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# GENETIC EVIDENCE FOR UNDETECTED ALLELES AND UNEXPECTED PARENTAGE IN THE GRAY-BREASTED JAY[1]

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*Abstract.* We performed protein electrophoresis to detect multiple parentage in broods of the Gray-breasted Jay (*Aphelocoma ultramarina*), a plural breeder that has been studied since 1969 in southeastern Arizona. We analyzed data from 43 nests (142 nestlings) for which we had blood, muscle, or feather samples from the nestlings and from at least one of the primary adults at a nest. Detection of multiple parentage was complicated by the apparent existence of an undetected (either null or masked) allele at the leu-gly-gly peptidase locus. The undetected allele resulted in offspring with single-banded phenotypes that were inconsistent with the phenotypes of adults with different single-banded phenotypes, assuming simple Mendelian inheritance. Including inconsistencies due to the undetected allele, we detected inconsistencies at 14 nests (33% of total), involving 29 nestlings (20%). We estimate that inconsistencies at seven nests (16%), involving 13 nestlings (8%) were due to causes other than the undetected allele. Inconsistencies at three nests were due to multiple parentage within broods, as the nestlings had double-banded phenotypes. The genetic data for these three nests did not allow us to distinguish between multiple paternity and multiple maternity. Data on clutch size and behavior indicated that multiple paternity was most likely. Inconsistencies at three nests in which nestlings had single-banded phenotypes were likely due to multiple paternity. Inconsistencies at five nests were most parsimoniously explained by an undetected allele present in three generations of one lineage and inconsistencies at two additional nests were likely due to an undetected allele. We attributed inconsistencies at one nest to nest usurpation. We argue that some females may gain a phenotypic benefit from mating with more than one male. Males that mated with a female may be more likely to feed her on the nest and to feed her nestlings.

*Key words: Allozymes;* Aphelocoma ultramarina; *genetics; Gray-breasted Jay; hidden variation; null allele; parentage; paternity; protein electrophoresis.*

## INTRODUCTION

Genetic tests by which particular individuals can be excluded as parents of particular young have increased our understanding of mating and rearing behavior of birds (see review by Westneat et al. 1990). The Gray-breasted Jay (*Aphelocoma ultramarina*, also known recently as Mexican Jay) has an unusually complex system of mating and rearing (Brown and Brown 1990) that requires genetic tests for a reasonable understanding of the system.

These jays live all year in large, nonmigratory, territorial groups that typically contain four to eight breeders plus a variable number of nonbreeding jays of all ages. Commonly, a nest is built by only two jays, the incubating female and a male that guards her around the time of laying. We refer to these as the primary male and primary female for that nest. Many individuals, including the primary male, may feed the female on the nest and the young. This situation provides many opportunities for multiple mating or brood parasitism by females and for takeovers of nests and/or mates. Brown and Brown (1990) review further details of the social organization of this species.

We used genetic techniques to detect possible multiple paternity and multiple maternity within broods, which we collectively refer to as multiple parentage. Many investigators of multiple parentage have examined electrophoretic variation in proteins (allozymes) (Gowaty and Karlin 1984, Mumme et al. 1985, Bednarz 1987, Westneat et al. 1987, Wrege and Emlen 1987, Hoffenberg et al. 1988, Bollinger and Gavin 1991). Lacking independent information to test the inheritance patterns of allozymes, investigators have made several assumptions regarding the genetic control and expression of protein variation. These as-

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sumptions are (1) electrophoretic bands are phenotypes that correspond to genotypes, (2) all alleles are expressed as electrophoretic bands (alleles are codominant and there are no null alleles), (3) alleles are inherited in a simple Mendelian fashion. In this paper we present evidence that indicates that these assumptions were not appropriate for one locus in our study of Gray-breasted Jays. Our estimate of the minimal rate of multiple parentage benefitted from our ability to test and reject the second assumption.

## METHODS

We obtained genetic and behavioral information from two areas in Cave Creek Canyon, on the eastern slope of the Chiricahua Mountains, near Portal, Arizona. One area was the Southwestern Research Station (SWRS), where groups of color-marked jays have been studied since 1969. The other area was along the lower section of the South Fork of Cave Creek, where jays were first marked in 1984. Gray-breasted Jays inhabit the pine-oak-juniper woodland that dominates the canyons. Of the groups we describe in this paper, the Bryce, Hillside, Station, Tank, and Up Canyon groups were in the SWRS study area and the Sunny Flat and Keyhole groups were in the South Fork area.

