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Extra-pair Fertilization, Mate Choice and Genetic Similarity in the Mexican Jay (*Aphelocoma ultramarina*)

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ABSTRACT

Inbreeding depression should select for the ability of females to avoid inbreeding or minimize its effects. We tested for a relationship between genetic similarity of social pairs and the occurrence of extra-pair fertilization (EPF) in the Mexican jay (*Aphelocoma ultramarina*), a bird species with known inbreeding depression and a high EPF rate (Brown and Brown 1998, Li and Brown 2000). Multi-locus minisatellite and microsatellite DNA fingerprinting were used to detect extra-pair young and measure genetic similarity between social parents. We found that 15 of 38 (39%) nests had at least one EPF and 21 of 115 (18%) young were the result of EPF. The mean DNA fingerprinting band-sharing score between social mates who had at least one EPF was significantly higher than the mean band-sharing score between mates who did not (0.35 vs. 0.26). The mean band-sharing score for non-EPF dyads (0.26) was similar to the background band sharing among non-relatives (0.23). The mean band sharing score for mates that had an EPF was significantly higher than that of non-relatives (background) and was significantly lower than that of half-siblings (0.51). Our results showed a significant positive relationship between genetic similarity of social mates and incidence of EPF at $P<0.01$.

TABLE OF CONTENTS

CHAPTER 1

Why do Birds Have Extra-pair Fertilizations?

A Literature Review

CHAPTER 2

An Empirical Study:

EPF in the Mexican jay is Positively Correlated with Genetic Similarity of Social Mates

LIST OF TABLES AND FIGURES

CHAPTER 1

CHAPTER 2

Chapter 1.

Why Do Birds Have Extra-pair Fertilizations?

INTRODUCTION

Because of relatively recent advances in molecular techniques, it has been shown that most avian species (86% in 130 studies) exhibit some degree of extra-pair fertilizations (EPFs) (Griffith et al. 2002). In socially monogamous species, an EPF is defined as the offspring of one putative parent and an extra-pair breeder. In most cases of passerine species the extra-pair parent is a male (Griffith et al 2002, Moller 1986, Westneat et al.1990). There are several hypotheses for EPF occurrence in birds, and each one may explain EPFs in some species. Most hypotheses for EPF can be placed into two categories: direct fitness benefits and indirect fitness benefits to the member of the social pair engaging in EPC (extra-pair copulation) that leads to EPF. For this study I define direct fitness benefits as behavioral or physiological influences that affect an adult individual's lifetime fitness (such as fecundity or lifespan). Indirect fitness benefits are those that affect the direct fitness of an individual's offspring (such as "good genes" or hybrid vigor through mate choice). Rates of EPF vary between species, as do proposed benefits of EPF at the individual level. In this study I focused on the indirect fitness benefits of EPF to adult females.

1. Benefits of EPF to Females

Table one lists different conditions and possible benefits to females for different hypotheses of EPF occurrence in birds.

Good genes, genetic diversity and heterozygosity

Some hypotheses, such as the "good genes" hypothesis are fairly straightforward; females recognize, and copulate preferentially, with males who possess phenotypic cues that are linked to fitness traits that may confer a benefit to all offspring, such as size, or confer a sexually selected trait that benefits only male offspring in attracting a mate ("sexy sons") (Weatherhead and Robertson 1979). This has been shown in the red caps of male yellow warblers (*Dendroica petechia*) (Yezerinac and Weatherhead 1997) and many other species (reviewed by Arnold 1983, Birkhead and Moller 1992, Kodric-Brown and Brown 1984, Hamilton1990). Another hypothesis, the increased heterozygosity hypothesis (Brown 1999), results in increased heterozygosity, and by extension, increased fitness in young (Acevedo-Whitehouse et al. 2002, Jazwinski 1996, Mitton et al. 1993). The increased heterozygosity hypothesis, as proposed by Brown (1997, 1999), suggests that females have the ability to detect males that are genetically dissimilar to themselves, and mate with them preferentially, even if they already have a social mate. For example, Foerster et al. (2003) showed that female blue tits (*Parus caeruleus*) increased heterozygosity of their offspring by preferentially having EPFs with genetically dissimilar males. This hypothesis relies on some aspect of male phenotype linked to genetic dissimilarity that females recognize. In the genetic diversity hypothesis, as defined by Griffith et al. (2002), females increase genetic diversity of their offspring through EPFs, but cannot detect genetic similarity between themselves and potential extra-pair partners. Thus in this scenario, all breeding males have an equal chance of being an EPF sire and all breeding females have an equal chance of having EPFs. Ideally, each offspring in a female's brood would have a different genetic father. In this way, a female maximizes the genetic diversity of all of her offspring thus enhancing the chances of survival of some offspring in different environmental conditions (Westneat et al. 1990, Williams 1975).

