of lambda DNA (digested with *Hin*dIII + *Hin*dIII/*Eco*RI + *Bst*EII; 13 bands between 21·2 and 2·3 kb; largest gaps between 21·2 and 8·4 kb and between 3·5 and 2·3 kb), as a control for possible differential mobility among samples (Galbraith et al. 1991), and then electrophoresed through a 0·8% agarose gel at 1·2–1·5 V/cm for about 50 h. We transferred size-fractionated DNA by Southern blotting to charged nylon membrane (Genescreen Plus) or to charged polyvinylidene difluoride membrane (Immobilon-N). Blots were air-dried overnight and then vacuum-baked for 2 h. To facilitate alignment of X-rays, we marked the corners of all blots with IBI Glo-Juice (International Biotechnologies, Inc., New Haven, CT), a product containing photophosphor, which emits small amounts of visible light after exposure to bright light sources.

Blots were rinsed in 5 × SSC (1 × SSC = 0·15 M NaCl, 0·015 M sodium citrate) and then prehybridized for 4–14 h at 65°C in a bag containing 12–24 ml of 7% SDS, 0·001 M EDTA, 0·26 M sodium phosphate, pH 8·0 and 1% bovine serum albumin (fraction V; Westneat et al. 1988). We probed the blots sequentially, with Jeffreys' 33.15, Jeffreys' 33.6 (J33.15 and J33.6; Jeffreys et al. 1985) and pSP2.5RI mouse probe homologous to the *Drosophila* periodic locus (PER for periodic; Georges et al. 1988) labelled by random primer extension (Pharmacia oligolabelling Kit; $>8 \times 10^8$ cpm/µg). Hybridizations were performed at 65°C for 14–20 h. Blots were washed at 65°C in 2 × SSC, 0·1% SDS. Filters were exposed to X-ray film for 2–14 days at −70°C using one intensifying screen (Dupont Cronex lightning plus). We stripped blots with 0·4 M NaOH at 42°C for 20–30 min for repeated probings. We probed blots first with the fingerprinting probes and finally with lambda (to provide size markers in each lane).

We scored the blots by eye with the aid of clear acetate sheets taped to the X-ray (Galbraith et al. 1991). We marked lambda bands from the blot being scored on an acetate sheet which we then

Table I. Two-by-two contingency table of variable names for the calculation of band-sharing coefficient and chi-squared with adjusted expected frequencies for statistical evaluations

Individual 1	Individual 2		
	Band present	Band absent	Row total
Band present	a	b	e
Band absent	c	d	f
Column totals	g	h	n

aligned (by the location of Glo-juice registration marks and the edges of blots themselves if clearly visible on X-ray film) and taped to the blot's fingerprinting autoradiograph. Scorable bands (bands that were sufficiently intense that they would be detectable in all lanes if present) were identified in each lane. We identified bands of comparable intensity that migrated approximately the same distance as the same fragment if found to be within 1 mm of each other (as measured from the nearest lambda size marker). We considered fragments that differed by greater than 1 mm to be distinct fragments. The 1 mm cut-off is based on empirical measurements; bands from individuals loaded twice on the same gel revealed that 96% ($N=128$) of the measurements differed by 0–0·5 mm. We did not score relatively faint bands that could have been obscured by similar-sized intense bands.

We compared banding patterns between dyads of individual birds in two-by-two contingency tables in which values for each of the categories along with row and column totals were determined (Table I). We analysed presence/absence data for all bands scored using SAS (Statistical Analysis System Release 6.04). We compared banding patterns of sample dyads using the descriptive statistic D

$$D=\frac{2\times a}{2\times a+b+c}$$

This provides an index that ranges from 0, no bands shared, to 1, all bands shared (Wetton et al. 1987). To test for the significance of high values of D one could use a χ^2 contingency table. However, the normal generation of expected frequency values for the chi-squared contingency table would not adjust for population-specific band-sharing levels. Unrelated individuals are expected to share some bands due to the finite number of alleles at any given locus (Lynch 1988). J. S. Quinn and R. Morrison (unpublished data) provide a way to modify the calculation of expected frequencies to take the average of band sharing between unrelated members of a population into account.

The calculation of this chi-squared with adjusted expected frequencies begins with the calculation of

$$\text{ø}=\frac{(a\times d-b\times c)}{\sqrt{e\times f\times g\times h}}$$

The ø coefficient is related to χ^2 as follows

$$\chi^2=n\text{ø}^2$$

We calculated an average ø for 96 dyads of unrelated individuals, assuming that members of dyads in which individuals were from different nesting groups were unrelated to each other. Violation of this assumption yields a more conservative test (type I error less likely). We use this average value of ø, hereafter denoted ø′, as a means to calculate chi-squared expected values adjusted to the band-sharing characteristics of the population. Expected frequencies (denoted a', b', c' and d') were calculated for each dyad as follows

$$a'=(e\times g+(\text{ø}'\times\sqrt{e\times f\times g\times h}))/n$$

by substitution

$$b'=e-a'$$

$$c'=g-a'$$

$$d'=h-b'$$

We used these expected frequencies in a chi-squared test with one degree of freedom. Computer simulations of this approach produced very close to the expected proportions of randomly generated values that exceeded the critical values for various levels of alpha (J. S. Quinn and R. Morrison, unpublished data). Additionally, these proportions were relatively insensitive to differences in the population average of the amount of band sharing between unrelated individuals (J. S. Quinn and R. Morrison, unpublished data).

