

Original Article

Tests of the kin selection model of mate choice and inbreeding avoidance in satin bowerbirds

Sheila M. Reynolds,^{a,b} J. Albert C. Uy,^{c,d} Gail L. Patricelli,^{c,e} Seth W. Coleman,^a Michael J. Braun,^{a-c} and Gerald Borgia^{a,c}

^aBehavior, Ecology, Evolution and Systematics Program, Biology/Psychology Building, University of Maryland, College Park, MD 20742, USA, ^bDepartment of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, 4210 Silver Hill Road, Suitland, MD 20746, USA, ^cDepartment of Biology, Biology/Psychology Building, University of Maryland, College Park, MD 20742, USA, ^dDepartment of Biology, University of Miami, Coral Gables, FL 33146, USA and ^eDepartment of Evolution and Ecology, University of California, 2320 Storer Hall, One Shields Avenue, Davis, CA 95616, USA

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In typically outbreeding species, females can avoid a reduction in offspring fitness by choosing unrelated sires. However, the kin selection model of mate choice suggests that it may be adaptive to mate with relatives to gain inclusive fitness benefits, especially in lekking species. Several studies have shown that females tend to mate with relatives, but the detailed behavioral data necessary to determine whether this reflects an active preference is difficult to acquire. We test the hypotheses that females actively preferred or avoided relatives in mate choice in satin bowerbirds (*Ptilonorhynchus violaceus*), a lekking species in which comprehensive observations of natural mate choice were obtained using automated video cameras positioned at bowers. We identified specific males that were sampled by individual females and assessed whether relatedness influenced their acceptance or rejection as mates. We found no consistent effect of relatedness on mate choice across years or among multiple stages of mate choice. In 2 of 6 years, females copulated with relatives at or above the half-sibling level significantly more often than expected, but this was attributed to females searching for mates in areas populated by relatives, and not to an active preference for relatives. Furthermore, we found no evidence for inbreeding avoidance through mate choice discrimination or sex-biased dispersal.

Key words: bowerbirds, inbreeding avoidance, kin selection, leks, mate choice, outbreeding, relatedness, sexual selection.

INTRODUCTION

The effect of relatedness on female mate choice is a key issue in sexual selection (Keller and Waller 2002; Kempenaers 2007; Mays et al. 2008). In typically outbreeding species, close relatedness between mates can have detrimental effects on offspring fitness, termed “inbreeding depression,” due to the expression of recessive deleterious alleles or the loss of heterosis (Charlesworth and Charlesworth 1987; Pusey and Wolf 1996; Keller and Waller 2002). Therefore, females are commonly expected to avoid mating with relatives (when possible), and this has been demonstrated in a number of species (reviewed in Pusey and Wolf 1996; Keller and Waller 2002).

However, mating with relatives may also benefit females because they can increase their inclusive fitness by helping their male relatives reproduce (Parker 1979; Smith 1979; Waser et al. 1986). This kin selection model of mate choice has received far less attention than the inbreeding avoidance model but has recently gained empirical support in studies in which females appear to favor relatives as mates (Bateson 1982; Barnard and Fitzsimons 1988; Burley et al. 1990; Ratti et al. 1995; Peacock and Smith 1997; Krokene and Lifjeld 2000; Duarte et al. 2003; Cohen and Dearborn 2004; Barber et al. 2005; Kleven et al. 2005; Sommer 2005; Schjørring and Jäger 2007; Thünken et al. 2007; Richard et al. 2009; Thurin and Aron 2009; Ryder et al. 2010; Wang and Lu 2011; Robinson et al. 2012).

The kin selection model of mate choice (Parker 1979; Smith 1979), also called the optimal inbreeding model (Lehmann and

Address correspondence to S. Reynolds. E-mail: sheila.reynolds@gmail.com.