Inbreeding avoidance

In the inbreeding avoidance scenario, females may be able to detect genetic similarity between themselves and their social mate or be able to recognize potential relatives by some other cue, such as vocal recognition of nestmates or other potential kin (Hopp et al. 2001) and be stimulated to pursue EPFs (Blomqvist et al. 2002, Bensch et al. 1994). The ability to recognize genetically dissimilar extra-pair males is not required (but may occur). Blomqvist et al. (2002) compared genetic similarity of social mates and EPF occurrence for three species of shorebirds and found that the mean band-sharing values for pairs with EPF was higher than that of pairs without EPF. The prediction in the case of inbreeding avoidance is that genetic similarity between social mates is positively correlated with EPF occurrence, and EPF sires should, on average, be less genetically similar to the female than the social male is. In this hypothesis females may recognize potential EPF sires as being less genetically similar to themselves than their social mates; however, if the genetic similarity of the female and her social mate is substantially greater than the background genetic similarity of the population, then the EPF sire may be less related to the female than the social male is simply by chance. The outcome predicted by the inbreeding avoidance hypothesis is the same as that of the increased heterozygosity hypothesis. Both hypotheses result in increased heterozygosity of offspring; however, the factors that may cause females to pursue EPFs are different.

Inbreeding avoidance and genetic compatibility

The genetic compatibility hypothesis, as described by Griffith et al. (2002), is a scenario where females maximize genetic compatibility between themselves and their mate by using male phenotypic cues to "detect genetic similarity between themselves and males" (Birkhead and Moller 1992). This definition is the same as the inbreeding avoidance hypothesis described above, and is inadequate. Genetic compatibility is not limited to degrees of genetic similarity. For example, there may be important gene complexes that are unique to certain populations or subpopulations that, if not passed on to offspring intact, could confer a fitness cost (outbreeding depression). In order to avoid confusion, it would be better to define the genetic compatibility hypothesis as such: females maximize genetic compatibility between themselves and males through detection of some aspect of compatible male genotype such as genetic dissimilarity and/or some male phenotypic cue that is linked to compatibility of a gene complex or other epistatic factor. The results of these two hypotheses may be the same (increased heterozygosity of offspring in the case of female recognition of genetic similarity) or entirely different (increased heterozygosity in the inbreeding avoidance hypothesis and unchanged or decreased heterozygosity of offspring in the case of the genetic compatibility hypothesis due to transmission of gene complexes to offspring).

Fertility

There are at least two hypotheses to explain EPF that invoke some amount of protection of the female against infertility of the male. In the bet hedging hypothesis females have EPFs to guard against infertility of their social mate, but they cannot assess the fertility of males (Wetton and Parkin 1991). In another fertility hypothesis females can assess the fertility of their social mate and have EPFs to maximize successful reproduction (Sheldon 1994).

2. Interspecific Variation in Rates of EPF

Which species are more likely to have EPF? There are certain predictions based on life history traits that have been proposed to favor EPF occurrence in different species.

Dispersal

Species with low dispersal rates should have higher EPF rates than species with high dispersal rates. This trait may have powerful influence on EPF rate, because in species with low dispersal rates close relatives may breed near each other, thus creating the potential for inbreeding depression. This effect may be magnified in cooperatively breeding species such as fairy wrens (*Malurus cyaneus*) (Dunn and Cockburn 1999) and Mexican jays (*Aphelocoma ultramarina*) (Brown 2001) because some offspring will not disperse at all but will remain with the natal social group for their lifetime. In both of these examples empirical evidence supports a negative correlation between dispersal and EPF rate (Dunn and Cockburn 1999, Li and Brown 2000).

Breeding density and breeding synchrony

Other factors that may influence EPF occurrence across species are breeding density (Hill et al. 1994, Moller and Birkhead 1993) and synchronous breeding (Stutchbury and Morton 1995, Stutchbury 1998a, 1998 b). Evidence for a positive relationship between breeding density and EPF has received criticism. Westneat and Sherman (1997) subjected the data from the Moller and Birkhead (1993) interspecific comparison to their own comparative analysis and found no relationship between EPF rate and breeding density. Similarly, the evidence for EPF and synchronous breeding put forth by Stutchbury and Morton (1995) has since been subjected to a more robust analysis by Westneat and Sherman (1997) controlling for phylogeny and other possibly confounding factors. No relationship between EPF rate and breeding synchrony was found. Stutchbury has since reanalyzed her own data and published two additional papers (Stutchbury1998a and 1998b) that again show a correlation between EPF and breeding synchrony. These papers have also been challenged, most notably by Westneat and Yezerinac (1998), on the grounds that the available empirical evidence suggests no relationship between EPF and breeding synchrony. In their review of interspecific variation of EPF, Griffith et al. (2002) list data for 12 different species that refute Stutchbury's hypothesis (whose 1998 study was based on 9 species).

