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Shared parentage and incest avoidance in the cooperatively breeding acorn woodpecker

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Abstract

Social groups of acorn woodpeckers (*Melanerpes formicivorus*) range in size from unaided pairs to 15 adults. Behavioural indicators of mate guarding, assumed incest avoidance and observations of egg-laying indicate that social organization ranges from monogamous pairs to groups with up to seven male and three female putative cobreeders plus up to 10 nonbreeding helpers. In addition, groups occasionally lack a putative breeder throughout the breeding season. Here we report results from multilocus DNA fingerprinting of 372 nestlings from 123 nests in groups with putative cobreeders of one or both sexes. No extra-group fertilizations were found. Putative cobreeding males within social groups shared paternity. However, the most reproductively successful male was, on average, almost three times as successful as the next most successful and additional males only occasionally sired offspring. In contrast, cobreeding females shared parentage equally. Helpers never bred incestuously when their opposite-sex parent (or another relative, such as their uncle) held breeding status in the group. However, during breeding male vacancies, 14 nestlings were produced when helper males bred incestuously with their mother. Both male and female helpers usually became successful cobreeders with their same-sex parent following replacement of the opposite-sex breeder(s) by unrelated individuals.

Keywords: acorn woodpecker, cooperative breeding, DNA fingerprinting, inclusive fitness, *Melanerpes*, reproductive skew

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Introduction

Acorn woodpeckers (*Melanerpes formicivorus*) have one of the most complex and variable social systems of any vertebrate. Groups consist of young that remain on their natal territory past reproductive maturity and of coalitions of helpers that disperse to fill a breeding vacancy (Koenig 1981; Koenig *et al*. 1995b). Regardless of group composition, there is only one active nest at a time and all group members cooperate in nestling care and year-round defence of their territory and the granary tree in which they store acorns. Prior genetic work has demonstrated that extra-group parentage is rare or nonexistent in groups consisting of simple pairs (Dickinson *et al*. 1995). Detailed observational studies have also confirmed that more than one female may lay eggs communally in the same nest (Mumme *et al*. 1983b; Koenig *et al*. 1995a) and have provided fragmentary evidence for multiple paternity (Mumme *et al*. 1985). Nonetheless, the mating system within larger groups, particularly those containing cobreeder males, has remained elusive.

Here, we present the results of a genetic analysis of parentage of acorn woodpeckers at Hastings Reservation in central coastal California, focusing on groups that contain multiple cobreeders of one or both sexes. Our goals are to describe the genetically effective mating system of this population and to compare this system with earlier conclusions drawn largely from behavioural evidence.

Materials and methods

Study population and assignment of social status

Acorn woodpeckers have been under continuous study at the Hastings Natural History Reservation, located in

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central coastal California, for over 25 years (MacRoberts & MacRoberts 1976; Koenig & Mumme 1987; Koenig *et al.* 1998). The current study population consists of ≈ 35 social groups and 160 individuals, almost all of which are marked with unique combinations of colour bands. The population is censused continuously to determine group membership. During the breeding season (mainly April to June), nests are found by looking for indications of breeding such as mate-following and by searching for birds in cavities that might be incubating eggs. During the nestling period, feeding observations are made to confirm group membership and to determine individual provisioning effort (Mumme *et al.* 1990). Mean (± SE) group size averages 4.4 ± 0.1 (n = 815 group-years) adults during the breeding season, but ranges up to 15.

Within groups, we identify three categories of individuals: (i) members of single-sex coalitions of siblings that immigrated into a territory following a reproductive vacancy; (ii) progeny of these coalitions while their parents (or other coalition members of both sexes) are still present; and (iii) progeny that have ostensibly inherited breeding status in their natal territory following the replacement of the opposite-sexed breeders with unrelated immigrants from elsewhere (Koenig *et al.* 1998).

Progeny living in their natal territories with their parents or other coalition members still present (category 2) do not appear to participate in the breeding activities (Koenig *et al.* 1998, 1999) of the group and are considered 'non-breeding helpers'. Groups that have at least two birds of the same sex in one or both of the other categories (1 and 3) are considered 'potential cobreeders' (males) or 'potential joint-nesters' (females).

In total, 55% of male and female helpers that survive to their first spring eventually obtain breeder status, but males are more likely than females (24 vs. 5%) to obtain breeder status on their natal territories and to share cobreeder status with their parent (Koenig & Mumme 1987; Koenig *et al.* 2000). Cobreeding status is common, with 47% of groups containing cobreeder males, 22% cobreeder females and 12% containing cobreeders of both sexes.