We captured the South Fork individuals in the winter and early spring of 1984 and 1985 using mist nets at sites baited with bread and sunflower seeds. We performed laparotomies to determine sex and took blood samples from a wing vein and muscle samples from the breast (Baker 1981). We noted no deaths or aberrant behavior resulting from these procedures. We captured the SWRS birds in traps during the summer and took two growing primary feathers from each. In both study areas, individuals were weighed, measured, and banded with colored bands and one U.S. Fish and Wildlife Service band. Adults were banded with a total of six bands; nestlings wore four bands. In this paper, we use three- and four-letter abbreviations of band combinations to refer to individuals. Nestlings at both areas were weighed and banded at 2–3 weeks of age, at which time two growing primary feathers were taken. We obtained enough tissue for 1–3 electrophoretic runs.

We found nests by searching appropriate habitat and by following birds seen carrying nest-lining material. We made behavioral observations at and away from nests. Based on these observations we determined the primary pair at each nest.

In winter we established feeding stations in each territory. We baited stations with sunflower seeds in January, February, and March and recorded agonistic interactions, using the methods of Barkan et al. (1986).

The samples of blood, muscle, and growing feathers were analyzed by starch-gel electrophoresis using standard techniques (Selander et al. 1971). We assayed 23 proteins, which revealed bands for 28 presumptive loci. Monomorphic loci (followed by Enzyme Commission numbers) were aconitase (4.2.1.3), alcohol dehydrogenase (1.1.1.1), fumarase (4.2.1.2), glyceraldehyde-phosphate dehydrogenase (1.2.1.12), $\alpha$-glycero-phosphate dehydrogenase (1.1.1.8), glutamate oxalate transaminase-1 and -2 (2.6.1.1), hemoglobin, isocitrate dehydrogenase (1.1.1.42), lactate dehydrogenase-A and -B (1.1.1.27), leucine aminopeptidase (3.4.11 or 13), malate dehydrogenase (1.1.1.37), malic enzyme-1 and -2 (1.1.1.40), mannose phosphate isomerase (5.3.1.8), 6-phosphogluconate dehydrogenase (1.1.1.44), phosphoglucomutase-1 and -2 (2.7.5.1), sorbitol dehydrogenase (1.1.1.14), and superoxide dismutase-1 and -2 (1.15.1.1). Five loci were polymorphic and reliably scored from feather samples: adenosine deaminase (ADA, 3.5.4.4), esterase (EST, 3.1.1.1), glucose phosphate isomerase (GPI, 5.3.1.9), leu-ala peptidase (LA, 3.4.11 or 13), and leu-gly-gly peptidase (LGG, 3.4.11 or 13). Albumin variants were present in plasma but rare. LA could not be scored from the muscle samples. Due to low levels of variation in ADA, GPI, and LA, we used the EST and LGG loci for resolving multiple parentage.

Using information from a three-generation pedigree, we provide evidence of an undetected allele at the LGG locus, which coded for a monomeric protein. An allele may be undetected either because it is a null allele with no expressed band, or because it is masked, that is, the band is so close to other bands that it is not detected with traditional starch-gel electrophoresis. For a monomeric protein, an individual heterozygous for a standard (i.e., detectable) allele and an undetected allele would be indistinguishable from an individual homozygous for the standard allele (Table 1). Null alleles can be revealed by the lack of a band in individuals homozygous for the null allele, but these individuals will be rare if the

TABLE 1. Genotypes associated with single- and double-banded electrophoretic phenotypes at a locus with two standard alleles (*A* and *B*) and an undetected allele (*O*).