The need for paternal care

Another life history trait that has been proposed to affect EPF rate is the need for paternal help in rearing offspring. If paternal care is necessary for the nest to succeed, and males can detect cuckoldry, females should be less likely to pursue EPFs. The need for male parental care has been shown to be negatively correlated with EPF rate (Mulder et al. 1994, Gowaty 1996). Thus, species in which the breeding female is capable of raising offspring by herself, or with help from other non-breeding individuals in cooperative breeding species such as fairy wrens (*Malurus cyaneus*) and Mexican jays (*Aphelocoma ultramarina*), should have higher average EPF rates than species in which paternal care is necessary. Indeed, both of the species mentioned above have extremely high EPF rates (approximately 90% in fairy wrens and 60% in Mexican jays) (Dunn and Cockburn 1999, Li and Brown 2000).

lifespan

EPF rate has also been shown to be negatively correlated with life span (Mauck et al. 1999, Wink and Dyrcz 1999, Arnold and Owens 2002). In species where male parental care is necessary, males of long-lived species are less tolerant of EPF because they have a good chance of mating several more times in their lifetime, so the loss of offspring in one (or a few) nests is not as severe as it is for males of short-lived species. This abandon-if-uncertain strategy only pays off if this behavior results in higher lifetime reproductive success for the male. Short-lived males may only have one chance to produce offspring, so they are forced to make the best of a bad situation and tolerate EPY in their nests. While there is evidence for the prediction of a negative relationship between lifespan and tolerance of EPF by males (Mauck et al. 1999, Wink and Dyrcz 1999, Arnold and Owens 2002), there are other possibilities. Males in long-lived species may actually be more tolerant of EPY than expected. If males have multiple years to mate, then tolerating some EPY in nests may be worth the fitness gained by having at least some paternity (provided that clutch size is greater than one) in all of their successful nesting attempts. The prediction for short-lived species can also be reversed. If the breeding season length permits multiple mating attempts in one year, it may benefit short-lived males to abandon early nests in which there is EPY in favor of new nesting attempts that season for which they may achieve exclusive paternity of offspring in a nest.

3. Focus of Thesis

While all of these hypotheses are plausible for some species under certain conditions, there is a dearth of empirical evidence for any of them. This is particularly true of hypotheses that address indirect fitness benefits to females for EPF that have the same predicted outcome, such as the increased heterozygosity of offspring and the inbreeding avoidance hypotheses, but have potentially different causal factors (female recognition of genetically dissimilar potential EPF sires versus recognition of genetic similarity of their social mate). This thesis tests whether the empirical evidence of our study is consistent with the inbreeding avoidance theory (Brown 2001). The prediction is that genetic similarity of social mates in Mexican jays is positively correlated with the frequency of EPF. Furthermore, the expected results for the increased heterozygosity of offspring hypothesis are that females will be less genetically similar to their EPF sires than they are to the social partner.

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Table 1. Different hypotheses for EPF occurrence in birds and the possible benefits of EPF to females who have them.

Hypothesis	**Description**	**Benefits**	**References**
"Good genes"	Females prefer sexual partners with phenotypic cues linked to fitness such as plumage, symmetry etc.	All offspring may receive increased fitness or only "sexy sons"	Arnold 1983, O'Donald 1983, Hamilton 1990, Weatherhead and Robertson 1979
Genetic diversity	Females prefer multiple sexual partners	Increased genetic diversity of brood	Griffith et al. 2002, Westneat et al. 1990, Williams 1975
Increased heterozygosity of offspring	Females recognize genetically dissimilar males including "rare males"	Increased heterozygosity of individual offspring	Bensch et al. 1994, Brown 1997, 1999
Inbreeding avoidance	Females recognize genetically similar social mates and may recognize genetically dissimilar extra-pair males.	Increased heterozygosity of individual offspring	Blomqvist et al. 2002, Bensch et al. 1994, Foerster et al. 2003
Genetic compatibility	Females can distinguish between incompatible and compatible male genotypes including genetic similarity and epistatic congruence	Offspring have increased fitness either through increased heterozygosity or retention of epistatic factors such as gene complexes	Zeh and Zeh 1996, Birkhead and Moller 1992 , Tregenza and Wedell 2000
Fertility: bet hedging	Females pursue EPF to insure production of offspring but cannot detect male sterility	Females maximize the probability of having at least some offspring regardless of social male's virility.	Lifjeld 1994, Wetton and Parkin 1991
Infertility recognition	Females detect sterility of social male and respond by seeking EPFs	Females with sterile social mates produce some extra-pair young	Gibson and Jewell 1982, Sheldon 1994

Chapter 2.