DNA fingerprinting

Whole blood samples (50 µL) taken from the brachial vein were added to a cell lysis solution (Longmire *et al.* 1988) and stored at −20 °C. DNA was extracted by incubating with Proteinase K at 55 °C and extracting several times in phenol and 24 : 1 chloroform/isoamyl alcohol, followed by ethanol precipitation. DNA (5 µg) was digested with a three times excess of *Hae*III at 37 °C for 3–5 h and loaded into a 0.9% agarose gel (25 cm). Electrophoresis was performed in 1× TBE at 38 V for ≈ 68 h until fragments < 3 kb were run off the gel. The fragments were then transferred to nylon filters by alkaline blot and cross-linked by UV light (312 nm, 0.2 J/cm^2). Jeffreys' probes 33.6 or 33.15 (Jeffreys *et al.* 1985) were hybridized to filters as either DNA (e.g. Haydock *et al.* 1996) or RNA (Carter *et al.* 1989) probes labelled with [^{32}P]CTP and exposed to X-ray film for 1–30 days. Filters were subsequently stripped using boiling 0.1% SDS and probed with the second probe. Five filters (40 of the 400 assigned offspring) were only probed with Jeffrey's probe 33.15 because the filter had deteriorated prior to use with the second probe. More detailed information on our fingerprinting protocols can be found elsewhere (Rabenold *et al.* 1990; Dickinson *et al.* 1995; Haydock *et al.* 1996).

Scoring fingerprints and assignment of parentage

All adults members of each group were run at least twice on a gel and on both sides of the potential offspring to aid in alignment during scoring. Fingerprints were scored by placing an acetate sheet over the autoradiograph and marking only whether a lane was for a nestling or an adult, but not the adults' social status. To increase scoring accuracy we first marked the bands (restriction fragments) in each nestling's lane that were easily discernible; we excluded faint bands and blurred bands at the bottom of the film. Each marked band in a juvenile lane was compared with potential bands in each adult lane and recorded as present, absent or unclear. Bands that were unclear for any of the individuals on the fingerprint were not considered further. After scoring the marked bands in the nestling lanes, the few remaining bands exclusively present in one or more of the adults were scored.

With respect to each nestling, we calculated: (i) the number of unattributable bands for all possible male-female dyads of adults; (ii) the number of parental specific bands for all possible male–female dyads assuming the first member of the dyad is a parent, followed by assuming the second member of the dyad is a parent; and (iii) band-sharing with each adult. An unattributable band in an offspring lane was one that was absent in both adults of the dyad. We summed the results across both probes for the fewest number of unattributable bands and the number of parental-specific bands. Band-sharing was calculated independently for each probe and for both probes combined.

We assigned parentage using the following criteria.

1. We accepted any male–female dyad from the group showing two or fewer unattributable bands (both probes combined) as a potential parental dyad (unattributable or novel bands are assumed possible due to mutation, Jeffreys *et al.* 1985; Westneat 1990).
2. In cases in which more than one dyad fulfilled criterion 1, we accepted the dyad showing the fewest unattributable bands as the parental dyad.
3. In the uncommon cases of ties in the number of unattributable bands, the offspring was assigned to the adult

Table 1 Formulae used to determine certainty of assignment of parentage

Quantity	Notation	Formula
Band sharing (where): S = band is shared A = band found in only the 1st individual B = band found in only the 2nd individual	s	$\frac{(S/(A+S)) + (S/(B+S))}{2}$
Mean allelic frequency* (s_u = band sharing between unrelated adults in population)	q	$1-\sqrt{(1-s_u)}$
Probability that an offspring's fragment is shared with parent's*	s_o	$\frac{1+q-q^2}{2-q}$
Probability that a fragment is shared with a full sibling†	s_s	$\frac{4+5q-6q^2+q^3}{4(2-q)}$
Probability that a offspring's fragment is shared with a father's son who mated incestuously with his mother‡	s_i	$\frac{5+2q-3q^2}{4(2-q)}$
Probability that a offspring's fragment is shared with a father whose son mated incestuously with his mother‡	s_f	$\frac{1+3q-2q^2}{2(2-q)}$

*Following Jeffreys *et al*. (1985) and Georges *et al*. (1988). †Following Haydock *et al*. (1996). ‡Following Parker *et al*. (1999).

with the highest band-sharing value. Band-sharing could not be used to assign parentage when group members were closely related, as was true in most cases we considered. Consequently, we relied almost exclusively on unattributable bands to assign parentage.

When we did use band sharing to assign parentage (assignment criterion 3), we paid particular attention to evaluating the possibility of incest when ambiguous dyads contained at least one helper. If a helper male breeds incestuously with his mother, no unattributable bands are expected to be produced in both the dyad of the helper male and his mother and the dyad of the helper's father and mother, because all of the paternal-specific bands in the helper have to have come from the helper's father. The converse holds if a helper female breeds incestuously with her father (Parker *et al*. 1999; Wright *et al*. 1999). However, relative to expected band-sharing between nonincestuous parents and offspring (s_o), band-sharing values are expected to be predictably high between the 'helper' that bred incestuously and the offspring (s_i) and predictably low between the helper's same-sex parent (i.e. a bird with breeder status) and the offspring (s_f) (Table 1).

