

Reproductive Roles in the Cooperatively Breeding Acorn Woodpecker: Incest Avoidance versus Reproductive Competition

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ABSTRACT: Incest is rare in the cooperatively breeding acorn woodpecker (*Melanerpes formicivorus*) despite a polygynandrous mating system in which nearly all group members are close relatives. Here we test the relative importance of avoiding matings between close relatives (incest avoidance) and within-sex competition for breeding opportunities (reproductive competition) in determining the mating system of acorn woodpeckers by examining how reproductive roles change following breeding vacancies. In 83% of cases in which helpers of the same sex were present in the group, reproductive vacancies were resolved when new unrelated immigrants filled the vacancy to the exclusion of resident same-sex helpers, who generally emigrated or did not breed while they remained in the group. Helpers of the opposite sex, especially when male, were significantly more likely to remain in their natal group and in about half the cases inherited and bred following reproductive vacancies. This result was not explainable by reproductive competition, since the number of immigrants was often less than or equal to the number of same-sex helpers in the group. Apparent incest resulted in 5% of cases. The time required to resolve reproductive vacancies was significantly longer for groups with helpers of the same sex as the vacancy. These results confirm that both incest avoidance and reproductive competition are important factors determining reproductive roles within groups of this highly social species.

Keywords: acorn woodpecker, cooperative breeding, incest avoidance, reproductive competition.

The role of incest avoidance—defined as the behavioral avoidance of matings between first-order or other close relatives—in determining reproductive roles within groups of highly social animals has long been controversial. This is partly because incest avoidance has until recently been considered to be strictly in the purview of human culture and thus capable of “distinguishing us unequivocally from related species” (Hammel et al. 1979). This has changed with studies documenting the rarity of incestuous matings in a variety of group-living species, including acorn woodpeckers *Melanerpes formicivorus* (Koenig and Pitelka 1979), baboons *Papio anubis* (Packer 1979), black-tailed prairie dogs *Cynomys ludovicianus* (Hoogland 1982), and white-footed mice *Peromyscus leucopus* (Wolff 1992). However, there are at least two additional problems impeding a general understanding of the role of incest avoidance in animal societies.

First, incest has been reported to be common in a variety of social vertebrates based solely on circumstantial demographic evidence, which cannot be considered reliable. For example, Brown (1974) estimated dramatically high levels of inbreeding in groups of the Mexican jay *Aphelocoma ultramarina* based on demographic inferences of low dispersal rates that proved to be unfounded (Brown 1987). More recently, the splendid fairy wren *Malurus splendens* of Australia was reported as breeding incestuously in 21% of social groups based on group composition (Rowley et al. 1986), but subsequent genetic work revealed the unexpected finding that females regularly mate outside their social unit, thereby avoiding incest in most cases (Brooker et al. 1990; Dunn et al. 1995). Nonetheless, other recent studies reporting low levels of genetic variation or high levels of band sharing within social groups continue to be interpreted as being due to high levels of inbreeding (Reeve et al. 1990; Lambert et al. 1994).

The second problem in developing a general understanding of the importance of incest avoidance is the potential for within-sex competition for breeding opportu-

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nities, or reproductive competition, to explain the low levels of incest frequently observed in animal societies. For example, Shields (1987) and Craig and Jamieson (1988) both pointed out that many of the apparent cases of incest avoidance previously documented for acorn woodpeckers (Koenig and Pitelka 1979; Koenig et al. 1984) could be parsimoniously explained by parental dominance and reproductive competition reducing the opportunity for inbreeding independent of any behavioral avoidance of incest per se. Similar arguments can be levied against many of the cases of apparent incest avoidance reported in the literature.

Here we address this issue by testing the relative importance of incest avoidance and reproductive competition in determining reproductive roles in the acorn woodpecker, a cooperatively breeding species with a polygynandrous mating system in which more than one male and female may breed within the same social unit (Koenig and Mumme 1987; Koenig et al. 1995). We draw on recent work using DNA fingerprinting to determine parentage directly, along with behavioral evidence and data on apparent reproductive suppression based on our long-term population study of this species.

Background

Group Composition and Reproductive Status

Groups of acorn woodpeckers at Hastings Natural History Reservation, Carmel Valley, California, consist of two to 13 individuals of both sexes and all ages. Copulations within groups are rarely observed, only a single nest is attended by a group at a time, and all birds typically contribute to incubation, brooding, and feeding of young (Mumme et al. 1990). Traditional pairing behavior is absent. Thus, there is generally little or no behavioral basis on which to assign reproductive status to a particular individual or set of individuals within a group.

We can, however, often divide group members into two categories: those born elsewhere that have immigrated into the group ("immigrants") and those born within the group ("helpers"). Immigrants are generally brood mates or at least offspring from the same group that are themselves closely related and disperse together in unisexual sibling units. Although definitive data on parentage was not available for most groups until recently, current molecular work has revealed that extragroup matings are rare: only one of 282 (<1%) offspring for which parentage has thus far been determined, including offspring produced by both socially monogamous (Dickinson et al. 1995) and socially polygamous groups (J. Haydock, W. D. Koenig, and M. T. Stanback, unpublished data), had a parent that was apparently not

a group member. Thus, we assume that helpers are the offspring of the group breeders and are therefore closely related to all other group members. Helpers typically remain in their natal group for ≥ 1 yr and may do so for their entire lives (Koenig and Mumme 1987).