RESULTS

Samples Analysed

To test for genetic monogamy and over-representation of one female's eggs in the respective incubated clutch, we examined relatedness among

all groups for which we had sufficient blood samples. We conducted genetic analyses on four groups (A3.1, A5.2, E2.1, E4.1) where the sampling included some adults (total of 21 of 36 in attendance as well as nestlings (total of 18 of 18 hatched). Distances between sampled nesting sites were as follows: A3.1 to A5.2: 900 m; A3.1 to E2.1: 4105 m; A3.1 to E4.1: 5474 m; A5.2 to E2.1: 3895 m; A5.2 to E4.1: 5632 m; and E2.1 to E4.1: 2211 m. Distances between the centre of an active nesting site and the outer circumference of a neighbouring site ranged from 200 to 2700 m with an average of 603 m. Guiras that were part of actively reproducing groups were rarely seen more than 300 m away from their nests.

*Alu*I digested DNA samples were blotted and probed with PER, revealing a simple pattern with a few (one to five) very strong bands (Fig. 1). Assuming that each band in a nestling was inherited from one parent or the other, adults sharing one or more bands with a particular nestling were suspected to be parents of that nestling. When one or more nestling bands were not present in any sampled adult, we concluded that at least one parent had not been sampled. Table II lists sampled nestlings and adults according to whether or not bands were shared between them, and whether at least one parent of a particular nestling had not been sampled.

*Hae*III digested DNAs probed with J33.6 or J33.15 revealed fingerprints with well-separated bands, which allowed easier fragment size discrimination, facilitating band-sharing analyses. Accordingly, all band-sharing scores are based on *Hae*III blots probed with J33.6 and J33.15. DNA fingerprints (*Hae*III) produced complex banding patterns with sufficient band separation to allow us to score between 133 and 155 different-sized bands per breeding group (bands from the two probings combined; bands of the same size detected by both probes were counted only once) with an average ($\pm$SE) of $41{\cdot}3 \pm 0{\cdot}7$ bands per individual.

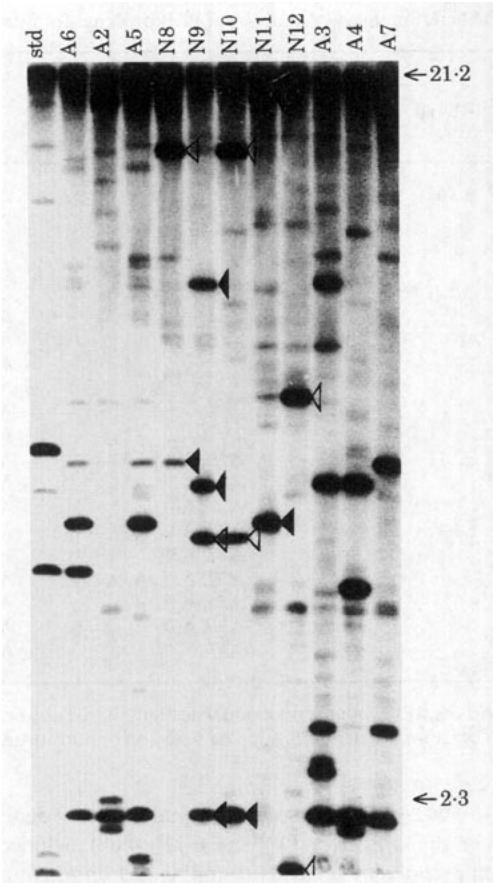

Figure 1. DNA fingerprints of *Alu*I digested DNA from a breeding group (A5.2) probed with PER. The lane designated 'std' is sample A30 from nest E2.1. Molecular size markers indicating the approximate fragment size range are in kilobase pairs. Black arrow heads indicate bands in nestling lanes that were present in one or more adult lanes. Open arrow heads indicate bands in nestling lanes that were not present in any sampled adult. The fragment size nearest the 2.3 kb marker in N9 and N10 was not informative (all adults lanes had that band) and was ignored.