An empirical study:

Evidence That Extra-pair Fertilization in the Mexican Jay (*Aphelocoma ultramarina*) is Positively Correlated to Genetic Similarity of Social Mates

INTRODUCTION

There are several possible indirect fitness benefits for having extra-pair fertilizations (EPF) in animals. In the "good genes" hypothesis, females recognize and copulate preferentially with males who possess phenotypic cues that are linked to fitness traits that may confer a benefit to all offspring, such as size, or confer a sexually selected trait that benefits only males in attracting a mate ("sexy sons") (Arnold 1983, Birkhead and Moller 1992, Hamilton1990, O'Donald 1983, Weatherhead and Robertson 1979). The genetic diversity hypothesis predicts that females will increase the genetic diversity of her brood by copulating with several different males (Griffith et al. 2002, Williams 1975, Westneat 1990). Other hypotheses, such as the increased heterozygosity of offspring hypothesis (Brown 1997,1999) and the inbreeding avoidance hypothesis (Brown 1997) result in increased heterozygosity, and by extension, increased fitness in young (Acevedo-Whitehouse et al. 2002, Foerster et al. 2003, Jazwinski 1996, Mitton et al. 1993). Another hypothesis is the genetic compatibility hypothesis wherein females guard against genetic incompatibility with their social mate by pursuing EPFs. Finally, females may pursue EPFs as a way to insure against sterility of their social mates (Lifjeld 1994, Sheldon 1994, Wetton and Parkin 1991).

The fitness costs of mating between genetically similar individuals, or inbreeding depression, are well documented in animals (Crnokrak and Roff 1999, Keller et al.1996, Pusey and Wolf 1996). When a female in a socially monogamous species has a limited number of males from which to choose in a short time, she may form a social relationship with a male that is genetically similar to her. In such cases, a female might improve the quality of some of her offspring through EPF.

Mexican jays live in discrete social groups (flocks) of between 5-25 individuals. Each flock defends their territory from adjacent flocks, and because Mexican jays do not migrate, their territories remain fixed throughout generations (Brown 2001). While flocks may periodically contain more of one sex than another, there was no pattern of sex bias within flocks over time (since 1969, Brown personal communication) in the study population. Mexican jays are cooperative breeders, and all individuals in a flock, regardless of sex or age, may exhibit helping behavior during their lifetimes. Flocks have established dominance hierarchies, usually with a dominant male and a dominant female (who may or may not be a reproductive pair) (Brown et al. 1997). An unusual characteristic of Mexican jays is that they have a plural breeding system. A plural breeding system is one in which more than one nest may be active simultaneously within a social group. In Mexican jays there can be up to 5 active nests in a social group, each with a different mated pair (Brown 1970,1987,1994). The dispersal rate of Mexican jays is one of the lowest of any North American bird species (Brown 2001). Approximately 50% of young remain in the natal territory for their lifetime and individuals that do disperse usually emigrate to an adjacent flock (Brown and Brown 1984).

Mexican jays have an unusually high rate of EPF. A study by Li and Brown (2000) reported that 32/51 (63%) nests had at least one EPF and 55/159 nestlings (40%) were EPFs. EPF sires for 46/55 (83.6%) EPF young were identified and 44/46 (96.1%) of all EPF sires were intra-group. Because Mexican jays have an extremely conservative dispersal rate (Brown and Brown 1984) and they live in groups known to contain close relatives (Brown and Brown 1981), the possibility of inbreeding and inbreeding depression is significant. Brown (2001) suggested that the high rate of EPF in Mexican jays might be related to the low dispersal rate of this species. We tested for a positive correlation between genetic similarity of social mates and incidence of EPF in the Mexican jay.

METHODS

Study Population

Behavioral data were collected from a population of Mexican jays near Portal, Arizona at the Southwestern Research Station of the American Museum of Natural History and the surrounding Coronado National Forest; Latitude 31.883 N, longitude 109.203 W. This population has been studied annually since 1969. We used ground and pole traps baited with peanuts and sunflower seeds to capture birds. Most birds were color banded for individual identification, and blood samples have been collected for all banded birds since 1990. For this study, we used behavioral and genetic data collected over 11 years (1993-2003) for nests for which we had identification and preserved blood samples for both parents as well as chicks that were alive at banding age (usually 14 days after hatching). Data from 10 different social groups (flocks) were used in this study (Table 1). The breakdown of the study population for this paper was: 1993, 2 families with 3 and 2 chicks respectively; 1994, 4 families with 5, 2, 2, and 3 chicks; 1995, 7 families with 4, 2, 3, 5, 3, 2, and 3 chicks; 1997, 7 families with 3,3,3,3,2,1, and 3 chicks; 1998, 5 families with 1,3,4,5,and 4 chicks; 1999 3 families with 2, 2, and 5 chicks; 2000, 3 families with 5, 5 and 5 chicks; 2001, 5 families with 3,3,1,1 and 3 chicks; 2002, 1 family with 4 chicks; 2003, 1 family with 5 chicks.

Adults were identified as nest owners (mated pairs) if they were seen bringing nest material to the nest site, building the nest, or incubating eggs (females). Males were usually identified during the nest-building phase. Males were also assigned to a female if they were observed closely following the female when returning to or leaving a completed nest, chasing other males away from the female and/or perching at the nest while the female was in the nest. Males were only assigned to a nest if the preceding behaviors were observed before egg laying was complete. Males were not assigned to nests that were observed after the female was in the incubating phase (all eggs were laid).