Perrin 2003), argues that, by actively preferring relatives as mates, females increase their inclusive fitness and this benefit can supercede reasonably high costs of inbreeding depression (Parker 1979; Smith 1979; Waser et al. 1986; Lehmann and Perrin 2003; Kokko and Ots 2006; Puurtinen 2011; Szulkin et al. 2013). Another model, the optimal outbreeding model (Bateson 1983), argues that breeding with individuals that are too distantly related can be detrimental to fitness due to the breakup of coadapted gene complexes, however, this model applies more to cross breeding between populations and is not expected to influence mate choice on the individual level (Tregenza and Wedell 2000). Critical to the kin selection model is the assumption that when a male mates with a relative, he does not lose other mating opportunities with additional females (Parker 1979; Smith 1979), that is, that the related female provides an “extra” mating for the male. Thus, males gain “extra” offspring, even if those offspring suffer from inbreeding depression. The benefits to females of choosing related mates, however, depend on the tradeoff between inclusive fitness benefits and inbreeding costs to her offspring’s fitness.

This model assumption has led some authors to argue that non-resource-based (NRB) mating systems (i.e., leks) provide the best opportunity for the kin selection model to lead to the evolution of female preferences for relatives (Dawkins 1979; Parker 1979; Smith 1979; Bateson 1983; Waser et al. 1986; Lehmann and Perrin 2003; Kempenaers 2007). Males in NRB systems provide nothing to females but sperm and there is no paternal care. Thus, males who mate with relatives are unlikely to be depleted of resources important for attracting additional mates. Furthermore, it has been argued that the inclusive fitness benefits of inbreeding may be enhanced in NRB species because high skews in male mating success result in many males who would otherwise not mate, and related females can rescue the inclusive fitness they would accrue through these males by mating with them (Dawkins 1979; Kempenaers 2007). However, the vast majority of studies of relatedness in mate choice have focused on species with resource-based mating systems (see Kempenaers 2007; Mays et al. 2008; Jaimeson et al. 2009), therefore not allowing this prediction to be tested effectively.

The kin selection model predicts that females have an active preference for relatives in mate choice. However, females may mate with relatives as a side effect of other processes. Detailed information is needed to differentiate between an active preference and other causes that would lead females to mate with relatives. For example, limited natal dispersal may give rise to spatial relatedness structure in which individuals tend to live near relatives. This may increase the encounter rates of relatives in mate choice (Jaimeson et al. 2009), so that even if females choose randomly with respect to relatedness, they will mate with relatives at a high rate because available males are biased toward relatives (Bohank 1999; Duarte et al. 2003; Francisco et al. 2007). Thus, it is important to identify the specific males available to females to discern whether females are searching for mates in areas dominated by relatives (a spatial effect of relatedness on mate choice) or if females are actively rejecting nonrelatives in favor of relatives. Knowledge of the consistency of female mate choice with respect to relatedness, both within and between mating seasons, may also be used to differentiate between active preferences and other processes: active preferences should be consistent within and between seasons. Of all the studies that have reported preferences for relatives in natural mate choice, none have assessed this pattern separately in multiple mating seasons or at multiple stages of mate searching. Additionally,

no study to date has been able to identify the specific males that were actually sampled and rejected by individual females in order to compare their relatedness to the relatedness of the female and her chosen mate (Szulkin et al. 2013).

Satin bowerbirds (*Ptilonorhynchus violaceus*) provide a unique opportunity for the detailed study of relatedness in mate choice in an NRB mating system. Satin bowerbird courtship and copulation occur at bowers on the ground that can be monitored with automated video cameras. This allows for an unparalleled and comprehensive record of mate searching and mate choice (Borgia 1995), including identification of specific males that are sampled and rejected by females (Uy et al. 2001). Satin bowerbird mating system and life history suggest that there are many opportunities to encounter relatives in mate choice, provided females do not disperse from their natal populations. Females are reproductive in their first or second year, whereas males retain their bowers at the same locations for multiple years, enhancing the possibility of females encountering their fathers during mate searching. Male mating success is consistent across years, so top males are likely to remain the most attractive males when their daughters reach maturity. Additionally, high skews in male mating success (Borgia 1985) and small clutches (Donaghey R, unpublished data) suggest that paternal half-siblings with no social recognition of each other comprise a large proportion of the population and may breed together.