Controlling for Background Band Sharing Among Non-relatives

To calculate expected frequencies of band occurrences for unrelated individuals, four adults from each breeding group were selected at random (with the exception of one degraded sample that was excluded) and used to generate a *Hae*III DNA fingerprint with Jeffreys' 33.15 and 33.6 (Fig. 2). The examination of levels of band sharing among non-relatives provides data necessary for testing the statistical significance of band sharing between putative relatives. The resulting data, for 96 dyads of individuals from different groups ($\bar{X} \pm$SE number of bands/individual: $43{\cdot}2 \pm 1{\cdot}0$), were used to calculate expected frequencies (presence or absence of bands in two compared individuals) to account for band sharing among non-relatives. We assumed that adults from different breeding groups were unrelated. The average values for the 96 dyads of unrelated adults were $D = 0{\cdot}27 \pm 0{\cdot}006$ and

Table II. Summary of analyses of PER probings for four breeding groups of guira cuckoos

Breeding group	Nestling	Adults with shared bands*	Adults lacking shared bands	Minimum number of parents not sampled	Fraction of adults not sampled in group
A3.1	N17	A15, A16	A13, A14	0	2/6
	N18	A14, A15, A16	A13	1	
	N19	A14, A15	A13, A16	0	
	N20	A14, A15	A13, A16	1	
A5.2	N08	A5, A6, A7	A2, A3, A4	1	2/8†
	N09	A3, A4	A2, A5, A6, A7	1	
	N10		A2, A3, A4, A5 A6, A7	1	
	N11	A2, A5, A6	A3, A4, A7	0	
	N12		A2, A3, A4, A5 A6, A7	1	
E2.1	N27	A29, A31, A33	A30, A32, A34	1	3/9
	N28	A29, A31, A33	A30, A32, A34	1	
	N35	A29, A31	A30, A32, A33, A34	1	
E4.1	N41	A37, A38, A39, A40	A36	1	8/13
	N42	A37, A39	A36, A38, A40	1	
	N43	A37, A38, A39, A40	A36	0	
	N44	A36, A37	A38, A39, A40	0	
	N45	A36, A38	A37, A39, A40	1	
	N46	A37	A36, A38, A39, A40	1	

*Adults with whom an individual nestling shared one or more bands (unless all sampled adults had that band).
†Total number of adults sampled based on opportunistic counts during occasional nest checks.

$\varnothing = 0{\cdot}042 \pm 0{\cdot}008$. *D*-scores of dyads of individuals from the same nests ($\bar{X} \pm \text{SE} = 0{\cdot}32 \pm 0{\cdot}02$) included some apparent relatives (unpublished data), thus elevating the average value. Based on the average *D*-score for unrelated adults from different nests, it is possible to calculate theoretically expected *D*-score values for first- ($r = 0{\cdot}5$), second- ($r = 0{\cdot}25$) and third-degree ($r = 0{\cdot}125$) relatives: $0{\cdot}27 + (0{\cdot}73/2) = 0{\cdot}63$ for first degree; $0{\cdot}27 + (0{\cdot}73/4) = 0{\cdot}45$ for second degree; $0{\cdot}27 + (0{\cdot}73/8) = 0{\cdot}36$ for third degree. The lowest statistically significant *D*-score in this study (see below) was 0·39, suggesting that statistically significant relationships were first or second degree.

Statistical testing of *D*-scores apparently separates first- and second-degree relatives from lower degrees of relatedness. However, it was not possible for us to distinguish, with a high level of certainty, first- from second-degree relationships on the basis of band-sharing analyses of the dyad in question. An individual was designated as the only sampled genetic parent of a particular nestling if: (1) that individual had the highest *D*-score of any adult compared with that nestling; (2) the *D*-score exceeded 0·54 (closer to the theoretically expected value for a first-degree relative); and (3) the *D*-score was significant at 0·001 or less.

Band-sharing Analyses

To illustrate the approach used to analyse relatedness within breeding groups, data from nest A3.1 are presented in some detail. Table III displays band-sharing coefficients (*D*) for members of breeding group A3.1. No *D*-scores for comparisons between adults at this nest were significantly greater than the background band-sharing level of unrelated adults (background level; $\alpha = 0{\cdot}05$). This suggests that none of these adults were first- or second-degree relatives. The *D*-scores for comparisons between adult A15 and three nestlings, N17, N19 and N20 were statistically significantly higher than the background level. Similar results were obtained for comparisons between adult A16 and nestlings N17 and N18. Although we cannot distinguish statistically between first- and second-degree relatives, these values were closest to the theoretically expected *D*-score for first degree relatives. The analysis suggests that A15 and A16 were the parents of N17. Additionally, results of an