We determined parentage of 282 offspring at 50 nests of 23 different groups containing at least one adult helper between 1992 and 1996 using standard multilocus DNA fingerprinting techniques (see below). Only groups containing the presumed parents or other first-order relatives of the helpers were included. Of these, we were able to unambiguously exclude all helpers as parents for all but eight cases. To quantify this, we counted the number of nestling-helper dyads for which fingerprinting or observations of egg laying demonstrated that the helper was not a parent. A total of 461 male offspring-helper dyads and 283 female offspring-helper dyads were tested. Of these, male helpers could be excluded as potential parents in 456 (98.9%) and female helpers in 281 (99.3%). Of those cases in which offspring could not be excluded as potential parents, only three, all involving a male helper and his mother at a single nest, were apparently truly incestuous, while the other five cases involved groups in which the putative parent was present and also could not be excluded as a potential parent. These latter cases were thus almost certainly the result of false inclusions. We conclude that incest by helpers living in groups along with related adults of the opposite sex is extremely rare (three of 744, or 0.4%, of dyads involving helpers).

Our ongoing molecular analyses, along with prior behavioral data, also confirm that the breeding system is polygynandrous, with up to at least three males and three females parenting offspring within a single nest (Koenig et al. 1995; J. Haydock, W. D. Koenig, and M. T. Stanback, unpublished data). Offspring are parented by immigrants that dispersed as sibling coalitions or by helpers that have inherited their natal territory following replacement of the related adult breeders of the opposite sex by individuals from outside the group; this latter situation is discussed in detail below. In general, however, immigrants are at least potentially breeders, while helpers are nonbreeders.

Hypotheses and Predictions

Approximately 31% of the adult population is made up of nonbreeding helpers (Koenig and Mumme 1987). What suppresses reproduction among this large fraction of the population? Developmental age is not a constraint, since both males and females are reproductively competent starting the year following their birth. Instead, two alternative, but not mutually exclusive, hypotheses may play a role: within-sex competition for breeding opportu-

Table 1: Predicted outcomes of reproductive vacancies

<i>Helpers present</i>	<i>Reproductive competition</i> (A)	<i>Incest avoidance</i> (B)
1. Opposite sex as the vacancy	Rapid replacement Helpers usually disperse	Rapid replacement Helpers inherit and breed
2. Same sex as the vacancy	Replacement only occurs when helpers are out-competed by a larger immigrant coalition Helpers inherit and breed	Replacement occurs but may be delayed Helpers disperse or, if they stay, do not breed

nities (reproductive competition; RC) and avoidance of inbreeding between close relatives (incest avoidance, IA).

Discriminating between these hypotheses is generally impossible for established groups, since a helper may fail to breed either because breeders of the opposite sex are close relatives (IA) or because an older, dominant breeder of the same sex suppresses the helper's opportunity to parent offspring (RC). The circumstances surrounding reproductive vacancies provide opportunities where one or the other of these factors is reduced, thereby allowing their relative importance to be estimated.

Death or disappearance of a group member generally results in no obvious change in the reproductive roles of the remaining individuals as long as at least one breeder of each sex remains alive in a group. In contrast, significant behavioral changes frequently accompany the death or disappearance of all breeders of one sex, an event termed a *reproductive vacancy* (Koenig 1981; Hannon et al. 1985). What happens to nonbreeding helpers, both of the same and of the opposite sex as a vacancy, following reproductive vacancies, provides a unique opportunity to contrast predictions of RC and IA (table 1; see also Hannon et al. 1985; Shields 1987). These predictions assume that, in the absence of a reproductive vacancy, helpers reap indirect fitness advantages by helping but that they gain relatively more by dispersing if they are able to obtain a breeding opportunity in the population (Koenig and Mumme 1987; Koenig et al. 1992).

Helpers of the Opposite Sex as the Vacancy Present (table 1, row 1). If IA alone determines reproductive roles (table 1, 1B), both helpers and remaining breeders benefit by

having the vacancy filled by unrelated immigrants as quickly as possible. Once this occurs, helpers should no longer be reproductively suppressed as a consequence of the opposite-sex breeders being close relatives. Thus, they should inherit and cobreed with remaining same-sex breeders.

Vacancies in groups containing only helpers of the opposite sex should also be filled quickly if RC alone suppresses reproduction by helpers (table 1, 1A). However, the loss of the parent of the opposite sex does not change the within-sex competitive environment for helpers, even after unrelated immigrants fill the vacancy. Only in cases when reproductive competition is unusually low might helpers remain and cobreed. Furthermore, as nonbreeding helpers, they gain less by remaining in the group because the offspring they might subsequently help raise would only be half-siblings. Thus, most helpers are predicted to leave the group if and when they are able to obtain a reproductive opportunity elsewhere.

Helpers of the Same Sex as the Vacancy Present (table 1, row 2). If RC alone determines reproductive roles (table 1, 2A), helpers should simply inherit and breed incestuously with the breeders of the opposite sex following the disappearance of same-sex breeders. No replacement by birds from outside the group should occur; rather, the vacancy is filled from within the group. One potentially significant caveat pertains to this prediction: because larger coalitions can often outcompete smaller ones in fights over vacancies (Hannon et al. 1985), helpers could potentially be evicted from groups containing vacancies by larger sibling coalitions (Shields 1987).