Satin bowerbird females have a multistep mate searching process (Uy et al. 2001; Coleman et al. 2004), which allows us to assess the influence of relatedness at different stages of mate searching. Females search among adjacent bowers in limited areas of the available habitat (Uy et al. 2001). They receive courtship from a subset of the males in their search areas and then typically copulate with one of these males. By testing the effect of relatedness at each of these sequential stages—choosing a search area, choosing males for courtship, and choosing males for copulation—and in multiple years, we can assess the consistency and possible causes of any relatedness effect. For example, if females tend to choose search areas populated by relatives, but do not favor relatives at later stages of mate searching, this would suggest that there is a spatial relatedness effect on mate choice but not an active preference for relatives. Conversely, an active preference for relatives would be supported if females favored relatives consistently throughout mate searching, or at the later stages of mate searching, when females have been able to assess males up close and make their final choices.

We assessed the role of relatedness in mate choice in a natural population of satin bowerbirds using 6 years of detailed information on female mate searching and mate choice. We tested several predictions of both the kin selection and inbreeding avoidance models. Here, we address the following questions: 1) Do females mate with relatives more or less often than expected by chance? 2) If so, how consistent is this pattern among 6 mating seasons in which females have opportunities to repeat their choices? Both the kin selection and inbreeding avoidance models predict that females have an active preference (for or against relatives), so their effects should be consistent across years and 3) If there is a relatedness bias, how does this bias develop across the 3 stages of mate searching? If a tendency to mate with relatives derives from a passive spatial relatedness structure, then females will search for mates in areas populated by relatives but should not show an active preference for relatives over other possible mates at later stages of mate searching.

Additionally, we test 3 other predictions of the inbreeding avoidance model. First, in several socially monogamous species, females who mate with relatives are more likely to seek additional

mates in the form of extrapair copulations than females mated to nonrelatives (Blomqvist et al. 2002; Eimes et al. 2005; Tarvin et al. 2005; Freeman-Gallant et al. 2006), possibly as a result of reduced fertilization success from mating with relatives (Tregenza and Wedell 2002). Similarly, inbreeding avoidance may explain why 18% of female satin bowerbirds copulate with more than 1 male (Cendes 2009). Thus, we test whether females who mated with multiple males were more related to their first mate than singly mating females. Second, females who mate with relatives may be less likely to return to those mates the following year if they experience inbreeding depression through reduced hatching success or fledging success (e.g., Bensch et al. 1994; Kempnaers et al. 1998; Van de Castele et al. 2003). So, we test whether females who returned to their mates across years were less related to them than females who switched mates. Third, inbreeding avoidance may explain why some females reject highly attractive males. The top 3 males (out of 34) at our study site typically mate with 50% of females, but some females who receive courtship from these males reject them. We assessed whether these females who reject top males are related to them more often than females who mate with them. Similarly, we test whether females whose search areas include top males but do not receive courtship from them were related to those males more often than females who do receive courtship.

The role of relatedness in mate choice depends on dispersal patterns because if dispersal effectively separates male and female relatives, they would not encounter each other during mate searching (Jaimeson et al. 2009). Little is known about satin bowerbird dispersal. A study by Nicholls and Austin (2005) found that populations along the New South Wales coast of Australia showed a genetic isolation-by-distance pattern with no barriers to gene flow between populations, suggesting that at least some dispersal occurs. Female-biased dispersal is common among passerine bird species (Clarke et al. 1997), but here we conduct the first test for sex-biased dispersal in satin bowerbirds.