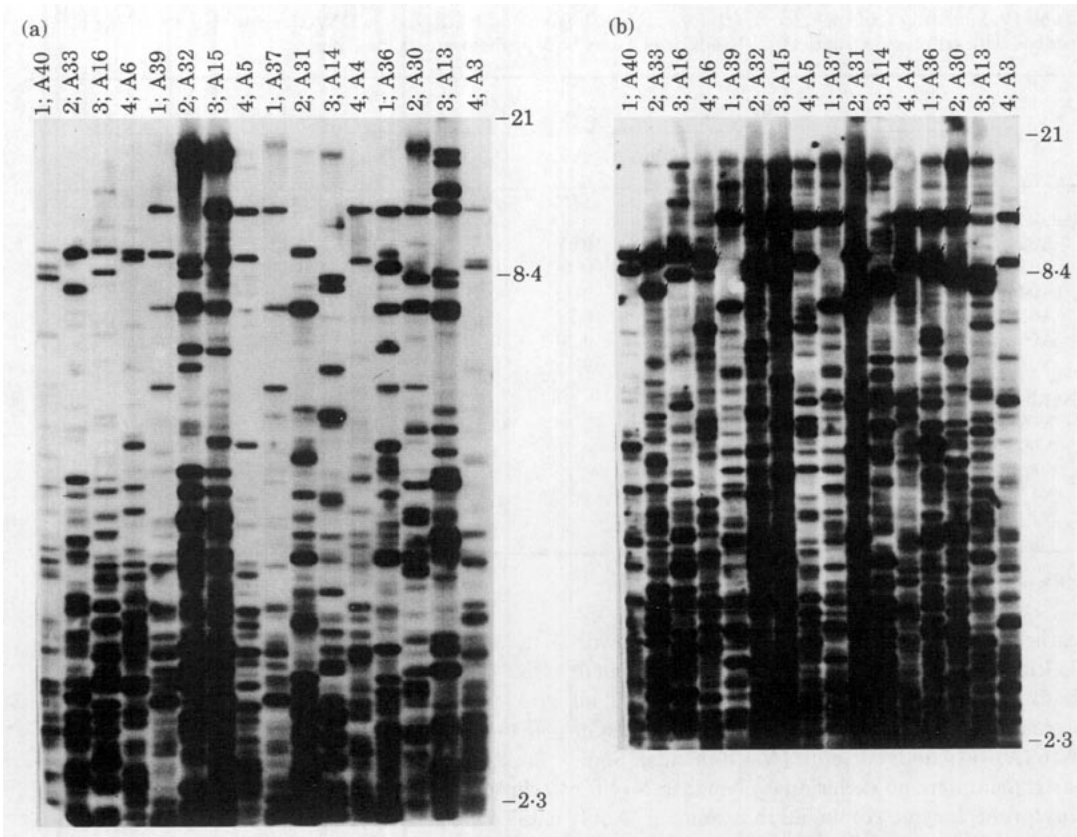

Figure 2. DNA fingerprints of four randomly selected guira cuckoo adults from each of four breeding groups labelled 1–4 (E4.1, E2.1, A3.1 and A5.2, respectively) followed by identities of sampled individuals. Guira cuckoo DNA was digested with *Hae*III and probed with (a) Jeffreys' 33.15 or (b) Jeffreys' 33.6. Small arrows in (b) indicate bands that matched exactly those bands in (a). Such bands were included only once in band sharing analyses.

Table III. Similarity coefficient scores (*D*) for individuals from nest A3.1. Sample DNAs were digested with *Hae*III and probed with Jeffreys' 33.6 and 33.15 (bands detected by both probes were counted only once)

	Adults				Nestlings			
	A13	A14	A15	A16	N17	N18	N19	N20
Adult								
A13	40	0·10	0·27	0·24	0·24	0·29	0·24	0·27
A14		43	0·26	0·32	0·30	0·25	0·21	0·28
A15			48	0·43	0·57***	0·35	0·64****	0·62****
A16				44	0·65****	0·62****	0·28	0·39
Nestling								
N17					36	0·49***	0·38	0·46**
N18						37	0·25	0·38
N19							42	0·52***
N20								42

In this and following tables, the diagonal gives total numbers of bands scored for each individual. Right of the diagonal are the *D*-scores (band sharing coefficient; Wetton et al. 1987).
*$P<0·05$; **$P<0·01$; ***$P<0·001$; ****$P<0·0001$.

Table IV. Similarity coefficient scores (*D*) for individuals from nest A5.2. Sample DNAs were digested with *Hae*III and probed with Jeffreys' 33.6 and 33.15 (bands detected by both probes were counted once)

	Adults						Nestlings				
	A02	A03	A04	A05	A06	A07	N08	N09	N10	N11	N12
Adult											
A02	33	0·24	0·30	0·37	0·35	0·17	0·38	0·28	0·32	0·17	0·15
A03		34	0·54***	0·39	0·29	0·49**	0·26	0·31	0·43*	0·17	0·22
A04			33	0·34	0·27	0·33	0·21	0·34	0·40	0·22	0·15
A05				43	0·54***	0·29	0·61****	0·22	0·21	0·24	0·20
A06					42	0·20	0·54***	0·20	0·36	0·36	0·25
A07						39	0·22	0·23	0·27	0·28	0·52**
Nestling											
N08							35	0·41	0·31	0·27	0·22
N09								38	0·73****	0·38	0·38
N10									41	0·45	0·39
N11										47	0·56***
N12											46

*$P<0·05$; **$P<0·01$; ***$P<0·001$; ****$P<0·0001$.