DNA Fingerprinting

We used multilocus minisatellite DNA fingerprinting (Jeffreys et al. 1985, Rabenold et al. 1990, Westneat 1990). Blood samples of approximately 100 µl were taken from birds either when they were chicks or when trapped as juveniles or adults, by venipuncture of the ulnar vein. Genomic DNA was extracted from blood using a standard proteinase K and phenol/chloroform/isoamylalcohol procedure followed by ethanol precipitation (Sambrook et al.1989). Three µg DNA were cut using the restriction enzyme *Hae* III. After digestion, the fragments were separated on 0.8% agarose gels for 65 hours at 20V. After Southern blotting, the

DNA was hybridized using Jeffreys' probe 33.15 (Jeffreys et al. 1985, Rabenold et al. 1990). The probe was radiolabeled with [^{32}P] dCTP and visualized using phosphor-imaging.

Extra-pair young for nests from 1990-1996 were identified in a previous study using microsatellites (Li and Brown 2000). All other genetic data for this study were generated using minisatellites. We tested whether results using minisatellites were consistent with those using microsatellites by comparing results using both molecular markers for parental exclusions from 17 nests from the years 1993 through 1995. We found one additional paternal exclusion using minisatellites. Results from the two methods were highly correlated: 14 of 15 (93%) paternal exclusions matched, and 44 of 45 (98%) non-exclusions matched. Together, 59 of 60 (98.3%) chick assignments of EPF or non-EPF matched between the two molecular markers. Confidence limits were generated for this proportion based on the binomial distribution and the F distribution (Zar 1999). The 95% confidence interval was 0.9108-0.9996.

Fingerprints of social mate pairs were scored blind by J.A.E. with respect to whether a dyad had an EPF in the nest or not. Scores were confirmed by an independent scorer also scoring dyads blind with respect to the tested hypothesis. Individuals were not scored across gels. Band-sharing values [Dice's Index, D] were calculated using the formula D= 2S/(A+B+2S) where S equals the number of bands shared between a dyad, A equals the number of bands unique to the first individual and B equals the number of bands unique to the second individual (Lynch 1988; Lynch 1991; Rabenold et al. 1990). Extra-pair young can be detected by identifying bands that the offspring has that are unattributable to either parent. In this study, putative fathers were excluded from parentage using two criteria: 1. If a chick had more than one band that was unattributible to either parent and 2. the social father and chick had a band sharing value less than the lower 95% confidence interval of the empirically generated distribution of band-sharing values for first-order relatives (0.52). The distribution of band-sharing values for first-order relatives was calculated from dyads of parents and offspring in which there were no unattributable bands. The distribution of band-sharing scores for non-relatives was generated by scoring dyads of individuals from different flocks that were not adjacent to each other (approximately 0.5- 1 kilometer apart). The distribution of band-sharing scores for half siblings was calculated from dyads of chicks from the same nest that shared the putative mother but had different fathers. In generating these distributions, some adults were used in the non-relative analysis and in the social pair analysis. In no cases were chicks that were used in the parent to offspring or the half sibling kin distributions used again (as adults) as a part of the social mate EPF/non-EPF analysis.

To test for non-independent segregation of DNA fragments (linkage) we used 7 families of 5 offspring with no EPFs and checked for band combinations that always transferred from parent to offspring as a pair or not at all. We found no such linked band combinations.

Because of the extremely conservative dispersal of Mexican jays, close relatives are usually present within a given group's territory (Brown and Brown 1981). Therefore, an estimate of the probability of missassigning an uncle as father is necessary. The probability of incorrectly assigning an uncle as a father can be calculated using the empirically derived mean background band-sharing value of 0.23 (our data) using the equation X (background band-sharing) = $2q-2q^2$ where q = allele frequency:

$0.23 = 2q-q^2$; $q = 0.12$ (Georges et al. 1988). From this value we derived the probability of missasigning an uncle as father = 0.017. Using the equations from Georges et al. (1988) and Rabenold et al. (1991), we also derived the expected mean band-sharing value for full siblings (first order relatives) as 0.63. This value was nearly identical to the empirically measured mean band-sharing value for parent to offspring (first order relatives) which was 0.65.

Because Mexican jays usually do not form a pair bond that lasts more than one season, we view each nesting attempt as a new datum for the EPF/non-EPF analysis. The results reported in Table 2 and Figures 1A and 1B reflect this methodology. We recognize, however, that some workers may object to using the same mated pair in the data set more than once on the grounds of pseudo-replication. To address this we chose, at random, one nesting event from each mated pair that was repeated in the data set. While doing this reduced our sample size from 38 nests to 31, the results were consistent with those reported above in that the mean band-sharing values of social pairs with EPF were higher than the mean band-sharing values for social pairs without EPF: Mann-Whitney U-test: $U(14)(17)=167$, $0.025<P<0.05$.

To test for a significant difference between band-sharing values for EPF and non-EPF categories we used a Mann-Whitney U test for ranked data. We also performed a correlation of EPF occurrence and genetic similarity of social pairs using a Chi-square contingency table and then tested for a positive linear trend using a Chi-square test for linearity (Armitage 1971).