METHODS

Field methods

We continuously monitored male displays and female mate searching throughout the mating seasons (late October–December) from 1997 to 2002 at Tooloom National Park, New South Wales, Australia (28°28'S, 152°26'E). Unbanded birds were captured and banded prior to each mating season and blood samples were taken as previously described (Reynolds et al. 2007). Behaviors at each bower were continuously monitored using automated video cameras activated by infrared motion sensors. Birds were identified on video by their leg band combinations (Borgia 1995; Uy et al. 2001). Reynolds et al. (2007) showed that this video record accurately indicates patterns of male mating success and paternity.

Relatedness estimation and classification

Pairwise relatedness estimates, r (Queller and Goodnight 1989), were calculated from 16 polymorphic microsatellite markers as described in Reynolds et al. (2009). Allele frequencies were estimated from a total of 248 adult birds caught in the study site (Reynolds et al. 2009). Following Reynolds et al. (2009), we classed pairs with $r \geq 0.13$ as related, corresponding to the r value expected for genealogical relationship at or above the half-sibling level (due to the variation of relatedness estimates, approximately 50% of half-sibling pairs have r estimates less than the theoretical expectation of

0.25; see Reynolds et al. 2009 for further details on the derivation of the 0.13 cutoff). Additionally, we identified possible parent–offspring (PO) pairs by the patterns of allele sharing between individuals. PO pairs share an allele at every locus by definition, so we classified pairs as PO if they shared alleles at 15 or 16 microsatellite loci, allowing 1 mismatch among loci to account for mutation or genotyping error. Full siblings may also share alleles at every locus, but they are not constrained to this pattern. Thus, our classification of PO pairs may include father–daughter pairs, mother–son pairs, and some full-sibling pairs. We further identified pairs that were possible father–daughter pairs using information on when birds were first banded. PO pairs in which the female was banded in a year after the male's first season as a bower owner were classed as possible father–daughter pairs because the male may have reproduced prior to the female's first year. Females who were banded prior to a male's first season as a bower owner were assumed not to be the daughter of that male because non-bower-holding males typically do not sire offspring (Reynolds et al. 2009).

Data analyses

We evaluated the sex bias in dispersal using the mean assignment index test (Paetkau et al. 1995; Favre et al. 1997). The corrected assignment index, A_{IC} , of an individual is calculated as the product across loci of the squared allele frequencies at homozygous loci or twice the product of allele frequencies at heterozygous loci minus the mean of these products for all individuals in the population (Goudet et al. 2002). A_{IC} represents the likelihood that an individual's genotype originated in the focal population, with lower A_{IC} being associated with immigrants to the focal population. Sex bias in dispersal is detected by a statistical difference between the mean A_{IC} of males and females, either using a t -test or a randomization test in which the sexes of individuals are permuted across A_{IC} scores 1000 times. This analysis included all reproductive individuals in the population, that is, all bower-holding males and copulating females observed between 1997 and 2002, for which we had complete genotypes at all loci.

We used 2 approaches to assess the effect of relatedness on overall mate choice and on different stages of mate searching. For each of our main hypotheses, we used mixed model logistic regressions and randomization tests. The logistic regressions (as described below) were useful for deriving overall results across years and for illustrating how each individual year contributed to those overall results. The randomization tests (as described below) were useful because their designs were tailored to specific aspects of our data and were more carefully controlled for relevant variation among individual males and females. However, the randomization tests were not adaptable to a multiyear overall analysis, so we report results of both analyses for each hypothesis.

We assessed the effect of relatedness on female mate choice each year in 2 ways. First, we conducted mixed model logistic regressions to determine the effect of relatedness on whether each possible male–female pair in a given year mated (a binomial response variable). We included relatedness (a binomial variable) as a fixed effect and male and female identities as random effects to control for the replication of individual males and females among pairs. We also conducted a multiyear logistic regression analysis with relatedness and year as fixed effects and male, female, and pair identities as random effects. Pair identity was included to control for the replication of pairs among years (in this and all other multiyear logistic regression analyses). Second, for each year, we conducted Monte Carlo randomization tests (Manly 1997) in which the

number of related copulating pairs observed was compared to a null distribution of 10 000 permutations in which males were randomly assigned to females. For these randomization tests, females who mated with more than 1 male in a year were assigned their last mate as their observed mate to control for pseudoreplication of females.