earlier blot (samples cut with *Alu*I and probed with J33.15) showed no bands in N17 that were absent in both A15 and A16; of 37 bands scored in N17, all were accounted for in A15 ($N=9$ shared bands) or A16 ($N=12$ bands) or both ($N=16$ bands). Similarly, there were no exclusionary bands in N17 for any probe/enzyme combination examined. Adult A15 yielded a non-significant *D*-score compared with nestling N18, as did adult A16 when compared with nestlings N19 and N20. Adults A15 and A16 are the genetic parents of nestling N17, but each mated with others to produce the remaining young. Adults A13 and A14 did not show high *D*-scores with any nestlings (Table III) and were apparently unrelated to them. If this interpretation is correct, N17 was a half-sibling to each of its nestmates. *D*-scores for N17 with N18 and N20 were statistically significant, but lower than the parent–offspring *D*-scores. N17 did not have a statistically significant *D*-score with N19. The coefficient of relatedness, *r*, between parents and offspring is 0·5, while *r* between full-siblings averages 0·5 (theoretical range = 0–1). The theoretical range in *r* for siblings is due to segregation. Similarly, expectations for band sharing in multi-locus DNA fingerprints are less exact for half-siblings, due to segregation. To examine the possibility that this low level of band sharing is due to segregation of chromosomes with a wealth of loci that hybridize with specific fingerprinting probes (segregation analysis was not possible due to insufficient samples), we examined the J33.15 and J33.6 fingerprints separately. While the *D*-score comparing N17 and N19 was low for J33.6 ($D=0·28$), it was significant for J33.15 ($D=0·44$; $P<0·025$) suggesting that N17 and N19 may have inherited one or more different homologous chromosomes from A15 which had many linked J33.6 loci and not as many J33.15 loci. Eighteen of 22 (82%) bands shared between nestlings N19 and N20 were also in common with adult A15. Excluding bands shared with A15, four of 26 (15%) bands were shared between N19 and N20 suggesting that these nestlings were half-siblings. The statistically significant *D*-score for the N19/N20 comparison in concert with that for the N20/N17 comparison provides indirect support for a half-sibling relationship between N17 and N19. Statistically significant *D*-scores for nestling dyads were closest to the theoretical value for second-degree relatives. We conclude that A15 produced young with A16 and two other mates, while A16 produced young with A15 and one other mate. Nestlings in nest A3.1 appeared to be either half-siblings or unrelated to each other.

The analysis of samples from nest A5.2 suggested only one parent–offspring relationship (Table IV). Statistically significant *D*-scores suggested that either A05 or A06 was a parent of N08. Since both A05 and A06 appeared to be males by DNA analysis (sexing of adult guira cuckoos in progress; see Quinn et al. 1990), and eight bands found in N08 were not present in A05 or A06, we can safely

Table V. Similarity coefficient scores (*D*) for individuals from nest E2.1. Sample DNAs were digested with *Hae*III and probed with Jeffreys' 33.6 and 33.15 (bands detected by both probes were counted only once)

	Adults						Nestlings		
	A29	A30	A31	A32	A33	A34	N27	N28	N35
Adult									
A29	39	0·34	0·53**	0·36	0·34	0·21	0·30	0·67****	0·42
A30		37	0·24	0·36	0·30	0·32	0·36	0·38	0·30
A31			37	0·23	0·47*	0·27	0·31	0·35	0·58****
A32				51	0·31	0·27	0·48	0·56**	0·28
A33					44	0·37	0·67****	0·30	0·44
A34						37	0·26	0·23	0·33
Nestling									
N27							40	0·54**	0·27
N28								42	0·33
N35									42

*$P<0·05$; **$P<0·01$; ***$P<0·001$; ****$P<0·0001$.

Table VI. Numbers of bands shared with suspected parent A, shared with suspected parent B, uninformative (shared with both suspected parents), and exclusive bands (absent in both suspected parents) in cases where both parents of nestlings may have been sampled

Nestling	Suspect parent A	Suspect parent B	A-shared	B-shared	Uninformative	Exclusive
N17	A16	A15	12	10	14	0
N27	A33	A32	16	10	12	2
N28	A29	A32	15	12	13	2

conclude that we sampled only one of the parents of N08. The data hint that A05 was a parent of N08, while A06 was a second-degree relative. A05 and A06 shared significant numbers of bands, as did two other adult dyads, A03/A04 and A03/A07. Adult A03 shared a significant, but low, number of bands with nestling N10 suggesting a second-degree level of relatedness. Similarly, although it is possible that A07 was a parent of N12, the *D*-score was low enough to suspect a second-degree relationship. Significant band sharing was detected among two dyads of nestlings: N09/N10 and N11/N12. These *D*-scores were closer to the value expected for first-degree relationships and may indicate full-sibships. Two other dyads were not significant, hence were classified as unrelated, although they may represent type II errors: N08/N09 ($D=0·41$, $P<0·1$) and N10/N11 ($D=0·44$, $P<0·1$).

In nest E2.1 the analyses suggest that one parent of each of the three offspring were sampled (Table V), yielding parent/nestling dyads A33/N27, A29/N28 and A31/N35. Adult A32 shared many bands with nestlings N27 and N28 but is probably not a parent. A32 had more bands than most samples ($N=51$), and accounted for most, but not all, of the bands not shared with the probable parents of the two nestlings (Table VI, Fig. 3). Furthermore, in the PER probing of *Alu*I fingerprints, A32 did not share bands with N27 or N28 and was not considered a likely candidate for being a genetic parent. Nestlings N27 and N28 had a statistically significant *D*-score (0·54), thus they apparently shared one parent (probably related to A32). This interpretation is supported further by a comparison of residual bands in N27 and N28 which were not found in their respective sampled parents; nine residual bands were shared by N27 and N28 out of a total of 12 and 16, respectively. This nest includes half-siblings and an unrelated nestling, and represents an example of genetically polyandrous or genetically polygynous mating.