RESULTS

A total of 116 chicks from 38 nests were genotyped, and 21 chicks from 15 nests were found to be the result of EPF. At 11 nests we were able to assign parentage to 11 extra-pair males for 15 chicks. In all cases there was only one EPF sire assigned for all EPF chicks in each nest. All EPF sires were part of the same flock as the mother. No EPF sires could be assigned to 6 EPF chicks from 4 nests.

Our results were consistent with our primary prediction that genetically similar social mates are more likely to have EPF. The mean band-sharing value of social pairs without an EPF was significantly lower than that of social pairs with at least one EPF: Mann-Whitney U(15)(23)=255, $0.005<P<0.01$. The mean band-sharing values with standard errors for all dyad types are reported in Table 2. Figure 1A shows the distribution of band-sharing scores for three classes of kin: first order relatives (parent to offspring), half-siblings and non-relatives. These empirically generated band-sharing distributions allowed us to infer levels of relatedness of individuals of unknown pedigree (e.g. social mates). For example, because the distributions of non-relatives and first order relatives do not overlap (Fig.1A), we can tell if a given pair of individuals is unrelated or probably first order kin (full siblings or parent-offspring). Figure 1B shows the distribution of band-sharing scores of EPF and non-EPF dyads. By comparing individual dyads in the band-sharing distributions of Fig.1B with the distributions of kin in Fig.1A we can infer levels of genetic similarity, and by extension, genetic relatedness, of social pairs that had EPF in the nest and those that did not.

We also tested whether the rate of EPF occurrence was dependent on genetic similarity of social pairs. A Chi-square contingency table was used to test for significance of the correlation between number of EPF nests in 3 categories of genetic similarity of social mates (Fig. 2). The correlation was significant: $0.05>P>0.025$. We also tested the data for a linear trend using a linear trend contingency table (Armitage 1971). The data are consistent with a linear trend: $0.025>P>0.01$. Using equation 24.9 (Zar 1999) we calculated a P value for the departure from linearity: $P>0.75$. There was also a trend for the proportion of all young that were EPF to increase with genetic similarity of social mates (Fig. 2). This finding is consistent with our hypothesis that EPF rate increases with genetic similarity of social mates.

If females have EPFs because they are genetically similar to their social mates, then we would expect the EPF sires to be less genetically similar to the female than the social male. There was a trend for EPF sires to be less related to the female (mean band-sharing of 0.29) than the social male (mean band-sharing of 0.34) (Figure 3). However, these results were not significant at $\alpha=0.05$; Mann-Whitney U Test (11) (11) $0.05<P<0.10$.

The inbreeding avoidance hypothesis and the theory of heterozygosity of mate choice (Brown1999, 2001) predict that females are less likely to have EPFs when they are genetically dissimilar to their social mate, therefore, the genetic similarity between non-EPF social pairs should resemble that of unrelated individuals. The calculated mean band-sharing value of 0.23 for unrelated individuals was similar to that for non-EPF pairs (0.26). Figures 1A and 1B show that the majority of the distribution of band sharing values for EPF dyads falls between (and overlaps) the distributions of non-relatives and that of half-siblings. Band-sharing scores for non-EPF social pairs were not significantly different from non-relatives (Normal approximation to Mann-Whitney U-test, sample size>40, U(23)(46)=495, Z=0.163, P>0.5; (Zar 1999). Band-sharing scores for social pairs with EPF were significantly lower than those of half-siblings U(15)(31)=228; 0.005<P<0.01) and significantly higher than those of non-relatives (Normal approximation to Mann-Whitney U-test: U(15)(46)=597, Z=4.81; P<0.001). The mean band sharing value for parent to offspring of 0.65 (Table 1) was nearly identical to the predicted value of 0.63 calculated from the mean background band sharing value of 0.23 (Georges et al. 1988).

DISCUSSION

Our finding that the frequency of EPF was positively correlated to genetic similarity of social mates is consistent with various theories of EPF based on increasing heterozygosity of offspring (Brown 1997, 1999) including the genetic compatibility hypothesis (Griffith et al 2002, Kempenaers et al. 1999, Tregenza and Weddell 2000) and the inbreeding avoidance hypothesis proposed for Mexican Jays (Brown 2001). Inbreeding is more likely when dispersal is reduced, and in the Mexican jay dispersal is more conservative than that of any other known bird species in North America (Brown 1994). Many individuals of both sexes breed on their natal territory and individuals that do disperse usually do so to an adjacent territory or one territory farther (Brown and Brown 1984). This highly philopatric dispersal pattern often results in territorial groups that contain relatives of breeding age (Brown and Brown 1981). Brown and Brown (1998) reported that approximately 5% of broods were inbred, and the costs of inbreeding were severe. Inbred pairs had smaller broods, and their young had lower rates of survival through their first year.