We fingerprinted two groups that produced offspring known to have only a female with breeder status and helpers that were all offspring of the female (a breeding male vacancy). We expected to find either incest or extra-group parentage in these two cases. No unattributable bands were found for both the dyad that contained the helper male and breeder female as well as the dyad with the helper's father (presumed dead for > 1 year) and the breeder female. We confirmed incest rather than extra-group mating in these cases by calculating band-sharing values and comparing these with the expected values (s_o, s_i, s_f, Table 1).

Certainty of assignment and independence of probes

The ability to assign parentage to only a single pair of acorn woodpeckers is dependent on band-sharing among potential parents, the mutation rate and the number of bands (fragments) scored. When band-sharing levels are high or close relatives are potential parents the ability to uniquely assign parentage decreases. We used the equations given in Table 1 and followed the methods of Haydock *et al*. (1996) to determine the probabilities of using unattributable bands to correctly and uniquely assign parentage among cobreeders and to exclude dyads containing helpers.

We scored band-sharing between the 33.6 and 33.15 probes for fingerprints of 10 individuals less related than cousins by cutting the same lane (individual) from two autoradiographs produced from the same filter, one probed with 33.6 and the other probed with 33.15. Lanes of the same individual were aligned according to the top of each lane and scored. Mean (± SE) band-sharing between the two probes was 0.07 ± 0.01, indicating low similarity in fingerprints derived from the two probes.

Statistical analysis

Statistical analyses were performed using the Statistical Analysis System (SAS Institute Inc. 1988). Loglinear analysis was used for categorical analysis using models that were fitted using maximum likelihood methods for which we report likelihood ratio statistics (G^2) (Stokes *et al*. 1995). Data sets of proportions of bands shared were arcsine transformed prior to running parametric statistical tests. Significance levels of $P < 0.05$ were accepted and all tests were two-tailed.

Results

DNA fingerprinting and certainty of assignment

Observed values for the proportions of bands shared were all within 0.03 of expected values and the number of parental specific bands were within 0.2 of expected values (Table 2). The greatest difference between observed and expected values occurred for full siblings, most likely because of our conservative method of band scoring which favoured calling bands ambiguous by position or intensity as present in adult lanes to reduce the possibility of false exclusion. Assuming correct parental assignment of the 400 offspring for which we determined parentage, the mutation rates for Jeffrey's probes 33.6 and 33.15 were 0.0027 and 0.0013, respectively. With these mutation rates and the average number of bands scored for Jeffrey's probes 33.6 and 33.15 (Table 2), two mutations are expected to be found in the same individual in one of 390 assignments of parentage. We observed two cases of 400 assignments.

Based on the expected values for the number of parental specific bands and for band sharing, exclusion probabilities for different combinations of nonparental dyads are given in Table 3. We were able to exclude the majority of dyads (90%) from being the parental dyad by finding at least three unattributable bands (Fig. 1). Of the remainder, most (92%) involved one dyad with fewer unattributable bands allowing parentage assignment on that basis. Only 24 (6.2%) offspring were assigned parentage based on band-sharing (assignment criterion 3). Fifteen involved distinguishing between dyads that contained only breeders, whereas nine involved distinguishing between dyads that contained helpers.

Extra-group parentage

We found no extra-group fertilizations or egg parasitism among the 386 nestlings from 127 nests produced in all groups that contained at least one male and one female with

	Jeffreys' 33.6	Jeffreys' 33.15
Number of offspring	360	400
Number of bands scored per offspring (b)	17.4 ± 0.3	24.2 ± 0.4
Band sharing between unrelated adults (s_u)	0.27 ± 0.01	0.32 ± 0.01
Allelic frequency (q)	0.14	0.17
Band-sharing between parents and offspring		
Expected (s_o)	0.60	0.63
Observed (father and his offspring)	0.60 ± 0.01	0.63 ± 0.01
Observed (mother and her offspring)	0.61 ± 0.01	0.62 ± 0.01
Band-sharing between full siblings		
Expected (s_s)	0.62	0.64
Observed	0.59 ± 0.01	0.62 ± 0.01
Number of parental specific bands		
Expected	7.0	9.0
Observed (father)	7.1 ± 0.2	9.2 ± 0.2
Observed (mother)	7.1 ± 0.2	8.9 ± 0.2

Table 2 Summary of fingerprinting results. Mean band-sharing among unrelated individuals (s_u) was determined for adults in the population known to be less closely related than cousins ($n = 137$ and 144 for probes 33.6 and 33.15, respectively). Expected values and allelic frequencies are based on band sharing between unrelated individuals and were calculated using the formulae in Table 1. Nestlings produced incestuously were excluded from calculations for the observed values of parental band-sharing and the number of parental specific bands