We conducted similar analyses to determine at what stages of mate searching relatedness played a role. Mate searching was divided into 3 stages: search area, courtship, and copulation. Each female's search area was defined as the minimum convex polygon that included all the bowers at which she appeared during the mating season. To test whether relatedness affected females' choices of search areas, we first conducted mixed model logistic regressions for each year in which, for each male–female pair, we modeled the likelihood of the male's bower being in the female's search area as a function of relatedness (a fixed effect) and male and female identities (random effects). Second, using Monte Carlo randomization tests (Manly 1997), we compared the relatedness of females to the males in their own search areas with their relatedness to males in other females' search areas. In this randomization analysis, we maintained search areas as defined units in order to control for the constraint that a search area must consist of spatially adjacent bowers and to preserve the observed level of variation in search area size. Our null hypothesis was that any female could have chosen any of the observed search areas regardless of her relatedness to the males in it. The samples for this test included all females who were observed at bowers in each year. We summed across females the number of relatives in their search areas as our test statistic and then compared this to a null distribution in which we randomized 10 000 times which female was associated with each search area.

In the second stage of mate searching, females choose to receive courtship from some but not all males in their search areas. To test whether relatedness affected females' choices of males for courtship, we again conducted 2 analyses. First, our mixed model logistic regression analyses included only pairs for which the male's bower was in the female's search area. We then modeled the likelihood that the female received courtship from the male as a function of relatedness (fixed effect) and male and female identities (random effects). For the randomization tests, we maintained each female's observed search area and the number of males from whom she received courtship, but we randomized 10 000 times which males were chosen for courtship. Our null hypothesis was that females could have chosen any male in their search area for courtship, regardless of relatedness. The samples for this test included all females whose search areas included at least 1 relative and at least 1 nonrelative and who did not receive courtship from every male in their search area. We counted across all females the number of relatives that were chosen for courtship as our test statistic.

In the third stage of mate searching, females typically choose 1 male for copulation from among the males that courted them. To assess the role of relatedness in this choice, we first conducted mixed model logistic regressions in which we included only pairs that courted, and we modeled the likelihood of a male being chosen as a mate as a function of relatedness (fixed effect) and male and female identities (random effects). The samples for the randomization tests included all females who mated after receiving courtship from at least 1 relative and at least 1 nonrelative, and who did not mate with every male that courted them. Similar to the previous randomization analysis, we randomized which of the courted males females chose for copulation and we counted the number

of relatives that were chosen for copulation across all females as our test statistic.

In addition to mate choice and mate searching, we tested the role of relatedness in several other aspects of female mating behavior. To assess the effect of relatedness on multiple mating by females, we identified all females who were observed to copulate with more than 1 male in a given year. We then tested whether multiply mating females were related to their first mates more or less often than singly mating females using chi-square tests for each year. Additionally, we tested if relatedness affects whether females return to their mates the following year using logistic regression. Male quality is known to influence female return rates (Uy et al. 2000), so we controlled for this effect by including male rank as an independent variable in the model. We included all female–mate pairs and the response variable for each female–mate pair was whether the female mated with that male again the following year. Females who did not mate the following year or whose mate was not present the following year were excluded. For rejection of top males, we tested whether females who copulated with any of the top 3 (highest numbers of copulations) males were related to them more or less often than females who received courtship from but did not copulate with them using chi-square tests. Similarly, we tested whether females who received courtship from top males were related to them more or less often than females whose search areas included these males but did not receive courtship from them using chi-square tests.