| | Electrophoretic phenotypes | | |
|---|---|---|---|
| | A | AB | B |
| Without undetected allele | *AA* | *AB* | *BB* |
| With undetected allele | *AO* | | *BO* |

frequency of the null allele is low. Hidden variation has been documented in numerous avian species (Aquadro and Avise 1982, Hackett 1989). Masked or hidden alleles present no problems in paternity analyses if they are masked by a band corresponding to one standard allele. But if a masked allele accounts for the undetected allele we found at the LGG locus, it is masked by two standard alleles, *A* and *C* (alleles and genotypes italicized). Thus, individuals suspected of being *AO* heterozygotes had the same A phenotype as those we assume were *AA* homozygotes. Further, individuals suspected of being *CO* heterozygotes had the same C phenotype as those we think were *CC* homozygotes. LGG is a typical peptidase, which results in fairly broad, fuzzy bands. We used electrophoretic conditions to obtain the best bands we could, including an agar overlay stain, but we did not have sufficient sample material to conduct an exhaustive search for masked alleles or hidden variation under varied electrophoretic conditions. Although such a masked allele is unusual, at the present time we do not have data to distinguish between this explanation and a null allele.

## RESULTS

### HARDY-WEINBERG PROPORTIONS

We tested phenotypic proportions for significance of departure from Hardy-Weinberg expectations using 29 birds aged three years or older from the SWRS study area in 1986. Because many group members were known to be offspring from previous years, it was not valid to test all members of the population. The South Fork study area did not contain enough adults to justify a test. The frequencies of observed phenotypes at EST (0.55 [A], 0.38 [AB] and 0.07 [B]) did not differ from Hardy-Weinberg proportions ($G = 3.45$, $df = 1$, $P > 0.05$). Likewise, the frequencies of observed phenotypes at LGG (0.52 [A], 0.40 [AB] and 0.08 [B]), did not differ from Hardy-Weinberg proportions ($G = 2.96$, $df = 1$, $P > 0.05$).

### GENETIC INCONSISTENCIES BETWEEN PRIMARY ADULTS AND YOUNG

We compared the EST and LGG phenotypes of nestlings to those of the incubating female and the primary male. At 43 nests we determined the phenotypes of the nestlings and at least one primary adult. At first we assumed that there were no masked or null alleles and that inheritance was Mendelian at all loci. At 14 (33%) of these nests, the phenotype of at least one nestling was inconsistent with the phenotype expected based on the phenotypes of the primary adults. We detected inconsistencies for 29 (20%) of the 142 nestlings sampled. There were 23 inconsistencies at only the LGG locus, four at only the EST locus, and two inconsistencies at both loci.

The relatively high frequency of inconsistencies at the LGG locus led us to examine our assumption that the three bands in the LGG phenotypes accurately represented three alleles. This assumption would be incorrect if there were actually a fourth allele that was undetected.

### EVIDENCE FOR AN UNDETECTED ALLELE AT LGG

Because of the long-term study of the SWRS individuals, we could construct a pedigree (Fig. 1) that contained 13 of the 25 young that were inconsistent with the primary adults at the LGG locus. The pedigree, based on five years of sampling from three generations, illustrates inconsistencies involving both primary males and primary females, mostly the latter. The phenotype of female YYR, a member of the Station group, was inconsistent with seven of the 14 nestlings in her nests. In the next generation, the phenotype of her presumed daughter (ROX) was inconsistent with three of the six nestlings in her nests (those with A phenotypes) and the phenotype of one of female YYR's sons (BBMB) was inconsistent with two of the four nestlings in his nest. An undetected allele, present in female YYR and passed to two generations of her descendants, would be the simplest explanation for 12 of the 13 inconsistencies in the pedigree. That the transmission ratio of the undetected allele was roughly 0.5 suggests that the allele was inherited in a simple Mendelian manner.

Other behavioral explanations of these 12 cases, brood parasitism, nest usurpation, and multiple