		Probability of ambiguity (N unattributable bands)		
Dyad type	Type of mating	0	1	2
Parent and offspring (full-sib helper)	Incestuous	2.0×10^{-3}	1.4×10^{-2}	4.5×10^{-2}
2 Full-sib helpers	Incestuous	2.7×10^{-3}	2.0×10^{-2}	7.3×10^{-2}
2 Half-sib helpers	Incestuous	3.9×10^{-7}	6.9×10^{-6}	6.0×10^{-5}
Parent and brother or sister	Cobreeder	6.3×10^{-5}	6.9×10^{-4}	3.7×10^{-3}
Parent and nonrelative	Extra-group	2.7×10^{-6}	4.0×10^{-5}	3.0×10^{-4}
Non-relative and nonrelative	Extra-group	6.8×10^{-13}	2.7×10^{-11}	5.4×10^{-10}

Table 3 Mean probabilities of ambiguous assignment of parentage based solely on unattributable bands using both probes. Probabilities are for a single nonparental dyad producing zero to two unattributable bands

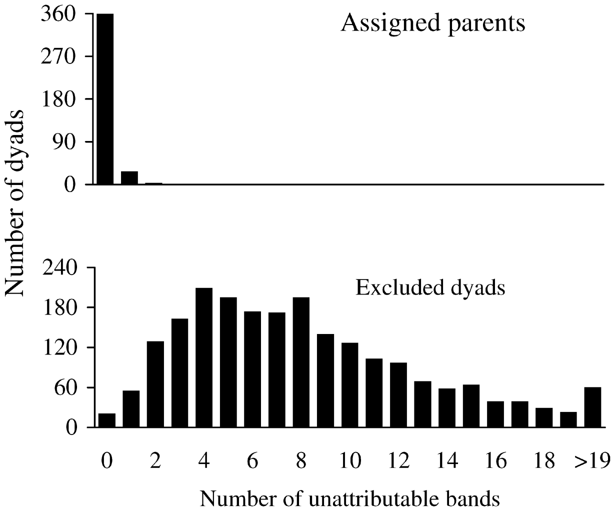

Fig. 1 Distribution of unattributable bands produced by considering every possible adult male–female dyad within groups as potential parents, summing across both probes 33.15 and 33.6 (in some cases only probe 33.15 was used, see Materials and methods). Dyads for nestlings produced in groups that had lacked a breeding male are excluded.

breeding status (Table 4). All of the nestlings were assigned to a dyad with two or fewer unattributable bands (Fig. 1).

Exclusion of incestuous mating by helpers

All 228 nestlings produced in groups with at least one bird with helper status and at least one male and one female with breeding status could be assigned to birds with breeding status. However, we also found helper–breeder and/or helper–helper dyads with two or fewer unattributable bands for 47 nestlings (Fig. 1). Based on finding fewer unattributable bands, 38 of these nestlings (36 with 0) were assigned to a dyad that contained two birds with breeding status. For the nine remaining nestlings, both the dyad with the helper and the dyad that consisted of two birds with breeding status produced no unattributable bands and assignment could not be made on this basis.

In these nine cases, the mean band sharing between the helper and the nestling suggested that the nestling and helper were full-siblings [expected (s_o) = 0.63; observed = 0.64 ± 0.03] rather than the helper's incestuously produced offspring [expected (s_i) = 0.71]. Band sharing between the nestling and the helper's same-sex parent (one of the birds in the breeder-breeder dyad) was lower than expected, suggesting that at least some of the helpers may have reproduced incestuously [expected if helper was parent (s_f) = 0.39; expected if helper's parent was also the nestling's parent (s_f) = 0.62; observed = 0.51 ± 0.04]. Six of these nine nestlings would have been the result of a helper female breeding with her father. However, females with helper status have never been observed to lay eggs (n = 222 eggs; Koenig *et al*. 1995a), and thus we consider this unlikely. The other three may have been the result of a helper male breeding with his mother. Considering these three nestlings, the helper and the nestling had band sharing that is about what was expected for full siblings in two cases (0.60 and 0.63, although band sharing with the third nestling was 0.73). Band sharing between the helper's father and the nestling was too high (0.57–0.66) to reject the possibility that the helper's father was not the sire of the three nestlings. Thus, band-sharing values and the complete absence of observed egg-laying by helper females indicates that none of these nine offspring was the product of incest. Because incest was not supported unambiguously for any of these offspring and because multiple dyads with zero unattributable bands are occasionally expected due to chance (Table 3), we assigned all nine offspring to the breeder–breeder dyads.