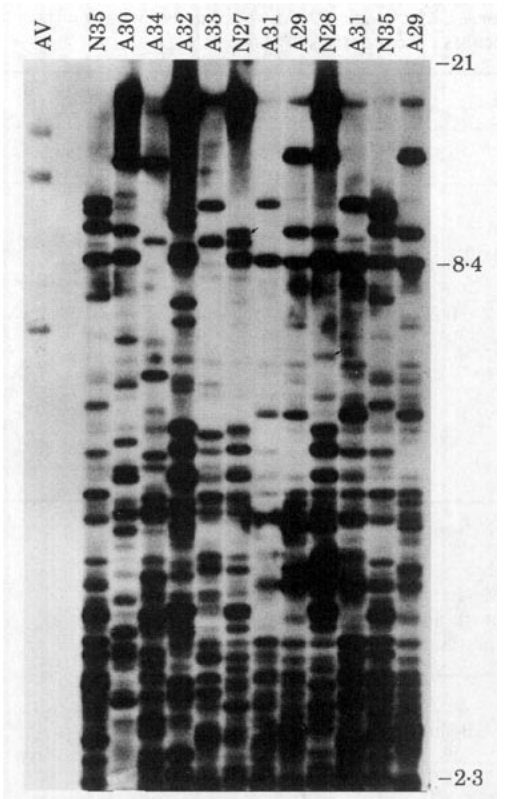

Figure 3. DNA fingerprints of *Hae*III digested guira cuckoo DNA (Nest E2.1) probed with Jeffreys' 33.15. The lane designated 'AV' is a lane with *Bam*HI digested adenovirus (150,000 cpm of labelled adenovirus was added along with the Jeffreys' 33.15 probe). Small arrows indicate: a band that was exclusive to nestling N27 (compared with A33 and A32); and a band that was exclusive to nestling N28 (compared with A29 and A32).

Two related adults, A37 and A39 at nest E4.1, shared many bands with four of the nestlings (Table VII), however, many of the nestlings' bands were not present in either adult. The high level of band sharing between A37 and A39 made it difficult to identify one of them as a parent of N41 or N43. Adult A37 was probably a parent of N42, however the other significant *D*-scores for adult/nestling dyads were closest to the theoretically expected value for second-degree relatives. It is likely that relatives of A37 and A39 as well as most parents of nestlings at this nest were among the eight unsampled adults. This prospect makes band-sharing assessment, according to specific hypotheses involving shared parentage, especially difficult. It is possible, however, to examine band sharing among nestlings according to lineages, or lines of descent.

A number of nestling dyads had statistically significant *D*-scores, although most were in the range expected for second-degree relatives. To determine whether a pair of apparently related nestlings were half-siblings, we looked for cases where a third nestling was related to only one member of the dyad with a statistically significantly high *D*-score. For example, the N44/N46 dyad yielded the highest nestling *D*-score (0·56) and the N41/N46 dyad was statistically significant ($D=0{\cdot}49$), however, the N41/N44 dyad was not ($D=0{\cdot}28$). A close examination of band sharing revealed that most bands shared between nestlings N41 and N44 were also in common with N46; nine bands were shared among N41, N44 and N46, 14 exclusively between N41 and N46, 15 exclusively between N44 and N46, and only three exclusively between N41 and N44. This argues that nestlings N44 and N46 shared bands through one lineage while N41 and N46 shared bands through another. Similar reasoning can be applied to other triplets (e.g. N41, N42 and N46; N41, N42 and N44) and these data suggest that six lineages contributed to the nestlings in nest E4.1 (Table VIII). This examination suggests that only N43 and N46 shared two lineages (i.e. could be full-siblings). However, the *D*-score for N43/N46 was only 0·42, suggesting the possibility that they are half-siblings whose non-shared parents are related to each other. While the analysis of this group is necessarily tentative, given the large proportion of unsampled adults, the evidence suggests that with the possible exception of the N43/N46 dyad, none of the nestlings were full-siblings. This conclusion is supported by the only moderately high *D*-scores among nestlings.

DISCUSSION

Complex breeding associations, which may characterize communal breeders, cannot be analysed completely without knowledge of genetic relatedness. We have provided such genetic data for the communally breeding guira cuckoo.