Why do female Mexican jays establish social relationships with genetically similar males if there is a fitness cost to doing so? There are at least two reasons. First, female mate choice is constrained by the social system of this species; the selection of males with which to mate (assuming female choice) is nearly always limited to those males within her flock (Brown 1994). Depending on the kin structure of the flock, the female may have no choice but to nest with a genetically similar male. Alternatively, a dominant male may choose to nest with a genetically similar female and mate guard her vigorously and disrupt any nesting attempts by her with other males (Brown 1987,1994, Brown et al. 1997). Since nearly all EPF sires in the study population were intra-group (Li and Brown 2001), a female's choice of EPF sire is limited, and may not necessarily be less genetically similar to her than the social male. In fact, the EPF sire may be more genetically similar to her than the social mate and our data show this to be true in some cases (Fig 3). In such cases, having EPFs may not result in more heterozygous young, but, because females do not depend on a social mate's parental care to raise a brood, there may be no fitness cost associated with having EPFs in this situation. This scenario is consistent with the inbreeding avoidance hypothesis.

While kin recognition has been shown in birds (Bateson 1982, Petrie et al. 1999, Russell and Hatchwell 2001), it is not needed to explain the findings of this study. Although our results are consistent with inbreeding avoidance by pre-insemination mechanisms (female recognition of genetically similar males), post-insemination mechanisms could also explain our findings. If all females in the study population were equally promiscuous, our results could be explained by female cryptic choice (Birkhead and Pizzari 2002, Pizzari and Birkhead 2000) or sperm competition (Birkhead et al. 1988, Pizzari et al. 2003). In both cases, more genetically compatible sperm could be favored over sperm more genetically similar to that of the female. In either case, females either cannot, or need not, assess genetic similarity of potential mates prior to insemination. Post-insemination mechanisms could be favored by fitness benefits gained through maximization of heterozygosity and genetic compatibility as cited above.

There was a slight difference between the measured mean background band-sharing value of the study population (0.23) and the mean band-sharing value between EPF sires and their female partners (0.26), but at $P>0.10$. This trend is actually a reverse of the expected under the increased heterozygosity hypothesis (where females seek out genetically dissimilar males) (Brown 1997), but the present sample size is small (11).

While inbreeding avoidance appears to be an important factor contributing to EPF occurrence in Mexican jays, it is probably not the only one. EPFs were identified in broods of genetically dissimilar mated pairs (band-sharing scores near the background of 0.23). It is possible that in some situations females pursue good genes for their offspring (Frederick 1987, Westneat et al. 1990) and use phenotypic cues correlated to overall fitness such as song repertoire (Hasselquist et al. 1996), plumage brightness (Moller and Birkhead 1994) or even age (Richardson and Burke 1999).

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Table 1. Breakdown of families sampled over ten years. Rows A-J correspond to social groups and boxed numbers refer to social pairs (families). There were 31 total families. Only families 1-4 were sampled more than once.

	1993	1994	1995	1997	1998	1999	2000	2001	2002	2003
A			11							
B	5	7,9	12	18				26		
C				2,19	2	2	4,4	4		31
D		10		23	1	1				
E	6	8	13							
F					24,25					
G			14,15	20,22	3	3	3	27	30	
H			16							
I			17	21						
J								28,29		

Table 2. Mean band sharing scores and standard errors for five categories of dyads: First order relatives, half-siblings, non-relatives, social pairs with EPF and social pairs without EPF.

Type of Dyad	n	Mean±SE
First Order Relatives	242	0.65±0.004
Half Siblings	31	0.52±0.010
Non-relatives	46	0.23±0.011
EPF	15	0.35±0.030
Non-EPF	23	0.26±0.022