Incestuous mating in groups with a missing male breeder

Since we began work on the acorn woodpeckers in 1974, there have been 14 groups in which there were no males present that had acquired a putative vacancy via dispersal or inheritance for throughout at least one breeding season (April to June). In 10 (71%) of these groups, females refrained from breeding, suggesting that they were reluctant to breed when only closely related males were available within the group as sires. The remaining four groups (29%) produced

Table 4 The number of groups, nests and nestlings for which parentage was determined. 'Groups' include all nests produced by the same set of breeders with each sex considered independently

	Parentage		
Categories	Groups	Nests	Nestlings
Cobreeding males	40	99	295
Joint-nesting females	13	29	102
Pairs with helpers	3	4	14
Breeder male missing (incest occurred)	2	5	14
Total sample size*	54	131	400

*Total sample sizes do not equal the column sums because some groups contained both cobreeding males and joint-nesting females.

Table 5 Parentage among cobreeding males based on DNA fingerprinting. Per cent sired is for all offspring produced by a set of cobreeding males ordered by relative success

Cobreeding group (Mean *n* nestlings ± SE)	Mean per cent sired ± SE (*n*)		
	α	β	δ*
2 ♂♂ (8.2 ± 1.0)	77 ± 4 (25)	23 ± 4 (25)	—
3–6 ♂♂ (6.1 ± 1.1)	69 ± 7 (15)	23 ± 5 (15)	7 ± 3 (15)
Brothers† (7.9 ± 1.0)	71 ± 4 (25)	26 ± 4 (25)	10 ± 6 (7)
Fathers & offspring† (6.6 ± 1.0)	78 ± 6 (14)	19 ± 6 (14)	5 ± 4 (7)

*In groups with four or more cobreeding males, at most three males sired offspring (*n* = 8 groups that produced 12 nests). †Row totals do not total 100% because means are calculated independently for each rank and sample size differs. Means indicate the relative success of an *n*th ranked bird in a group with at least *n* breeders.

nestlings, however, we were only able to genetically determine parentage for two of these groups because we did not have DNA samples for all the individuals involved. A total of 14 nestlings was produced in these two groups from five nests (Table 4). For nine of these young, from three of the nests, the only dyad that could have produced the offspring involved a breeding female and her helper offspring. For the remaining five nestlings, which were produced in two nests of one of the groups, the offspring were compatible with mating between male and female helpers. In one nest, a helper male sired three offspring with his sister in a joint nest with his sister and mother. In the second group, a helper male produced two offspring at a single nest with his sister.

Band-sharing between the nestlings and assigned parents confirmed that incest occurred in these groups lacking a male with breeding status. Offspring produced incestuously by a helper male that mated with his mother had high band-sharing with their mother (0.73 ± 0.02, $n = 9$) as well as their father (0.71 ± 0.02, $n = 9$). Band-sharing between the helper and the nestling was equal to the expected value for a helper breeding incestuously [expected (s_i) = 0.71]. The band-sharing between the offspring and the helper's father (the missing breeder) was also similar to the expected value [expected (s_f) = 0.39; observed = 0.36 ± 0.03, $n = 8$; the missing breeder was not included on the fingerprint for one offspring]. Both band-sharing values differed significantly from the expected values if the breeding male had somehow escaped detection and sired the offspring with the breeding female [observed = 0.73 and 0.36, expected (s_o) = 0.63, $t = 4.3$ and 11.2, $P < 0.01$ for both]. Band-sharing values also confirmed incest for the five offspring attributed to helper–helper parentage.

Reproductive success of cobreeding males and joint-nesting females

On average, the most successful male sired approximately three offspring in a nest for every one sired by the second most successful male (Table 5). Up to three males sired offspring in a group, and in one case three males shared paternity in a single nest. Sharing of paternity was found for 25 of 40 sets of potential cobreeders (63%). However, only the three oldest birds shared parentage when groups contained at least four males with breeding status ($n = 8$). Considering nests for which we determined parentage for at least two offspring, complete monopolization occurred in 60 nests (66%), whereas shared paternity was found for 31 nests (34%). The mean per cent sired by the most successful male varied little regardless of the number or relationship of the potential cobreeders (Table 5).

Joint-nesting females produced similar numbers of offspring, at least when two cobreeders were involved (Table 6). These results are consistent with earlier observational data on maternity by joint-nesting females. Sharing occurred whether the potentially joint-nesting females were sisters or a mother and her daughter. However, we only determined parentage for one nest in the latter category. When all nests produced by a set of cobreeders are considered together, parentage was shared in 12 of 13 cases (92%). Considering only those nests in which parentage was determined for at least two offspring, complete monopolization was found in only five nests (19%) while shared parentage was found for 22 nests (81%). Groups having three females with cobreeding status are rare (2.6% of groups) and only two of the three females produced young within each of the two nests that produced at least three offspring. However, direct observation confirms that three females can lay eggs in such nests (Koenig *et al*. 1995a).

The proportion of offspring sired by the most successful cobreeding male was significantly greater than that parented by the most successful joint-nesting female ($t = 4.7$, d.f. = 44, $P < 0.001$, statistics are for unequal variances). In addition, cobreeding males were less likely to share parentage than were joint-nesting females, both within nests or when considering all the offspring produced by a set of cobreeders (nests: $G^2 = 19.7$, d.f. = 1, $P < 0.001$; sets of cobreeders: $G^2 = 4.9$, d.f. = 1, $P < 0.05$).