Band-sharing analyses have allowed the probable identification of a number of single parents, however we were unable to eliminate putative parents from consideration on the basis of exclusive bands in the young. Because of difficulties capturing some of the adults, we were able to identify both parents of only one nestling (N17). The paucity of nestlings for which we sampled both parents is

Table VII. Similarity coefficient scores (*D*) for individuals from nest E4.1 whose sample DNAs were digested with *Hae*III and probed with Jeffreys' 33.6 and 33.15 (bands detected by both probes were counted only once)

	Adults					Nestlings					
	A36	A37	A38	A39	A40	N41	N42	N43	N44	N45	N46
Adult											
A36	49	0·35	0·30	0·42	0·40	0·32	0·34	0·25	0·16	0·38	0·31
A37		41	0·31	0·63****	0·26	0·51***	0·58****	0·50***	0·46**	0·19	0·39
A38			44	0·38	0·28	0·24	0·36	0·29	0·24	0·26	0·22
A39				45	0·34	0·50***	0·44*	0·48**	0·38	0·28	0·41
A40					42	0·28	0·30	0·25	0·25	0·34	0·27
Nestling											
N41						46	0·53***	0·49***	0·28	0·30	0·49**
N42							45	0·33	0·53***	0·35	0·35
N43								39	0·39*	0·20	0·42*
N44									38	0·20	0·56***
N45										41	0·28
N46											47

*$P<0·05$; **$P<0·01$; ***$P<0·001$; ****$P<0·0001$.

Table VIII. Hypothetical lineages for nestlings in nest E4.1 according to *D*-scores among nestling dyads (lineages responsible for each nestling are designated with × in the appropriate cell)

	Nestling					
Lineage	N41	N42	N43	N44	N45	N46
1		×		×		
2	×	×				
3	×		×			×
4			×	×		×
5					×	
6					×	

likely owing to a shortage of females among our samples. Preliminary research using a lesser snow goose, *Chen caerulescens*, probe derived from the Z-chromosome (Quinn et al. 1990) indicates that of 17 captured adult guira cuckoos that have been evaluated, 13 were male (unpublished data). This is probably owing to a sampling bias, and possibly to different behavioural propensities of males and females to approach handreared lure birds in the trap as has been found in the closely related smooth-billed ani (Loflin 1983). We contend that in most cases, we identified only male parents.

DNA fingerprinting is a powerful tool for examining relatedness among individuals. Related individuals share DNA fingerprint bands by common descent (Jeffreys et al. 1985). However, background band sharing among unrelated individuals in a population introduces uncertainties regarding the origin of shared bands and must be taken into account (Lynch 1988, 1991). By testing patterns of band sharing with a chi-squared statistic with adjusted expected frequencies (J. S. Quinn and R. Morrison, unpublished data), we were able to reject the null hypothesis that high levels of band sharing were due to chance (or to the background level of band sharing). We assumed that significant levels of band sharing were due to common descent, and thus, implied relatedness.

The identification of parent–offspring relationships was not positive. However, the assignment of parentage was central to the evaluation of the monogamy hypothesis only for nests A3.1 and E2.1. Because we caught mostly males, and were able to sample 10 of the 15 adults observed attending those nests (four of six males and six of nine males for each nest, respectively), it is likely that we sampled at least the male parent of each offspring. None the less, we cannot absolutely refute the possibility that an uncle or aunt, or a full-sibling from a previous brood, was misidentified as a parent.

In an attempt to distinguish half- from full-siblings it was necessary to use additional evidence which varied in strength (Table IX). The strongest evidence falsifying a test of full-sibship was evidence from nest A3.1 showing that nestlings shared one parent (with N17 for whom both parents were

Table IX. Types of evidence against full-sibling relationships for dyads with statistically significant *D*-scores

Dyad	Evidence against full-sibship				Conclusion
	Share 1 parent not both*	Share 1 parent, probably not both†	Probably share 1 parent, not both‡	Share 1 lineage, not 2 lineages§	
N17/N18	×				Half-siblings
N17/N19	×				Half-siblings
N17/N20	×				Half-siblings
N19/N20		×			Half-siblings
N9/N10					Full-siblings
N11/N12					Full-siblings
N27/N28			×		Half-siblings
N41/N42				×	Half-siblings
N41/N43				×	Half-siblings
N41/N46				×	Half-siblings
N42/N44				×	Half-siblings
N43/N44				×	Half-siblings
N43/N46					Full-siblings
N44/N46				×	Half-siblings

*Both parents of one nestling identified, one adult was excluded as the parent of the other nestling in dyad.
†One adult identified as parent to both nestlings, analysis of unaccounted bands revealed little band sharing among nestlings.
‡Two different adults identified as parents of each respective nestling; unsampled second parent probably the same individual based on high levels of band sharing of residual bands (those not found in common with sampled parents).
§Based on comparisons of groups of three nestlings, one of whom shared significantly great numbers of bands with both (the other two shared few bands (see text)).

known) and not the other. Nestlings sharing one sampled parent were classified as half-siblings if they shared few residual bands (bands not shared in common with the sampled parent). Similarly, nestlings of different sampled parents (second parent not sampled) were considered half-siblings if they had significant *D*-scores and shared many residual bands (e.g. N27/N28, nest E2.1). The final, and perhaps weakest, source of evidence was based on comparisons of nestling triplets, in which one nestling had significant *D*-scores with both of the other nestlings, while the remaining two nestlings shared few bands with each other. This suggests that these nestlings shared only one, not both, lineages (e.g. nest E4.1), but this evidence is potentially subject to errors resulting from segregation. Cases lacking evidence against full-sibship were designated 'full-sibling' (Table IX), although this designation was inconclusive.