All statistical analyses were performed in Statistica 6.0 (Statsoft Inc., Tulsa, OK), except for the logistic regressions, which were conducted using R (R Core Team, Vienna, Austria) and the mean assignment index test and randomization tests, which were written as Excel macros (Microsoft, Redmond, WA).

RESULTS

Sex-biased dispersal

We genotyped 119 females and 48 males (5712 total male–female pairs) that were observed at bowers from 1997 to 2002 (Table 1). We tested for sex-biased dispersal using an assignment index test in which a larger AIC value indicates that an individual is more likely to have been conceived in the focal population. Our results showed that female mean AIC ($n = 112$, mean = -0.108 ± 1.99 SD) and male mean AIC ($n = 48$, mean = 0.026 ± 1.96 SD) were not significantly different ($t = 0.394$, $P = 0.694$), suggesting that dispersal is not sex biased in satin bowerbirds. A permutation test also showed no significant difference in AIC between males and females ($P = 0.693$). Therefore, related males and females are not expected to be geographically separated through sex-biased dispersal and may encounter each other during mate searching. Although dispersal may still separate male and female relatives even when it is not sex biased, each female had on average 4.4 ± 2.7 SD (range: 0–12) related males present in the study population each year.

Relatedness and mate choice

On average, 15.7% of all possible male–female pairs and 22.2% of copulating pairs were related at or above the half-sibling level ($r \geq 0.13$) each year (Table 1). Among all male–female pairs in which both birds were observed in the same year, 15 (0.26%) were PO pairs (mean $r = 0.48 \pm 0.09$ SD), and 6 of these were possible father–daughter pairs (see Methods). Across all years, we observed only one PO pair (out of 15) for which the male was in the female's

Table 1
Sample sizes and numbers of related pairs in each year

Year	No. genotyped birds (no. copulating birds)		No. unique pairs	No. copulating pairs	No. (proportion) related		
	Females	Males			All pairs	Copulating pairs	No. possible PO pairs
1997	86 (63)	32 (23)	2752	88	387 (0.14)	11 (0.13)	7
1998	84 (75)	30 (25)	2520	92	367 (0.15)	22 (0.24)	5
1999	72 (51)	29 (16)	2088	64	346 (0.17)	12 (0.19)	8
2000	67 (31)	25 (14)	1675	35	289 (0.17)	8 (0.23)	3
2001	67 (46)	29 (14)	1943	48	303 (0.16)	14 (0.29)	6
2002	51 (23)	22 (12)	1122	27	180 (0.16)	7 (0.26)	1
All	119 (108)	48 (34)	5712	245	834 (0.15)	46 (0.19)	15

For multiply mating females, this table includes all mates.

search area. She received courtship from him but did not mate with him.

In 2 of 6 years, 1998 and 2001, relatedness was significantly positively associated with mate choice (logistic regressions: 1998, $n = 2520$ pairs, $P = 0.020$; 2001, $n = 1943$ pairs, $P = 0.020$; Table 2 and Figure 1). In other words, females mated with relatives more often than expected by chance (randomization tests: 1998, $n = 74$ females, $P = 0.066$; 2001, $n = 42$ females, $P = 0.004$; Table 2), although the 1998 randomization result was only marginally significant. In the remaining 4 years, there were no significant effects of relatedness on mate choice (all $P > 0.196$, Table 2). In the overall, multiyear logistic regression analysis, relatedness did not significantly affect whether pairs copulated (logistic regression: $N = 12\,181$ pair-years, $P = 0.628$; Table 2).