Table 6 Parentage among joint-nesting females based on DNA fingerprinting and by observing egg-laying. Per cent sired is for all offspring produced by a set of joint-nesting females

Joint-nesting group (Mean *n* nestlings ± SE)	Mean per cent parented ± SE (*n*)		
	α	β	δ
2 ♀♀ (8.4 ± 1.6)	58 ± 2 (11)	42 ± 2 (11)	—
3 ♀♀ (5.0 ± 3.0)	56 ± 6 (2)	38 ± 13 (2)	6 ± 6 (2)
Sisters* (8.0 ± 1.7)	59 ± 2 (11)	40 ± 2 (11)	6 ± 6 (2)
Mother & her offspring (6)	50 (1)	50 (1)	—
Observed† (9.1 ± 1.6)	59 ± 3 (9)	41 ± 3 (9)	0

*Rows do not total 100% because means are calculated independently for each rank and sample size differs. Means indicate the relative success of an *n*th ranked bird in a group with at least *n* breeders. †See Koenig *et al.* (1995a) for parentage based on observations at nests during egg-laying.

Discussion

These results generally confirm the biological inferences of parentage drawn from our earlier indirect, mostly behavioural, data on this species. Multiple males compete and share in the production of offspring both within and between nests produced by the group. Cobreeding occurs among coalitions of siblings that have dispersed to obtain breeding status and between a parent and its same-sex offspring that has ascended to breeding status following the replacement of the opposite-sexed breeders. Extra-group mating does not occur and we detected no cases of females laying eggs in the nests of other groups. Incest by helpers occurs, but only rarely and in cases when reproductive vacancies have failed to be filled from outside the group. We observed no cases of incest in groups without reproductive vacancies. These patterns of parentage are consistent with incest avoidance being the primary determinant of whether birds are breeders or nonbreeding helpers within a group, and reproductive competition being the primary determinant of relative reproductive success among cobreeders (Koenig *et al.* 1998).

Considerable variation occurs in the degree to which reproduction is shared within groups, especially among cobreeding males. This complexity and variation in mating patterns offers the opportunity to address a range of hypotheses related to the benefits and costs of social behaviour, including reproductive partitioning theory, the relation of nestling feeding effort to parentage, and the effectiveness of mate guarding and female choice in determining parentage. These topics are addressed in more detail elsewhere and discussed only briefly here.

Reproductive partitioning

In acorn woodpeckers, joint-nesting females share reproductive success equally. In contrast, there is considerable bias or reproductive skew among cobreeding males, with the most successful cobreeding male being three times as successful as the next most successful. In groups with more than three males, only the older birds with breeding status sired offspring. However, among the males that bred, neither age nor condition determined which male was the most successful and there was often switching of the most successful breeder between nests (Haydock & Koenig, unpublished data). Switching is not consistent with the most popular set of models currently used to explain patterns of reproductive partitioning that assume control by one behaviourally dominant individual (Emlen 1996; Reeve *et al.* 1998). Thus, reproductive skew among males is probably not attributable to dominance relationships. Rather, it may instead be due to relatively low reproductive output combined with largely random process of mating that produces nonindependence of paternity within nests (Haydock & Koenig, unpublished data).

Behavioural indices of reproductive status

Prior to egg-laying, cobreeder males in groups with two breeder males guard the breeding female almost continuously (Mumme *et al.* 1983a). Patterns of mate guarding are similar for cobreeders that are siblings and those that are a father and a son. However, in groups with more than three cobreeder males, the youngest males sometimes do not participate in mate guarding (R.L. Mumme, unpublished data), presumably correlating with their extremely low probability of paternity. Rare observations of attempted copulation indicate that one of the most likely functions of mate guarding in acorn woodpeckers is to prevent other cobreeders from copulating successfully (Mumme *et al.* 1983a). Of eight attempted copulations, only one was apparently successful during a brief lapse of guarding by the second cobreeder, while the other seven were disrupted by the second cobreeder.

The rarity of observed copulation in acorn woodpeckers may be related to female choice. Females in groups with cobreeder males may only solicit in the presence of a single male, a rare event, because it is the only way to copulate successfully with the male of her choice. Alternatively, assuming that female breeders secretively copulate with each male breeder, copulation may effectively hide paternity information from the other breeder males. This may account for the similarity in feeding effort among males with breeder status, regardless of the number of young they have sired in the nest (Haydock & Koenig, unpublished data). Males may have little choice but to guard continuously to prevent cobreeders having sole access to the breeding female and to subsequently assume that their occasional solitary access to the female gives them some paternity. Male dominance relationships may be ineffective, explaining why the oldest male breeder, the presumed dominant, is not necessarily the most successful breeder. The only recourse for a male that is denied opportunity of parentage may be to destroy the nest and force the group to renest, giving themselves a new chance at parenting offspring (Koenig 1990).