In two cases, two adults shared significant numbers of bands with the nestlings being considered (N08 and N28). Parentage was assigned to the adult with the higher *D*-score. Conclusions about the genetic mating system, as well as the relatedness among nestlings remain unaltered if the other parents (those with the lower, but significant, *D*-score) were assigned parentage. Other cases in which parentage was somewhat ambiguous were not dependent upon the assignment of parentage for conclusions about relatedness among nestlings.

Our expectations of genetic monogamy were not supported in this study. The prediction of monogamy dictates that related nestlings should be full-siblings and that each parent should have only one mate. Evidence supporting the monogamous mating system was almost non-existent. Analyses of relatedness among 34 nestlings dyads indicated two probable and one possible full-sibships (9%; nest A5.2, N9/N10 and N11/N12; nest E4.1 N43/N46). The remaining nestling dyads appeared to be unrelated (59%, $N=20$) or half-sibships (32%, $N=11$).

We can infer that genetically polygynous and/or genetically polyandrous matings have occurred in nests containing half-siblings. Indirect evidence for polygamous mating in nest E2.1 was based on the significant band sharing between N27 and N28, combined with the identification of two different putative parents (A29 and A33, respectively). Evidence for both polyandrous and polygynous

matings can be found in group A3.1. We conclude that A15 and A16 were both involved in polygamous relationships, thus representing polygynandrous mating. Alternative explanations supporting genetic monogamy would necessitate a complex scenario involving monogamous parentage by unsampled siblings of A15 and A16, and four additional unsampled adults that were not observed in association with this nest. Breeding group A5.2 provided little relevant information. The identification of one probable parent–offspring combination (A5/N8) and two independent related dyads of nestlings (N9/N10 and N11/N12) did not allow further analysis of this family. The lack of other parent–offspring dyads suggests that there were undetected adults associated with this group. This group was observed only opportunistically during occasional nest checks and it is likely that the number of adults was underestimated. Examination of the lineage of the inter-related nestling triplet at nest E4.1 suggested that related nestlings were half-siblings. A shortage of sampled adults at this nest precluded further verification.

The prediction that a dominant female and her mate are responsible for most incubated eggs dictates that the majority of young should comprise a group of inter-related full-siblings. The high frequency of unrelated nestlings, as judged by low levels of band sharing, signifies that individual dominant females do not contribute the majority of incubated eggs in general. Group sizes in this study were larger than typical groove-billed ani groups (Vehrencamp et al. 1986), ranging from 8–13 adults. An increased number of reproductive females probably elevated the complexity of dynamic interactions among laying guira cuckoos relative to the smaller (primarily two female) groups of anis that Vehrencamp studied. Further study is required to evaluate the possibility that dominant female guira cuckoos mating with multiple mates garner more than their proportional share of incubated eggs through matings with multiple mates.

Individual adults in our study were differentially successful, some having a greater proportion of offspring in the nest than others. Of 21 adults tested, 52% ($N=11$) were related to one or more of the nestlings at their respective nests. Furthermore, there was considerable variance in *D*-scores among dyads of nestlings from the same nest, some being related, others apparently not. Thus, there are numerous asymmetries in the degrees of relatedness among nestmates and between adults and nestlings. One consequence of such asymmetries may be overt competition among adult and possibly nestling guira cuckoos. Members of the Crotophaginae demonstrate shared parental care, as well as competition for nest space for their eggs and nestlings (Vehrencamp 1978; Loflin 1983; Macedo 1991). Conspicuously uncooperative behaviour is evident during several phases of nesting in guira cuckoos. Most obvious is egg tossing and egg burial; circumstantial evidence indicates that infanticide (Macedo 1991), and possibly murder among nestlings, may be important factors in the high mortality suffered by chicks during the first week after hatching. Variability in the degree of cooperative behaviour, possibly an indication of the lack of cooperation by some, occurs during the nestling phase, as adults feed the chicks and attend the nest (Macedo 1991). In five of seven focal nests, there was significant heterogeneity among adults in their chick-feeding effort (Macedo 1991), and in the two nests observed for nest-attendance, some adults spent very little time near the nest. A similar pattern has been well documented for dunnocks, where males with high mating success were found to invest more in the feeding of chicks than those that had little access to the female during mating (Burke et al. 1989).

Behavioural research of communal breeders has revealed the cooperative and competitive nature of such breeding systems. However, the lack of specific genetic information has restricted the conclusions of most studies since it is often impossible to determine the reproductive success of specific individuals with certainty. We encourage researchers to consider the likelihood and limitations of incomplete sampling before committing to the expensive proposition of DNA fingerprinting. However, DNA fingerprinting of well-sampled groups, used in conjunction with behavioural observations, will result in a better understanding of communal breeding.

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