Relatedness and mate searching stages

Given that females tended to mate with relatives more often than expected by chance in 2 years, and did not strongly avoid mating with relatives in any year, we investigated when this bias in favor of relatives occurred among 3 different stages of mate searching. We assessed all years because a relatedness effect at an early stage of mate searching may have been wiped out at later stages of mate searching in years that showed no overall effect. Our randomization tests were 1 tailed because we had the directional prediction based on the mate choice analyses that females tended to mate with relatives and not avoid them (Gibbons and Pratt 1975). Females' search areas included more relatives than expected by chance in 2 years, 2001 and 2002 (randomization tests: 2001: $n = 67$ females, $P = 0.034$; 2002: $n = 51$ females, $P = 0.050$; Table 2). The corresponding logistic regression results were similar although only marginally significant in those 2 years (2001: $n = 1943$ pairs, $P = 0.061$; 2002: $n = 1122$ pairs, $P = 0.069$; Table 2). In analyzing the effect of relatedness on female choice of search area, it was only in the randomization test that we could explicitly control for the spatial constraint that all possible search areas must be composed of spatially adjacent bowers. Therefore, with respect to the search area hypothesis, we give greater weight to the randomization results than the regression results. The relatives within females' search areas were mostly related at the half-sibling level (mean r among these relatives was 0.19 ± 0.07 SD) and they included only one possible father across all years. In the combined analysis across all 6 years, female's did not tend to include relatives' bowers in their search areas (logistic regression: $N = 12\,181$ pair-years, $P = 0.246$; Table 2). Females did not choose relatives for courtship from among the males in their search areas more often than expected (logistic regressions: all $P \geq 0.132$;

randomizations: all $P \geq 0.055$; Table 2). Females did not choose relatives for copulation from among the males that courted them more often than expected (logistic regressions: all $P \geq 0.120$; randomizations: all $P \geq 0.156$; Table 2).

Relatedness and multiple matings

We tested the hypothesis from inbreeding avoidance models that females who mate with relatives are more likely to mate with additional males than females who mate with nonrelatives to avoid costs of inbreeding depression. Each year, among genotyped females that mated, 20% (range: 12–33%) mated with more than 1 male, and the mean number of mates per female was 1.2 ± 0.47 SD males (range: 1–4). We found that female satin bowerbirds that mated with more than 1 male were not more likely to be related to their first mate than singly mating females (chi-square tests: all $P \geq 0.131$; Supplementary material 1). Furthermore, females who mated with 2 males were not more related to their first mate than to their second mate (sign tests: all $P \geq 0.074$; Supplementary material 1).

Relatedness and returning to previous mates

The inbreeding avoidance model also suggests that females who mated with relatives were more likely to switch mates the following year if they experienced low hatching success due to inbreeding depression than females who did not mate with relatives. Relatedness to their mate did not predict whether female satin bowerbirds returned to the same males the following year in 4 of 5 years (logistic regressions: all $P \geq 0.238$; Supplementary material 1). Contrary to the expectation of the inbreeding avoidance model, females who mated with relatives in 2000 were more likely to return to those males in 2001 than females who mated with nonrelatives (logistic regression: $N = 19$, relatedness estimate = 3.19 ± 1.3 SE, $P = 0.013$; Supplementary material 1). This effect of relatedness is strong because the proportion of deviance explained by this logistic regression model was much higher than if the model excluded relatedness (with relatedness: Nagelkerke's $R^2 = 0.430$; without relatedness: Nagelkerke's $R^2 = 0.002$). However, because the year in which these females returned to their related mates, 2001, was the year in which we observed the highest frequency of related mates, it is difficult to differentiate whether the tendency of females to return to their related mates in 2001 was a cause or a consequence of the overall tendency to mate with relatives in 2001. Given that this was the only year (of 5) in which we observed a significant return rate to related mates, we are inclined to favor the latter explanation.