The similarity of reproductive success among cobreeding females indicates that dominance relationships are ineffective at skewing reproductive success for females. Older and younger female cobreeders are equally successful in parenting young because neither female can prevent the other from destroying her eggs, forcing synchronization of egg-laying (Mumme *et al*. 1983b; Koenig *et al*. 1995a). This lack of effective dominance by both males and females in determining reproductive success lessens the usefulness of optimal skew models to explain patterns of reproductive partitioning in this society.

Incest and inbreeding avoidance

Of 400 offspring, only 14 (3.5%) were apparently the product of incestuous matings. All of these were produced in groups lacking a male that had acquired breeding status via the conventional routes of dispersal or inheritance on the death of an opposite-sex breeder. In addition, we could not clearly exclude nine of 228 offspring (3.9%) from being the product of incest in groups that contained at least one bird with helper status and at least one male and one female with breeding status. Multilocus fingerprinting has been criticized as being unsuitable for genetically demonstrating incest avoidance (Cockburn 1998; McRae & Amos 1999), but Parker *et al*. (1999) have shown that multilocus fingerprinting is as reliable as other genetic methods for excluding incest and has the advantage of allowing assignment of parentage in cases of incest by comparing band sharing values among potential parents. Our results confirm the effectiveness of multilocus fingerprinting in determining parentage and excluding incest, even in large cooperative groups consisting of close relatives. Band-sharing values were also effective in confirming that incest had occurred in the groups that lacked a male with breeding status.

The rarity of incestuous breeding is consistent with the hypothesis that incest avoidance is important in structuring acorn woodpecker societies (Koenig *et al*. 1998). Breeding vacancies sometimes exist for up to 3 years, but even when birds of both sexes are present, the group normally forgoes breeding. The lack of incestuous breeding is also consistent with observations of mate guarding (Mumme *et al*. 1983a), in that helper males do not usually participate with breeder males in either attendance or following of the breeding female.

Of 24 groups with female vacancies throughout at least one breeding season, offspring were produced only once. In contrast, groups with male breeding vacancies produced offspring in four of 14 groups ($G^2 = 4.5$, d.f. = 1, $P < 0.05$, includes data from three groups for which we did not genetically determine parentage). Of the two groups in which incest was confirmed, both followed a breeding male vacancy rather than a female vacancy. This difference is possibly a result of fitness differences between male and female helpers. Ecological constraints on dispersal are greater for helper males than for helper females (Koenig & Mumme 1987), suggesting that it may be more advantageous for helper females, but not males, to delay breeding and put effort into dispersal. Incestuous breeding could be advantageous to both established breeder males and females that have lost their mates. However, whereas helper males are willing to mate incestuously with their mother, helper females are not willing to mate with their father.

Behavioural and physiological evidence of an incest taboo has been found for most cooperatively breeding birds (Cockburn 1998) and genetic analysis has confirmed that incestuous young are rare (< 5%) in many species (Rabenold *et al*. 1990; Haig *et al*. 1994; Bruce *et al*. 1996; Delay *et al*. 1996; Haydock *et al*. 1996; Heer 1996; Quinn *et al*. 1999). Presumably incest is avoided due to inbreeding depression. However, incestuous mating is common in rails and inbreeding depression has been found in the moorhen, *Gallinula chloropus* (McRae 1996). Incestuous breeding has also been claimed for some social mammals (Reeve *et al*. 1990; Keane *et al*. 1996), apparently without suffering inbreeding depression. Indirect evidence based on the frequency that potentially incestuous groups forgo reproduction indicates that the costs of inbreeding are great in acorn woodpeckers (Koenig *et al*. 1998). Unfortunately, our current genetically verified sample of incestuous offspring is too small to provide a direct estimate of inbreeding depression.

Extra-group mating

Females apparently do not have the option of reproducing successfully by seeking extra-group fertilizations, even when no male breeder is present in the group. Such opportunities certainly exist, even without leaving the territory, because extra-group birds commonly intrude into territories during the breeding season. Extra-group parentage is uncommon in most other cooperatively breeding species also (Cockburn 1998), but it has been found to occur regularly in Australian fairy wrens *Malurus* sp. (65–76% of offspring; Brooker *et al*. 1990; Mulder *et al*. 1994; Mulder 1997), western bluebirds *Sialia mexicana* (19%; Dickinson & Akre 1998), white-fronted bee-eaters *Merops bullockoides* (9–12%; Wrege & Emlen 1987) and white-browed scrubwrens *Sericornis frontalis* (11%; Whittingham *et al*. 1997). In the splendid fairy wren (*M. splendens*), Brooker *et al*. (1990) attributed the high rate of extra-group matings to inbreeding avoidance because social mates frequently were close relatives. However, later work on fairy wrens has shown that the prevalence of mating outside the group may be due to female choice based on when potential mates moult into breeding plumage, an indicator of male quality (Mulder *et al*. 1994; Dunn & Cockburn 1999). In the white-browed scrubwren (Whittingham *et al*. 1997), the majority of extra-group mating was by females that were members of socially monogamous pairs, consistent with the hypothesis of fertilization insurance or the effectiveness of mate guarding in multimale groups.