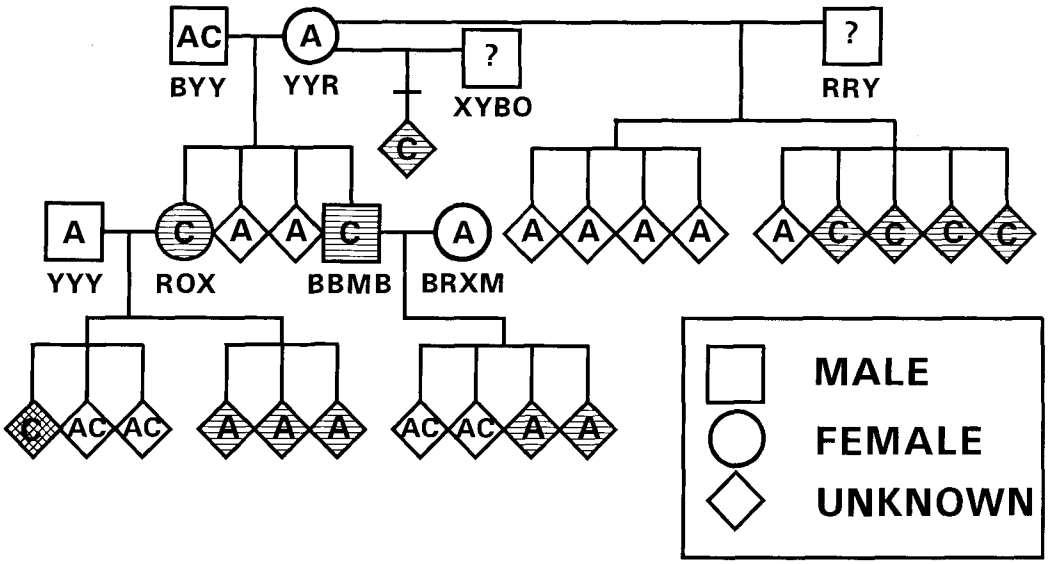


FIGURE 1. Pedigree showing electrophoretic phenotypes (A, AC, C) at the LGG locus in Gray-breasted Jays. Shaded symbols indicate offspring with inconsistent phenotypes that were probably due to an undetected allele passed by female YYR. The cross-hatched symbol indicates an offspring with an inconsistent phenotype that was not due to an undetected allele passed by female YYR. Abbreviated band combinations identify individuals. A question mark indicates an undetermined phenotype.

mating by females, require an extremely high frequency of these behaviors, especially female susceptibility to parasitism, across two generations of this lineage. Further evidence that the inconsistencies were due to an undetected allele is the fact that they were detected exclusively at the LGG locus. If behaviors such as multiple mating by females had been common, we would have expected to have detected inconsistencies at the EST locus as well.

## NEST USURPATION

Usurpation of a nest by a pair other than its builders has long been known (Brown 1963) and is not uncommon in Gray-breasted Jays. Most usurpations occur early in the establishment of a nest, before eggs are laid, and the clutch is presumed to have been laid by the usurper. The following case, however, seems to involve incubation by the usurper of the original female's eggs.

Female OMR, in the Hillside group in 1984, had built nest Hillside-5 with her mate (MOX). Apparently in response to interference from dominant birds (including female OOB), they gave up Hillside-5 and began another nest (Hillside-7). Female OOB, a yearling, incubated nest Hillside-5 a couple of weeks later. Her electrophoretic phenotype, however, was inconsistent with all of the nestlings (Table 2). Further indication that OOB is unlikely to have laid the eggs in this nest is that yearling birds have otherwise not been observed as incubators or mates of incubators in our population. Even at age two, only about 20% of females have attempted to breed.

The pair (MOX and OMR) that built nest Hillside-5 may have been genetic parents of the nestlings; however, we did not have genetic data for MOX or OMR. After about 10 days at their new nest (Hillside-7), a different female (BXY) harassed them there. In response to this harass-

TABLE 2. Electrophoretic phenotypes for a nest (Hillside-5) of Gray-breasted Jays in Arizona in which a female that usurped the nest apparently incubated eggs laid by the original female. Nestling phenotypes that are inconsistent with the incubating female are shown in boldface.

| Individual | Sex | Age[1] | Phenotypes | |
|---|---|---|---|---|
| | | | EST | LGG |
| OOB | F | Ad | AB | C |
| MBR | | J | A | ***A*** |
| MYX | | J | A | ***A*** |
| RMR | | J | A | ***A*** |
| YXO | | J | A | ***A*** |

[1] Ad = adult, J = juvenile.