If IA determines reproductive roles (table 1, 2B), the group is still bisexual following the vacancy but the benefits of reproduction are lower due to presumed inbreeding depression. Thus, it is in the interest of the remaining breeders to recruit an unrelated replacement from elsewhere as soon as possible. It is also in the interest of the helpers to the extent that they could subsequently obtain indirect fitness benefits by helping to raise half-siblings. However, there is at least one major potential cost to helpers of having the vacancy filled. Specifically, new immigrants are likely to view the helpers as potential rivals for food and other resources, if not reproduction itself, and may try to expel same-sex helpers. Because of this, helpers of the same sex as vacancies may benefit by delaying the filling of vacancies, leading to a conflict with the remaining breeders (Hannon et al. 1985; Koenig et al. 1988). Consequently, IA predicts that replacement by individuals outside the group will occur, but that it may be delayed. As long as helpers remain in the group, they should not breed, even after the vacancy is filled from

outside the group. Eventually, most helpers are expected to disperse to fill vacancies elsewhere in the population.

Study Area and Methods

We studied acorn woodpeckers at Hastings Natural History Reservation, Monterey County, central coastal California, between 1974 and 1996. Birds were banded with unique combinations of color bands and observed from blinds using appropriate optical equipment. Group composition was based on censuses conducted approximately bimonthly and on systematic nest watches. More than 2,500 birds have been banded to date. Additional details can be found in an earlier publication (Koenig and Mumme 1987). For analysis of reproductive vacancies, we compiled data on group composition and the outcomes of 197 vacancies recorded in our study population between 1974 and 1995. Of these, 62 were male vacancies and 124 were female vacancies; in 11 additional cases breeders of both sexes disappeared simultaneously or nearly so. These latter cases are not discussed further here. As of 1996, 56 of the male vacancies and 116 of the female vacancies had been resolved.

Parentage was determined using multilocus DNA fingerprinting. Blood was taken from the brachial vein of birds when banded and genomic DNA was isolated using standard procedures. Jeffrey's minisatellite probes (Jeffreys et al. 1985) were used to yield banding patterns of nestlings that were compared with those of all adult members within the group. Because extragroup parentage is rare (see above), we considered only adult group members as potential parents. Presence of novel fragments and, secondarily, band-sharing coefficients were used to exclude all adult members of a group as parents of any particular offspring except for one male and one female. Additional details of these procedures can be found elsewhere (Dickinson et al. 1995; Haydock et al. 1996). Two-tailed probability values are presented whenever possible.

Results

We first summarize how reproductive vacancies were resolved. These results are consistent with the predictions of IA, but do not reject the alternative hypothesis of RC being primarily responsible for the observed patterns of reproductive suppression. We then specifically address the alternative predictions outlined above by looking at the fate and reproductive status of helpers following reproductive vacancies; the relative size of immigrant coalitions compared with the number of same-sex helpers that were present in the group when vacancies arose and

that were still present when the vacancy was resolved; and length of time required to resolve vacancies.

Resolution of Vacancies

Vacancies are ultimately resolved when one or more birds assume breeding status to replace the missing breeders in the group. When only helpers of the opposite sex are present, vacancies must be filled by immigrants from elsewhere. When helpers of the same sex are present, vacancies may be filled either by immigrants or by helpers inheriting from within the group. These latter cases are thus of particular interest.

The majority of reproductive vacancies in which helpers of the same sex as the vacancy were present were resolved by new, unrelated immigrants of the same sex as the missing breeders joining the group (table 2, line 1). This was particularly true for female vacancies, 91% of which ended when one or more new female immigrants filled the vacancy. In 66% of these cases, all female helpers left the group prior to a subsequent breeding attempt (line 1a). In the remaining 34%, one or more female helpers stayed for at least one subsequent breeding season (line 1b). On one occasion the remaining breeder male eventually disappeared and was replaced by an unrelated bird allowing one of the helper females to inherit and breed (line 2). In one case the original breeder female returned to the group (line 3) and in one additional case the territory was abandoned without the vacancy ever being resolved (line 4). Finally, we observed two cases (4%) in which the outcome was apparent incest between the remaining breeder males and the helper females, who were their presumed offspring (line 5).

The various resolutions were somewhat different for male vacancies, although the majority (64%) were again resolved when one or more new, unrelated birds of the same sex immigrated into the group (table 2, line 1). In all these cases, all helper males left prior to the subsequent breeding season. The biggest difference from female vacancies was in the relatively high frequency (27%) of cases resolved when new unrelated females joined the group after the original breeder female disappeared (two cases) or emigrated elsewhere (four cases)(line 2). These cases, at least the latter four of which apparently involved "abdication" on the part of the breeder female, were followed by the remaining helper males inheriting breeding status in their natal territory. As with female vacancies, resolutions involving apparent incest were rare, occurring in only two (9%) cases (line 5).