Figure 1A

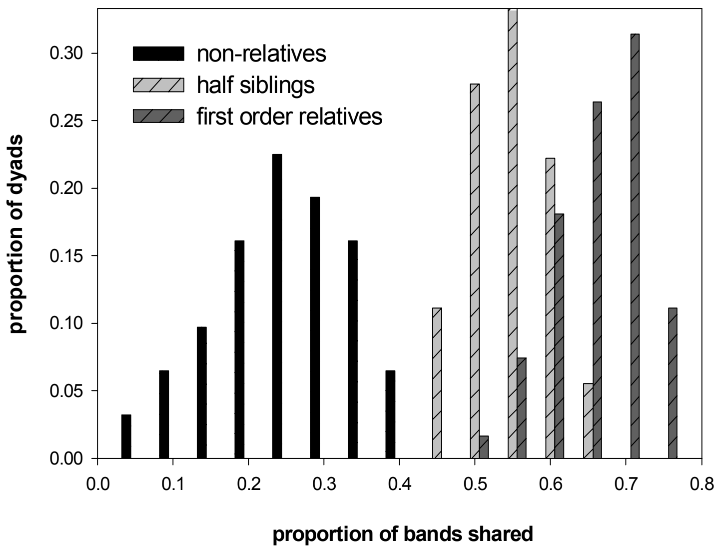


Figure 1B

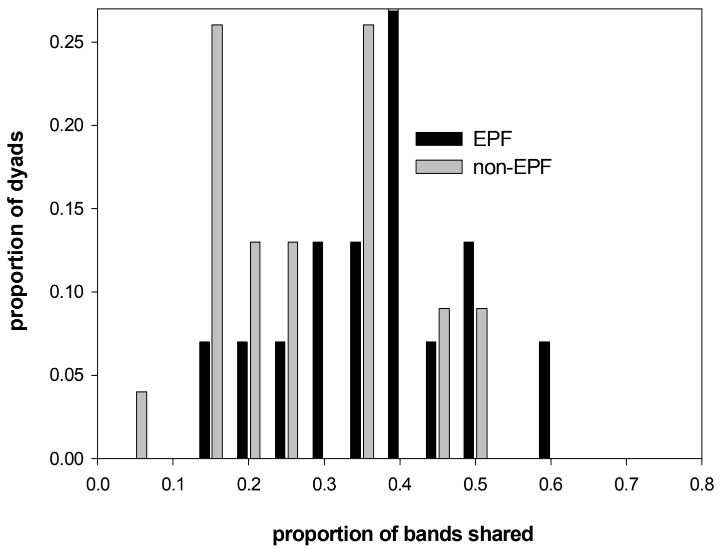
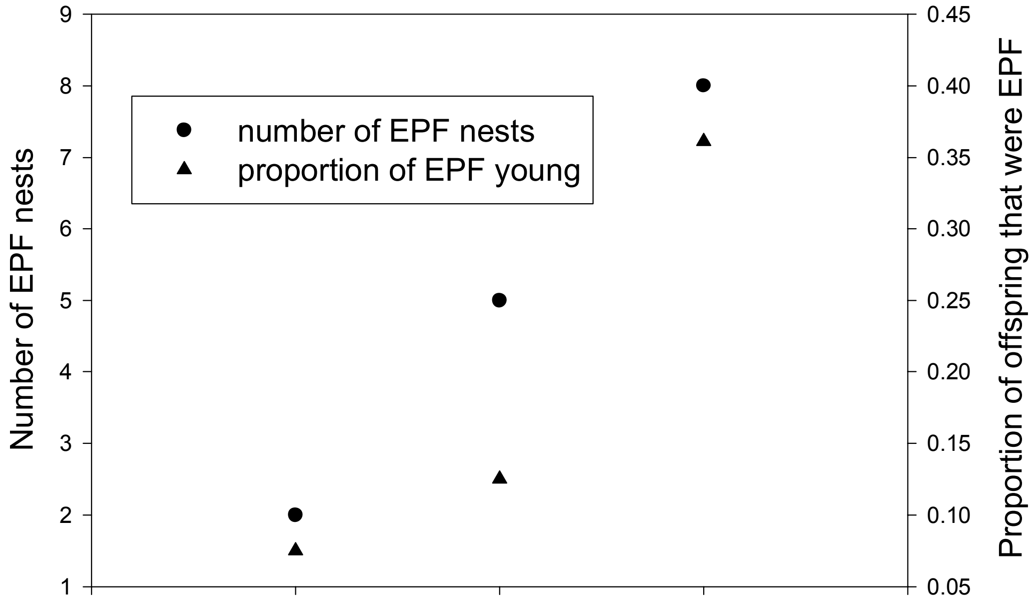
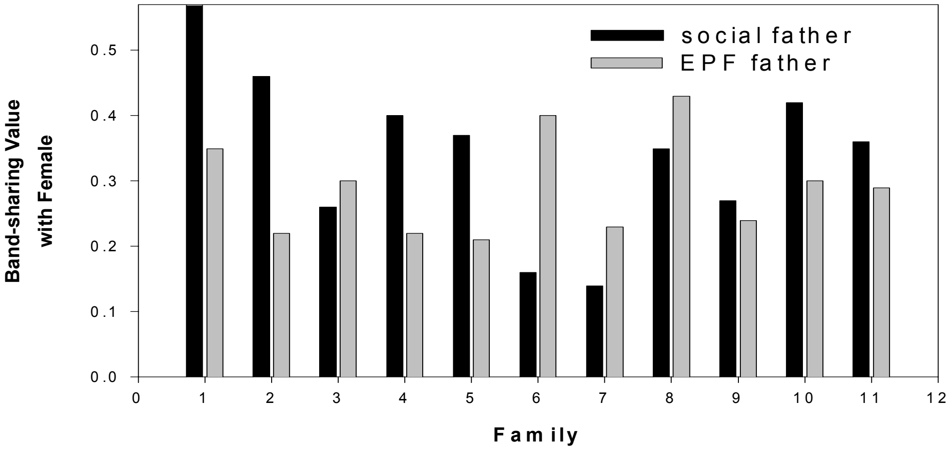


Figure 2



Band-sharing category	0.05-0.21	0.22-0.35	0.36-1
Number of nests	13	13	12
Number of young	40	40	36

Figure 3


social father
EPF father
Band-sharing Value with Female
0.0
0.1
0.2
0.3
0.4
0.5
0
1
2
3
4
5
6
7
8
9
10
11
12
Family