TABLE 3. Electrophoretic phenotypes of individual Gray-breasted Jays at nests with multiple parentage within broods, as indicated by inconsistent nestlings with double-banded phenotypes (shown in boldface). These data cannot distinguish between multiple paternity and brood parasitism. For each nest, the primary female and primary male are listed first, followed by the nestlings. Additional group members for which we knew phenotypes are listed after all nests for the group.

| Group-Nest | Individual | Sex | Age[1] | Phenotypes EST | Phenotypes LGG |
|---|---|---|---|---|---|
| Sunny Flat-1 | OOY | F | Ad | B | A |
| | YXY | M | Ad | B | A |
| | MXMR | | J | B | A |
| | OMXM | | J | B | A |
| | RBMX | | J | B | A |
| | XBRM | | J | B | A |
| | XMOR | | J | B | ***AB*** |
| Sunny Flat-3 | OOY | F | Ad | B | A |
| | YXY | M | Ad | B | A |
| | MOMX | | J | ***AB*** | ***AB*** |
| | ORBX | | J | B | A |
| | XMOM | | J | B | A |
| | XOBR | | J | ***AB*** | ***AB*** |
| Sunny Flat | MMR | M | Ad | A | B |
| Sunny Flat | YRO | F | Ad | AB | A |
| Bryce-3 | XMB | F | Ad | B | A |
| | MMM | M | Ad | B | AC |
| | BXMR | | J | ***AB*** | AC |
| | RRXO | | J | ***AB*** | AC |
| | XMOB | | J | B | AC |
| Bryce | ORM | M | Ad | AB | A |
| Bryce | MBM | M | Ad | AB | A |
| Bryce | OBB | M | Ad | A | A |
| Bryce | BBMB | M | Ad | A | C |
| Bryce | BBMR | F | Ad | B | A |
| Bryce | BRXM | F | Ad | B | A |

[1] Ad = adult, J = juvenile.

ment, female OMR apparently laid in Hillside-5, rather than Hillside-7.

An alternative explanation for this case is that OOB carried an undetected allele at LGG. She was not, however, a member of the family in the pedigree (Fig. 1) that carried the undetected allele. Her LGG phenotype was C, but none of the four young carried a *C* allele. If she was a carrier of an undetected allele, the probability of passing that allele to all four offspring would have been 0.06 ($0.5^4$).

## PARENTAL EXCLUSION: DOUBLE-BANDED PHENOTYPES

At three nests (7% of total), involving five nestlings (4% of total) and two breeding pairs (females OOY in Sunny Flat and XMB in Bryce), there were inconsistencies that could not be due to an undetected allele because the nestlings had double-banded electrophoretic phenotypes (Table 3). These three nests provide the best evidence of multiple parentage within broods.

Two of these nests were in the Sunny Flat group in 1985. The nestlings from these nests and the principal adults in the group are listed in Table 3. The incubating female at each nest was OOY. The group contained another breeding female (YRO), two males (YXY and MMR), three nonbreeding yearlings and, for a brief period, an unbanded one- or two-year old. Male YXY was consistently dominant over male MMR in agonistic interactions in the breeding season. Behavioral data, although not conclusive, indicated that the dominant male paired alternately with both breeding females. Both females nested twice. Considering the phenotypes of the two breeding females, neither male could be the exclusive father of all the nestlings in either nest of OOY. No combination of resident adults in this group could have been the parents of XM, the inconsistent offspring in nest Sunny Flat-1. In nest Sunny Flat-3, YXY can be excluded as the father of the two young with AB phenotypes, but he could have fathered the other young. Male MMR can be excluded as the father of all Sunny Flat-1 young and two Sunny Flat-3 young, but he could have fathered the two nestlings in Sunny Flat-3 (MOMX and XOBR) that were inconsistent with male YXY.

A third nest (Table 3) with unambiguous multiple parentage occurred in the Bryce group. Although the EST phenotypes of both primary adults (XMB and MMM) were B, two of the three young in their nest were AB. Of the other four males in the group for which we had genetic information, BBMB was consistent with the nestlings in question, assuming that XMB was the true mother (Table 3). Either of the other two females (BBMR and BRXM) could have been the mother, if they had mated with BBMB, but not if they mated with the primary male, MMM. We did not have genetic data for the highest-ranking male in the group (YOO). Both primary adults were relatively young and subordinate; the male and female were two and three years old, respectively. The data on dominance interactions taken in the preceding months (Table 4) showed MMM to be the lowest ranking of six males of breeding age. The female's rank could not be established because she was missing from the group in winter.