In summary, the majority of reproductive vacancies in which nonbreeding helpers of the same sex as the missing bird were present were resolved when immigrants from outside the group replaced the missing individual

Table 2: Resolutions of reproductive vacancies of groups containing nonbreeding helpers of the same sex as the missing bird

Resolution	Male vacancies		Female vacancies		All vacancies	
	No.	%	No.	%	No.	%
1. Replaced from outside the group	14	63.6	48	90.6	62	82.7
a. All same-sex helpers left	14	63.6	35	66.0	49	65.3
b. ≥ 1 same-sex helpers stayed	0	0	13	34.0	13	17.3
2. Remaining breeder left and replaced from outside the group; same-sex helper stayed	6	27.3	1	1.9	7	9.3
3. Original bird returned	0	0	1	1.9	1	1.3
4. Abandoned before breeding	0	0	1	1.9	1	1.3
5. Apparent incest	2	9.1	2	3.8	4	5.3
6. Total reproductive vacancies	22	100	53	100.1	75	99.9

Note: Percentages may not total exactly to 100 due to rounding.

and the same-sex helpers dispersed or left the group, as predicted by the IA hypothesis (table 1, 2B). Various other outcomes were observed, but only four resulted in known or apparent incest, even though in all cases groups were at least temporarily composed of closely related males and females (i.e., adult breeders and their opposite-sex offspring). This amounts to 5% of all vacancies reported in table 2, and 14% of the 29 such vacancies (nine male and 20 female) that lasted at least 1 mo and included part or all of a breeding season. Thus, incest avoidance is not a result of lack of opportunity.

Of the four cases of apparent incest listed in table 2, three occurred prior to our DNA fingerprinting studies. Thus, only one of the four cases was confirmed by genetic analysis of parentage. Subsequently, we have confirmed an additional case of incest in 1997. Thus, we have now documented two groups committing incest following reproductive vacancies.

Dispersal of Helpers Following Vacancies

We determined the fate of helpers, both individually and on a group basis, depending on their sex relative to that of the reproductive vacancy. For the former, helpers were counted as having stayed if they remained in the group through one or more breeding seasons following the resolution of the vacancy. For the latter, helpers were counted as having stayed if at least one remained through a breeding season following resolution of the vacancy.

Male helpers were far more likely to stay when vacancies were of the opposite sex (table 3), as predicted by IA (table 1, col. B). For female helpers, the proportion remaining following male and female vacancies did not differ significantly. However, the data for individuals were again in the direction predicted by IA, with more helpers remaining following vacancies of the opposite sex.

Reproductive Status of Helpers Following Reproductive Vacancies

What is the subsequent reproductive status of helpers that remained in their natal groups following a reproductive vacancy (table 3, col. 1)? Most of our data on this point come from females, for which reproductive status can be inferred from clutch size and by behavioral observations during egg laying. However, we were also able to acquire data on a several males using DNA fingerprinting. We restricted our analysis to groups in which at least one breeder of the opposite sex as the vacancy remained in the group, thereby reducing sample sizes somewhat from those listed in table 3.

Table 3: Fate of helpers following the resolution of reproductive vacancies

Sex of helpers/vacancy	Helpers stayed	Helpers left	% that stayed	P value*
Females:				
Individuals				.09
Male vacancy	10	32	23.8	
Female vacancy	16	87	15.5	
Groups†				.21
Male vacancy	7	14	33.3	
Female vacancy	16	37	30.2	
Males:				
Individuals				<.001
Male vacancy	15	33	31.3	
Female vacancy	79	51	60.8	
Groups†				<.001
Male vacancy	8	14	36.4	
Female vacancy	46	10	82.1	

* P values based on Fisher exact tests.

† Whether all helpers of the appropriate sex left or whether one or more remained through a subsequent breeding season.

Table 4: Reproductive status of helpers that remain following the resolution of a vacancy

Sex of helpers/vacancy	Helpers			% that bred	P value*
	Bred	Did not breed	Do not know		
Females:					
Individuals					.004
Male replaced; breeder female remains†	6	1	0	85.7	
Female vacancy; breeder male remains	1	9	3	10.0	
Groups					.002
Male replaced; breeder female remains†	5	0	0	100.0	
Female vacancy; breeder male remains	1	9	3	10.0	
Males:					
Individuals					.03
Female vacancy (observed)	5	10	54	33.3	
Female vacancy (expected)	7.97	7.03	...	53.1	
Groups					...
Female vacancy	5	1	33	83.3	

* P value for females by Fisher exact test; for males expected values and P value derived by randomization test; see text.

† The female breeder was the mother of the helper for four of these birds from three groups; the other two birds from two groups were unrelated to the breeder female with which they cobred.

Females laid eggs in six of seven (86%) such cases following a male vacancy (table 4). In contrast, only one of 10 (10%) females remaining following a female vacancy laid eggs. The difference was even more extreme on a group basis—that is, considering whether at least one or no helper female remaining in her natal group laid eggs following male or female vacancies (table 4).

For males, we obtained DNA fingerprinting data from 15 individuals that remained following the replacement from outside the group of the related adult female in the group and were present along with at least one older, adult male during a subsequent nesting attempt. Of these, five (38%) individuals (in four of five groups) were known to have sired offspring. One additional male helper not reported in table 4 was known to have successfully sired offspring as a cobreeder with his father following the disappearance of his mother based on allozyme data.

We tested the proportion of males successfully breeding following female vacancies against expected values using a randomization test in which all males in the group had an equal probability of siring offspring, thereby eliminating any bias due to reproductive competition. Ten thousand trials were performed to determine the distribution of the number of helper males (out of the 15 for which we had DNA data) that would be expected to successfully sire young under this null hypothesis. The results (fig. 1) suggest that in the absence of any reproductive competition among the male group members, the mean number of helper males expected to have parented offspring is 7.97 and the probability of five or

fewer males having sired offspring is 3%. Thus, although the data demonstrate that male helpers can sire offspring following replacement of their mothers from outside the group, reproductive competition apparently lowers their reproductive success below what it is expected to be if there were no reproductive skew among cobreeder males.