Extra-group mating, especially by females in groups with male breeding vacancies, may also be rare because males perform all of the nocturnal incubation (Koenig & Mumme 1987). Consequently, females cannot successfully produce a nest without the aid of a male breeder. Frequent egg destruction by cobreeder females ensures synchronization in laying during joint-nesting (Mumme *et al*. 1988; Koenig *et al*. 1995a) and occasional egg destruction by cobreeder males counteracts lack of opportunities for mating (Koenig 1990). In combination, these behaviours indicate that breeding in acorn woodpeckers requires both acceptance and cooperation by group members. Females that accept or seek copulations outside of the group would most likely lose the cooperative benefits of other group members and risk the destruction of their eggs.

We also detected no cases of females laying eggs in the nests of other groups. Intraspecific brood parasitism is probably rare in territorial bird species because females are unlikely to have knowledge of or access to other nests (Reyer *et al*. 1997). Furthermore, even if parasitic eggs are laid, they may be rejected (Petrie & Møller 1991). It is likely that all of these factors contribute to the lack of extra-group brood parasitism in acorn woodpeckers. Eggs that we placed in empty nest cavities during the breeding season are normally removed by a breeder from the group (R.L. Mumme, unpublished data). Even if extra-group females are capable of detecting where and when a resident female is about to begin a nest, eggs placed in a nest cavity before the resident female lays her first egg are likely to suffer the same fate. Furthermore, access to nest cavities that are defended by all group members is likely to be difficult.

Polygynandry and the mating system

Multiple parentage by both males and females is relatively common in acorn woodpeckers (Table 7). The frequency of multiple parentage among males based solely on the assumption of incest avoidance was approximately twice the adjusted frequency of actual cobreeding based on our fingerprinting results (46.5 vs. 24.9%). For females, almost all cobreeders mothered young, resulting in only a small decrease comparing potential with adjusted frequencies of cobreeding (21.5 vs. 20.0%). The adjusted frequency of multiple parentage is slightly higher for males than for females when nests are grouped by the set of cobreeders (24.9 vs. 20.0%) or if considered on a nest by nest basis (18.6 vs. 16.8%). Multiple parentage by three or more individuals is rare for both males and females, but for females this is mostly because groups with potential cobreeders are rare, whereas for males it is due to lack of breeding success for potential cobreeders (Table 7).

Table 7 Per cent potential cobreeding from 1973 to 1998 ($n = 816$ group-years) vs. per cent adjusted cobreeding based on genetic data. Potential values are based on the assumption of incest avoidance and or demographic records of dispersal of sibling coalitions. Adjusted values are obtained by using our fingerprinting data plus, for females, observations during egg-laying to modify potential frequencies according to actual proportion that successfully cobreed across nests for the tenure of the set of cobreeders

Group composition	Potential frequencies	Adjusted frequencies
Cobreeders of either or both sexes with or without helpers	55.3	40.0
Single breeding pair with helpers	21.4	36.7
Unaided pairs	19.4	19.4*
Breeder missing	6.7	6.7
Two cobreeding males	28.9	23.0
Three cobreeding males	11.5	1.9
Four cobreeding males	4.0	0.0
Five or more cobreeding males	2.1	0.0
Cobreeding males	46.5	24.9
Two joint-nesting females	18.9	19.2
Three or more joint-nesting females	2.6	0.8
Cobreeding females	21.5	20.0
Cobreeders of both sexes	12.1	3.1

*See Dickinson *et al*. (1995) for parentage of unaided pairs.

The acorn woodpecker is one of only a few species for which cooperative polygynandry has been demonstrated genetically along with guira cuckoos *Guira guira* (Quinn *et al*. 1994), alpine accentors *Prunella collaris* (Heer 1996), pukekos *Porphyrio porphyrio* (Jamieson *et al*. 1994; Jamieson 1997) and Smith's longspurs *Calcarius pictus* (Briskie *et al*. 1998). This social system sets these species apart from the majority of other cooperatively breeding species that are generally monogamous or only involve cobreeding males. Breeding outside the social group occurs commonly in a few cooperatively breeding species but rarely in most. The causes of this variability in cooperative mating systems remain to be fully explained. The acorn woodpecker, with a social system ranging from simple monogamous pairs to complex social groups, offers an unparalleled opportunity for understanding the selective basis of such variation.

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