TABLE 4. Frequencies of outcomes of agonistic interactions among the six adult male Gray-breasted Jays in the Bryce group at a feeding station in January 1986.

| Won | Lost | | | | | |
|---|---|---|---|---|---|---|
| | YOO | MBM | ORMX | BBMB | OBB | MMM |
| YOO | — | 9 | 7 | 13 | 15 | 40 |
| MBM | 1 | — | 10 | 19 | 14 | 24 |
| ORMX | 3 | 0 | — | 11 | 10 | 30 |
| BBMB | 0 | 0 | 0 | — | 8 | 22 |
| OBB | 0 | 0 | 0 | 0 | — | 22 |
| MMM | 0 | 0 | 0 | 0 | 0 | — |

## POSSIBLE PARENTAL EXCLUSION: SINGLE-BANDED PHENOTYPES

In addition to the 12 inconsistencies arising from single-banded phenotypes that may have been due to an undetected allele passed from female YYR (Fig. 1), we detected 12 other inconsistencies, at six nests, in which nestlings and adults had single-banded phenotypes. In such cases, inconsistencies could be due to nest usurpation, undetected alleles, or multiple parentage. Of the six nests, we inferred that one, described earlier, was usurped. Behavioral and genetic information presented below indicate that birds in two of the nests likely contained undetected alleles and that multiple paternity was likely at three of the nests.

Inconsistencies at two nests not in the pedigree could have been due to either multiple paternity or an undetected allele at the LGG locus (Table 5). Male YBX, in the Up Canyon group, had nests in 1985 and 1986 in which four of seven nestlings had LGG phenotypes that were inconsistent with his. This male was subordinate to other males in his group.

Inconsistencies at three nests were most likely due to multiple paternity. One case was a nestling (extreme lower left in Fig. 1) that had an LGG phenotype (C) inconsistent with male YYY (phenotype A), the primary male at the nest. This inconsistency cannot be explained by an undetected allele passed from female YYR. This was probably a case of multiple paternity; the male could not have had an undetected allele that had been passed from female YYR because he was not descended from her. Another male, XMO, an experienced breeder, was associated with the primary pair before laying and had a phenotype that was consistent with the nestling in question, as well as the other nestlings (Table 5). XMO had been the lowest ranking male at the feeding station the previous winter (Table 6), whereas the phenotype of the male that had been the highest ranking, XRM, was inconsistent with the nestling in question (Table 5).

Male YMX was inconsistent at the LGG locus,

TABLE 5. Electrophoretic phenotypes of individual Gray-breasted Jays at nests with multiple paternity or undetected alleles. Nestlings with single-banded phenotypes (shown in boldface) are inconsistent with the phenotype of the primary male. For each nest, the primary female and primary male are listed first, followed by the nestlings. Additional male group members for which we knew phenotypes are listed after the Tank and Keyhole nests.

| Group-Nest | Individual | Sex | Age[1] | Phenotypes | |
|---|---|---|---|---|---|
| | | | | EST | LGG |
| Up Canyon-5 | YBX | M | Ad | B | C |
| | OBRR | | J | B | ***A*** |
| | MYY | | J | AB | ***A*** |
| Up Canyon-1 | YBX | M | Ad | B | C |
| | MRXO | | J | B | ***A*** |
| | RBRX | | J | AB | ***A*** |
| | ORMO | | J | B | AC |
| | BRO | | J | B | AC |
| | XBM | | J | B | AC |
| Tank-4 | XBB | F | Ad | B | AC |
| | YMX | M | Ad | B | A |
| | X26 | | J | B | A |
| | X27 | | J | B | ***C*** |
| | X28 | | J | B | A |
| | MOB | | J | B | AC |
| Tank | OOX | M | Ad | B | C |
| Tank | XMO | M | Ad | B | AC |
| Tank | XRM | M | Ad | B | A |
| Tank | YYY | M | Ad | B | A |
| Keyhole-4 | RRR | F | Ad | B | A |
| | OOO | M | Ad | A | AB |
| | BXB | | J | AB | A |
| | OMX | | J | ***B*** | A |
| | MXO | | J | ***B*** | A |
| Keyhole | MMY | M | Ad | AB | AB |
| Keyhole | XYBY | M | Ad | B | AB |
| Keyhole | YYX | M | Ad | B | A |

[1] Ad = adult, J = juvenile.