Few males remained in their natal groups following

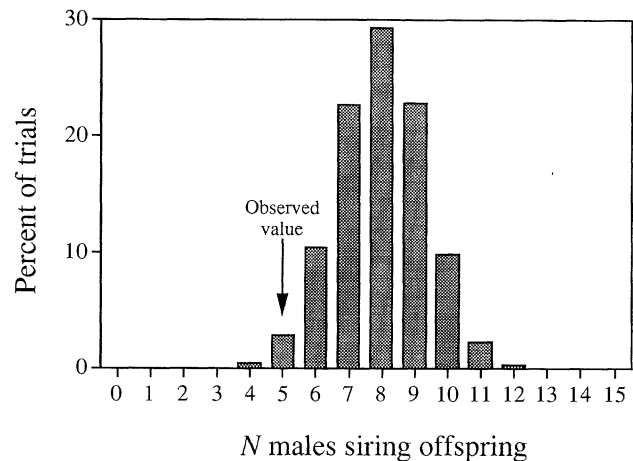


Figure 1: Results of a randomization test to determine the number of males (out of 15 from five groups) expected to have sired offspring given equal probability of parentage (no reproductive competition) and the number of offspring fingerprinted from the different groups. The mean of the 10,000 trials was 7.97; 302 trials (3.02%) resulted in ≤ 5 males siring offspring, which was the number of helper males observed to have sired offspring by DNA fingerprinting.

Table 5: Relative size of immigrant coalitions compared to the number of same-sex helpers present or known to be alive

Relative number of birds	Start of vacancy				End of vacancy			
	Male vacancy		Female vacancy		Male vacancy		Female vacancy	
	No.	%	No.	%	No.	%	No.	%
N helpers $>$ N immigrants	4	28.6	20	41.7	1	7.1	11	22.9
N helpers = N immigrants	3	21.4	20	41.7	3	21.4	16	33.3
N helpers $<$ N immigrants	<u>7</u>	50.0	<u>8</u>	16.7	<u>10</u>	71.4	<u>21</u>	43.8
Total	14		48		14		48	

male vacancies; all but two of the 15 individuals listed in this category in table 3 were in groups in which the related adult females in the group left or disappeared as well. Consequently, we omitted this category from table 4.

Relative Size of Immigrant Coalitions versus Number of Helpers

Larger coalitions of siblings are more competitive and more likely to fill vacancies than smaller coalitions (Hannon et al. 1985). This manifestation of reproductive competition offers an alternative explanation for how incest is avoided following many reproductive vacancies (Shields 1987). If this is an important confounding factor, at least two predictions follow. First, helpers in groups with same-sex vacancies should be evicted only by larger immigrant coalitions. Second, because large sets of helpers should only be evicted by large coalitions of siblings while any size of coalition could fill vacancies when no helpers of the same sex was present, the size of coalitions filling vacancies should be positively correlated with the number of helpers of the same sex present at the start of the vacancy.

The first prediction is examined in table 5. Combining the sexes, the size of the immigrant coalition was less than or equal to the number of helpers present in the group at the start of the vacancy in 47 of 62 (76%) cases, and less than or equal to the number of helpers known to be alive when resolution occurred in 31 of 62 (50%) cases. Thus, reproductive competition can be eliminated as a significant factor in the 50% of vacancies in which a larger number of helpers are replaced by a smaller coalition of immigrants.

To test the second prediction, we restricted analyses to cases in which vacancies were resolved when one or more immigrants of the same sex joined the group and correlated the number of same-sex helpers present at the start and at the end of the vacancy with the size of the coalition filling vacancies. For female vacancies, neither corre-

lation was significant (helpers at start: $r_s = 0.03$, $P > .7$; helpers at end: $r_s = 0.05$, $P > .6$; both $N = 114$). For male vacancies, there was a barely significant correlation between the size of immigrant coalitions and the number of male helpers present when the vacancy ended ($r_s = 0.32$, $N = 47$, $P = .03$) but not with the number of male helpers present when the vacancy started ($r_s = 0.20$, $N = 47$, $P = .18$).

Contrary to the hypothesis that helpers leave only when evicted by larger immigrant coalitions, we observed 10 cases (three male and seven female) in which the helpers were known to have dispersed to another group prior to when immigrants filled the vacancy. We suspect that this was generally the case—that is, that most coalitions of immigrants join groups and resolve vacancies only after same-sex helpers voluntarily leave the group.

Time to Resolution and Latency to Breeding Following Vacancies

Most vacancies are resolved within a few months: 55.4% of male and 49.6% of female vacancies were resolved within 1 mo and the mean (\pm SD) time vacancies went unresolved was 97 ± 160 d ($N = 56$) for males and 138 ± 254 d ($N = 115$) for females (fig. 2). However, some vacancies (12.5% male and 8.7% female) were not resolved within 1 yr and a few required several years for resolution. The maximum duration was 1.7 yr for a male vacancy and 3.8 yr for a female vacancy.