TABLE 6. Frequencies of outcomes of agonistic interactions among the adult male Gray-breasted Jays in the Tank group at a feeding station early in 1986.

| Won | Lost | | | | |
|---|---|---|---|---|---|
| | XRM | YYY | MRX | RXB | XMO |
| XRM | — | 10 | 9 | 8 | 6 |
| YYY | 0 | — | 13 | 17 | 5 |
| MRX | 0 | 0 | — | 19 | 17 |
| RXB | 0 | 0 | 4 | — | 5 |
| XMO | 0 | 0 | 0 | 0 | — |

but not the EST locus, with one (X27) of the four nestlings in his nest (Tank-4, Table 5). At least one yearling male in the group (OOX) had a phenotype that was consistent with that of the nestling in question (Table 5). Also consistent was male XMO, a floater that lived in an area bordered by the Tank group and that joined the group the following year. Two other males (XRM and YYY; Table 5) that had consorted with the female earlier in the year were inconsistent with nestling X27. Male YMX had been the highest-ranking male in the group at the feeding station.

The remaining inconsistency revealed by single-banded phenotypes was at the EST locus (Table 5). In a nest in Keyhole (Keyhole-4) in 1986, two of three nestlings had B phenotypes, which were inconsistent with the A phenotype of male OOO. He was loosely bonded to female RRR. None of the other three males in the group could be excluded from paternity (Table 5). Limited dominance data (24 interactions) indicated that OOO was subordinate to two of these males (MMY and XYBY) and was not observed interacting with the third. We found no evidence suggesting the existence of an undetected allele at the EST locus.

## DISCUSSION

Our results show that multiple parentage is a significant aspect of the biology of Gray-breasted Jays. Our most conservative analysis, based on double-banded exclusions, detected multiple parentage in 7% of broods (Table 3). We considered multiple parentage likely at another 9% of the nests, but we could not rule out undetected alleles (Table 5). Some of the pedigree inconsistencies, conservatively attributed to an undetected allele, actually could have been due to multiple parentage. Estimation of the probability of detection of multiple parentage (e.g., Westneat et al. 1987) would have resulted in estimates of the frequency of multiple parentage considerably above 10%. We did not make such estimates because of their imprecision with our sample sizes and because of the large number of inconsistencies that may not have been due to multiple parentage.

Were the cases of multiple parentage we detected due to brood parasitism or multiple mating by females? The cases involving females RRR (Keyhole group) and XBB (Tank group) (Table 5) strongly suggested multiple mating by females, but were inconclusive because of the possibility of undetected alleles. Three inconsistencies that definitely could not have been due to undetected alleles were due to multiple parentage. The clutch sizes in these three cases were five or fewer eggs, which is typical for nests with one laying female. Clutches of six have been observed only twice from a single female and on the rare occasions when two females attended a nest (J. L. Brown, unpubl. data). Multiple mating by females has been routinely observed in the field; brood parasitism has never been observed (J. L. Brown, unpubl. data). Thus, we have genetic and behavioral indications of multiple paternity but no indication of brood parasitism. The only case we observed of a female incubating eggs she likely did not lay involved a possible nest usurpation (Table 2).

Westneat et al. (1990) reviewed extra-pair copulation (EPC) in birds, considering the reproductive tradeoffs for males and the costs and benefits for females. Many of the factors discussed by Westneat et al. (1990) apply to Gray-breasted Jays, even though there are several differences between Gray-breasted Jays and the species they reviewed. For example, we have not referred to multiple mating by female jays as EPC because some females may not have traditional monogamous pair bonds. Furthermore, the contending males inhabit the same rather than different territories in Gray-breasted Jays. We focus on possible advantages to females from encouraging or tolerating secondary males, as we have not observed forced copulation in this species.