Mean latency is relatively short (0.9–2.1 mo) when helpers of the same sex are absent but relatively long (4.3–8.1 mo) when helpers of the same sex are present (table 6). In order to statistically disentangle the relationship between sex of vacancy and sex of helpers, we performed a three-way ANOVA in which sex of vacancy, presence of helpers of the same sex, and presence of helpers of the opposite sex were included as main factors potentially influencing latency. Results using data from 171 vacancies (56 male and 115 female) were unequivocal: there was no significant effect as a result of the sex of

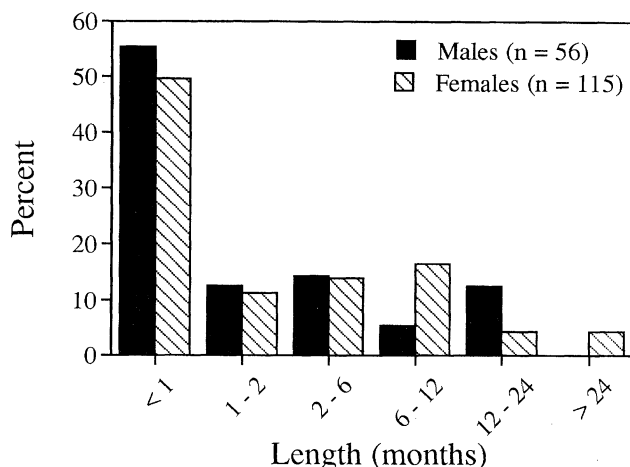


Figure 2: The distribution of the estimated length of time to resolution for male and female vacancies. Mean \pm SD is 97 ± 160 d for males and 138 ± 254 d for females.

the vacancy ($F = 0.7$, $df = 1$, 163 , $P = .41$), the presence of helpers of the opposite sex ($F = 0.4$, $df = 1$, 163 , $P = .53$), or of any of the two-way or three-way interactions between factors (all $P > .30$). In contrast, the effect of helpers of the same sex as the vacancy was highly significant ($F = 17.0$, $df = 1$, 163 , $P < .001$). In a multiple classification analysis, the mean latency controlling for sex of vacancy and presence of helpers of the opposite sex increased from 2.0 mo in groups without helpers of the same sex, to 7.0 mo in groups with helpers of the same sex. Thus, for both male and female vacancies the time required for resolution is significantly lengthened by the presence of helpers of the same sex as the vacancy.

Given that some reproductive vacancies require ≥ 1 yr to resolve, it follows that groups sometimes miss breeding opportunities while reproductive vacancies go unfilled, except in those rare cases in which incest apparently occurs (table 2, line 5). The number of such "skipped" years can be dramatic. Two groups were

Table 6: Mean \pm SD (N) months required to resolve vacancies as a function of helpers

Male helpers	Female helpers	Male vacancies	Female vacancies
0	0	1.5 ± 4.3 (26)	2.1 ± 3.6 (42)
≥ 1	0	4.3 ± 5.7 (9)	2.0 ± 3.5 (21)
0	≥ 1	$.9 \pm 1.5$ (8)	6.5 ± 10.0 (15)
≥ 1	≥ 1	7.2 ± 6.5 (13)	8.1 ± 12.0 (37)

known to have skipped three successive breeding seasons due to a failure to resolve reproductive vacancies, and in 1995, an extraordinary year for this phenomenon, six of 38 groups (15.8%) contained helpers, but not breeders, of one sex and either did not attempt to breed or failed directly as a result. (One additional group with this composition committed mother-son incest.) Given that these groups contained birds of both sexes, this lost reproductive potential is attributable largely, if not entirely, to incest avoidance. The demographic consequences of this phenomenon are quantified elsewhere (W. D. Koenig, M. T. Stanback, and J. Haydock, unpublished manuscript).

Discussion

Incest is generally presumed to be deleterious because of inbreeding depression; specifically, there is an increase in the expression of deleterious recessive alleles or overdominant loci resulting in reduced viability and fecundity (Wilmsen Thornhill 1993). Inbreeding depression is well documented in plants and in various artificial or laboratory populations (e.g., Darwin 1876; Wright 1977; Ralls et al. 1979; Charlesworth and Charlesworth 1987). However, evidence for inbreeding depression in natural populations of vertebrates is rare (Ralls et al. 1986; Pusey and Wolf 1996). The few studies that have demonstrated significant inbreeding depression in wild or semiwild populations, including those of van Noordwijk and Scharloo (1981) on great tits (*Parus major*), Jiménez et al. (1994) on white-footed mice, and Keller et al. (1994) on song sparrows (*Melospiza melodia*), are almost equally balanced against studies finding no evidence of inbreeding depression, including Hoogland (1992) on black-tailed prairie dogs and Keane et al. (1996) on dwarf mongoose (*Helogale parvula*).

As a result, the significance of the observation that incest is rare in almost all populations of vertebrates remains controversial. Even assuming inbreeding depression is significant and real, environmental factors may overwhelm its effects (Caro and Laurenson 1994; Caughley 1994), and alternative hypotheses may explain the rarity of incestuous matings (Shields 1987; Craig and Jamieson 1988).

In many species, the absence of incest is not surprising: many migratory bird species, for example, have highly fluid populations in which close relatives only rarely find themselves in potentially incestuous situations (e.g., indigo buntings *Passerina cyanea*; Payne 1991). This is not the case for most cooperatively breeding species, which are usually highly sedentary and may have relatively restricted dispersal (Zack 1990) greatly increasing the probability that close relatives will encounter each

other when mating. The demographic cost of incest avoidance under such circumstances can be significant (Hammel et al. 1979), especially if population growth rates are relatively low (Mills and Smouse 1994). Eventually, such costs are expected to lead to an increase in the degree of inbreeding tolerance (Waser et al. 1986).

In acorn woodpeckers in California the potential for incestuous matings is widespread. Birds live in family groups of up to 15 individuals and are polygynandrous, with up to seven cobreeding males competing for matings with up to three joint-nesting females. Cobreeding sets of birds are usually close relatives, either siblings or parent/offspring or a combination of the two. Communal offspring fledged by groups typically remain as nonbreeding helpers for up to several years and a significant number eventually inherit and assume reproductive status within their natal group (Koenig and Mumme 1987).

Previously, we reported that incestuous matings are rare (Koenig et al. 1984). However, as pointed out by Shields (1987) and Craig and Jamieson (1988), this observation by itself provides only weak evidence that incest avoidance is the critical force determining reproductive roles, since reproductive competition between individuals of the same sex can explain many or most of the observed patterns without resorting to active inbreeding avoidance mechanisms. Here we provide direct evidence supporting our previous speculation concerning the mating system of the acorn woodpecker, focusing in particular on its implications for the importance of incest avoidance.

Results of DNA fingerprinting of groups demonstrates that extragroup reproductive activity in the form of either extragroup fertilizations or egg dumping by females outside the group is rare (Dickinson et al. 1995). Results also support our previous conjecture that immigrants, which often join groups in unisexual coalitions, can successfully share both paternity and maternity within groups, while helpers living in groups with related adults of both sexes are nonbreeders.

More critical for testing the importance of incest avoidance (IA) and reproductive competition (RC) is the fate of helpers after all breeders of one sex or the other die or disappear from a group, creating a reproductive vacancy. Reproductive vacancies are often filled from outside the group following an extended fight, or power struggle, during which sibling coalitions from numerous groups fight for control of the territory (Koenig 1981; Hannon et al. 1985). In general, RC predicts that when helpers of the opposite sex are present, they are more likely to disperse, whereas if helpers are of the same sex as the vacancy, they should inherit their natal territory and breed; IA predicts the opposite pattern (table 1).

These contrasting predictions allow us to examine the relative importance of these two hypotheses for explaining the patterns of mating observed in acorn woodpeckers.

These contrasts are summarized in table 7. Apparent incest was rarely observed; rather, vacancies were most often filled by new immigrants of the missing sex or, especially for male vacancies, by apparent abdication of the breeder female allowing a new immigrant female to join the group and subsequently breed with the former male helpers. However, a small proportion of reproductive vacancies were resolved by apparent incest, thus offering some support for the hypothesis that reproductive competition may overwhelm the costs associated with incest, at least in rare cases. As of 1997, we have confirmed incest by two groups using DNA fingerprinting (J. Haydock and W. D. Koenig, unpublished data).

On the side of IA, helpers were significantly more likely to remain in natal groups and were known to have parented offspring in about half the cases following vacancies of the opposite sex. About two-thirds of vacancies were resolved without incest when an immigrant coalition of birds joined the group and all same-sex helpers left. It was generally unlikely that incest was avoided as a side effect of a superiorly large coalition of immigrants forcing out a smaller number of helpers. In at least 10 of these cases, helpers were known to have dispersed to another group before immigrants filled the vacancy, supporting the hypothesis that helpers were the direct cause of any delay in filling the vacancy and that immigrants were able to fill the vacancy only after helpers dispersed and found reproductive opportunities elsewhere.

Finally, the time required to resolve vacancies was significantly longer for groups with helpers of the same sex, and such groups experienced significantly reduced fecundity compared with groups experiencing vacancies that did not contain helpers of the same sex. Since these groups all suffered vacancies, the lower probability of attempting to breed and the lower reproductive success is attributable specifically to incest avoidance—that is, difficulties in resuming reproductive activity associated with groups consisting of only breeders of one sex and only related helpers of the other sex. The demographic consequences of this reduction are considerable (W. D. Koenig, M. T. Stanback, and J. Haydock, unpublished manuscript).

These results demonstrate that, consistent with the predictions of incest avoidance, helpers are able to inherit and breed following the disappearance of related adults of the opposite sex but not of the same sex. Thus, IA, independent of RC, plays a strong, significant role in determining reproductive roles in acorn woodpecker societ-

Table 7: Summary of results comparing reproductive competition and incest avoidance

Phenomenon	Data supporting		Source
	Incest avoidance	Reproductive competition	
Resolution of vacancies	95% resolved without incest	Subsequent incest has been confirmed in two groups	Table 2
Dispersal of helpers	More likely to remain in natal group when vacancies were of the opposite sex	39% of males and 76% of females left when vacancy was of the opposite sex	Table 3
Breeding of helpers	6 of 7 female and 5 of 15 male helpers bred following a vacancy of opposite sex; only 1 of 10 females known to have done so following a vacancy of same sex	Half of helpers not known to have successfully parented young following vacancy of opposite sex; observed proportion of helper males breeding following such vacancies 37% less than expected by chance	Table 4
Size of immigrant coalitions compared to number of same-sex offspring	Coalition size of offspring greater than or equal to that of immigrants in 50%–76% of cases; low correlations between number of helpers and size of immigrant coalition; in 10 cases helpers observed to have emigrated prior to immigration by new coalition	Half of immigrant coalitions greater than number of offspring present when vacancies were resolved; correlations between number of male helpers when resolved and size of immigrant coalition significant	Table 5
Time required to resolve vacancies	Significantly longer for groups with helpers of the same sex as the vacancy	...	Table 6
Lost reproductive potential	Significant for groups with helpers of the same sex as the vacancy	...	W. D. Koenig, M. T. Stanback, and J. Haydock (unpublished manuscript)