Two potential benefits of multiple mating, especially important in plural breeders, are genotypic benefits and phenotypic benefits (Westneat et al. 1990). A paired female might obtain a genotypic benefit if she can observe other males living in their group to assess dominance status, nest attentiveness, and other behavioral and morphological characteristics that might indicate high quality. If there is little or no cost to multiple mating, paired females may mate with additional males of high quality. In this study, we found two cases (nests Bryce-3 and Keyhole-4) of multiple mating by females that were paired with low-ranking males. These females may have gained a genotypic benefit. We also found three cases of multiple mating by females that were paired to the highest (nests Tank-4 and Sunny Flat-3) or second-highest (nest Tank-1) ranking males. These females probably did not gain a genotypic benefit, assuming that dominance rank

at the feeding station in winter provides an index of male quality.

Multiple mating also may result in feeding of the female and her nestlings by the males with which she mated, which would be a phenotypic benefit. Multiple matings that result in a phenotypic benefit may not result in a genotypic benefit. Although a phenotypic benefit of EPC is unlikely for most birds (Westneat et al. 1990), the unusual social organization of Gray-breasted Jays makes such a benefit more likely. We have often observed feeding of young in a nest by two or more adult males that associated with the female when she was receptive. Burke et al. (1989) reported feeding of young by polyandrous Dunnock (*Prunella modularis*) males that shared paternity of broods.

One of the case histories in which multiple parentage within a brood was documented suggested a phenotypic benefit to the female. In the Sunny Flat group, it was likely that one male (MMR) was the father of two nestlings in a nest at which he was not the primary male (Table 3). During the period that female OOY was laying in that nest, her consort (male YXY) was presumably spending some of his time feeding the incubating female he had consorted with earlier. Male YXY would seem to have been the higher-quality male, because he dominated the other male, consorted with both females in 1985, and consorted with females in 1984 and 1986, unlike male MMR. These observations suggest that female OOY copulated with a male other than the one she had consorted with even though the other male was apparently of lower quality than her mate. She may have gained a phenotypic benefit by increasing the likelihood that male MMR would feed her during incubation and feed her nestlings.

The conclusion that there was an undetected allele at the LGG locus seems inescapable. This conclusion raises questions concerning studies that have inferred multiple parentage based on single-banded phenotypes of nestlings and adults. Three studies excluded parents based solely on single-banded phenotypes. Gowaty and Karlin (1984) reported that the phenotypes of seven Eastern Bluebird (*Sialia sialis*) nestlings were inconsistent with the phenotypes of one of the putative parents; all of the inconsistent phenotypes of the nestlings and adults were single-banded. Bednarz (1987) concluded that two males in a breeding trio of Harris' Hawks (*Parabuteo unicinctus*) could not have been father and son because they had different single-banded phenotypes at two presumptive loci. Karlin et al. (1990) inferred multiple parentage at one Eastern Bluebird nest because of differing single-banded phenotypes of the putative father and one of the offspring. In several other studies many, but not all, of the inconsistencies were due to nestlings and adults with different single-banded phenotypes (Mumme et al. 1985, Wrege and Emlen 1987, Hoffenberg et al. 1988). Whether undetected alleles occurred in any of these studies in unknown.

The possibility of undetected alleles in electrophoretic studies complicates investigations of parentage. Undetected alleles occurring at low frequencies may not be revealed by the common practice of testing for Hardy-Weinberg proportions. Unusually high frequencies of single-banded phenotypes (i.e., homozygote excess) or the detection of combinations that should be rare (e.g., individuals having different single-banded phenotypes at two or more loci [Bednarz 1987]) should stimulate further investigation. Investigations of multiple parentage based on electrophoretic data should include at least summaries of the data indicating inconsistencies, rather than simply reporting the frequency of parental exclusions (e.g., Bollinger and Gavin 1991). Whenever feasible, detected inconsistencies involving single-banded phenotypes should be followed up by sampling additional nestlings of the parents and offspring to examine pedigrees. Polyacrylamide electrophoresis may be better than starch-gel electrophoresis for resolving bands (Romagnano et al. 1989). If protein electrophoresis continues to be used to detect multiple parentage, the possibility of undetected alleles will have to be added to the list of problems inherent in this technique (Mumme et al. 1985, Romagnano et al. 1989).

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