ies. Nonetheless, our results support an important role of RC as well (table 7). This is not surprising: behavioral data on egg destruction by joint-nesting females, mate guarding and nest destruction by cobreeding males, and fights over the filling of reproductive vacancies all provide ample evidence that RC is pervasive in acorn woodpecker societies (Koenig 1981, 1990; Mumme et al. 1983; Hannon et al. 1985; Koenig et al. 1995). Given the known significance of RC based on prior work, it is striking that the comparisons made here indicate that IA is on par with RC in terms of its importance in determining reproductive roles of acorn woodpeckers within groups.

Acorn woodpeckers live under conditions of severe environmental constraints limiting the ability of offspring to disperse and breed independently in the population

(Koenig and Mumme 1987; Koenig et al. 1992). As a consequence, reproductive vacancies are often filled quickly, sometimes within hours (Hannon et al. 1985). However, these ecological circumstances can lead to conflict between helpers of one sex and breeders of the other following reproductive vacancies (Hannon et al. 1985; Koenig et al. 1988). Assuming that incest is generally avoided, remaining breeders in such groups face the choice of attempting to force out helpers, thereby decreasing the helpers' survivorship and thus the breeder's own direct fitness but increasing the chances of filling the vacancy and breeding in the future, or of allowing the helpers to remain and potentially forsaking reproduction in one or more subsequent years.

Faced with such options, both of which apparently entail significant fitness costs, theoretical considerations

would predict that inbreeding tolerance would be broadened and that incest would frequently result even if inbreeding depression were relatively great (Waser et al. 1986). The fact that apparent incest occurs so rarely is thus all the more striking and indicates that the costs of inbreeding in this system are particularly great.

Unfortunately, we are unable to provide a direct estimate of inbreeding depression in acorn woodpeckers for at least two reasons. First, apparent incest is rare; only four of 75 (5.3%) reproductive vacancies in which offspring of the same sex were originally present in the group resulted in apparent incest between the remaining breeders of the opposite sex and one or more of the helpers (table 2). Earlier work documented 10 cases of known or probable inbreeding, five of which resulted from limited dispersal (i.e., related breeders dispersed independently to a group and were never resident in the same group prior to the incestuous mating; Koenig et al. 1984). No obvious inbreeding depression was evident in these cases. However, only two of these were behaviorally unambiguous, and we have thus far been able to confirm only two cases of incest with DNA fingerprinting. Such confirmation is important, as demonstrated by the splendid fairy wrens discussed earlier, in which a majority of females mate outside their social group rather than incestuously within the group (Brooker et al. 1990; Dunn et al. 1995).

This absence of a direct estimate of inbreeding depression is offset in part by our evidence that the costs of incest avoidance, based on the lower reproductive success of groups suffering reproductive vacancies containing helpers of the same sex as the missing individual, are significant, both to the individual groups involved and to the reproductive potential of the population as a whole (W. D. Koenig, M. T. Stanback, and J. Haydock, unpublished manuscript). Assuming that individuals behave to maximize their fitness, it follows that inbreeding depression must be considerable.

These results support other recent evidence that suggests that inbreeding depression may be significant, at least under conditions of environmental stress (Keller et al. 1994), and that incest avoidance can be important above and beyond the role of reproductive competition (Wolff 1992). Such findings indicate that the rarity of incest in natural populations of vertebrates is not simply a by-product of other unrelated ecological factors. Furthermore, contrary to arguments suggesting that the genetic consequences of inbreeding may be minor or even positive under some conditions (Shields 1987; Craig and Jamieson 1988), incest appears to be actively avoided in acorn woodpeckers and most, although not all, other species in which this phenomenon has been critically examined. Inbreeding apparently has a potentially impor-

tant effect on the viability of acorn woodpeckers and most, if not all, natural populations of vertebrates.

There remain but a small number of vertebrates for which incest is believed to be a regular part of their mating system. The most convincing thus far is the dwarf mongoose, where extensive behavioral and genetic data indicate a unique social organization in which inbreeding avoidance plays only a small role and inbreeding depression appears to have little effect (Keane et al. 1996). Two others are the African naked mole-rat (*Heterocephalus glaber*), where extensive analysis of genetic variation (Reeve et al. 1990; Honeycutt et al. 1991) combined with behavioral evidence from laboratory populations (Sherman et al. 1991) suggests that colonies may be severely inbred, and the pukeko (*Porphyrio porphyrio*), a New Zealand rail for which high levels of band sharing lends some support to the behavioral inference that incest is common (Craig and Jamieson 1988; Lambert et al. 1994). The ecological factors facilitating incest in these taxa, but not in the vast majority of other species in which incest is rare despite (in many cases) comparably intense ecological constraints on dispersal, are unknown but remain a critical mystery in the attempt to understand the role of inbreeding in natural populations.

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