Table 2
Results for logistic regression and randomization analyses for 4 hypotheses

Hypothesis	Test	1997 results ^a	1998 results ^a	1999 results ^a	2000 results ^a	2001 results ^a	2002 results ^a	All years results ^a
Overall mate choice Females mate with relatives more or less often TEBC	Logistic regression	-0.318 ± 0.35 <i>P</i> = 0.358	0.607 ± 0.26 <i>P</i> = 0.020	0.258 ± 0.35 <i>P</i> = 0.456	0.511 ± 0.44 <i>P</i> = 0.242	0.822 ± 0.35 <i>P</i> = 0.020	0.616 ± 0.48 <i>P</i> = 0.196	0.561 ± 1.16 <i>P</i> = 0.628
	Randomization ^b	6/9 (63) <i>P</i> = 0.390	17/11 (74) <i>P</i> = 0.066	9/9 (51) <i>P</i> = 1.00	6/5(30) <i>P</i> = 0.823	14/6 (42) <i>P</i> = 0.004	6/4 (23) <i>P</i> = 0.412	
Mate searching Females' search areas include more relatives TEBC	Logistic regression	-0.170 ± 0.20 <i>P</i> = 0.387	0.275 ± 0.19 <i>P</i> = 0.139	0.295 ± .021 <i>P</i> = 0.156	0.190 ± 0.21 <i>P</i> = 0.362	0.467 ± 0.25 <i>P</i> = 0.061	0.477 ± 0.26 <i>P</i> = 0.069	0.139 ± 0.12 <i>P</i> = 0.246
	Randomization ^c	38/43 (86) <i>P</i> = 0.828	45/38 (84) <i>P</i> = 0.107	41/33 (72) <i>P</i> = 0.072	64/59 (67) <i>P</i> = 0.235	27/19 (67) <i>P</i> = 0.034	28/21 (51) <i>P</i> = 0.050	
Females choose relatives for courtship more often TEBC	Logistic regression	-0.267 ± 0.46 <i>P</i> = 0.565	0.986 ± 0.65 <i>P</i> = 0.132	-0.151 ± 0.56 <i>P</i> = 0.787	-0.217 ± 0.32 <i>P</i> = 0.499	0.087 ± 0.67 <i>P</i> = 0.896	-0.254 ± 0.50 <i>P</i> = 0.609	-0.138 ± 0.20 <i>P</i> = 0.500
	Randomization ^d	27/26 (21) <i>P</i> = 0.390	41/ 37 (29) <i>P</i> = 0.055	34/ 32 (24) <i>P</i> = 0.249	26/ 30 (26) <i>P</i> = 0.903	21/ 21 (11) <i>P</i> = 0.590	17/16 (14) <i>P</i> = 0.374	
Females choose relatives for copulation more often TEBC	Logistic regression	-0.100 ± 0.44 <i>P</i> = 0.820	0.399 ± 0.35 <i>P</i> = 0.259	0.0272 ± 0.46 <i>P</i> = 0.952	0.592 ± 0.50 <i>P</i> = 0.237	0.571 ± 0.56 <i>P</i> = 0.311	0.555 ± 0.57 <i>P</i> = 0.330	0.341 ± 0.22 <i>P</i> = 0.120
	Randomization ^e	11/11 (12) <i>P</i> = 0.609	22/20 (27) <i>P</i> = 0.264	12/11 (18) <i>P</i> = 0.339	8/6 (17) <i>P</i> = 0.163	14/12 (9) <i>P</i> = 0.156	7/5 (8) <i>P</i> = 0.217	

TEBC, than expected by chance.

^aFor logistic regression analyses, we report the slopes for the effect of relatedness on the outcome (±SE) and the *P*-value for the slope. For randomization analyses, we report observed/expected frequencies (sample size) and *P* value.

^bObserved and expected values represent the number of females that mated with relatives. Expected values were calculated from the observed proportions of all pairs that were related (see Table 2).

^cObserved and expected values represent the total number of relatives within all females' search areas. Expected values indicate the median of the null distribution for the randomization test.

^dObserved and expected values represent the total number of relatives from whom females received courtship. Expected values indicate the median of the null distribution for the randomization test.

^eObserved and expected values represent the total number of relatives with whom females mated. Expected values indicate the median of the null distribution for the randomization test.

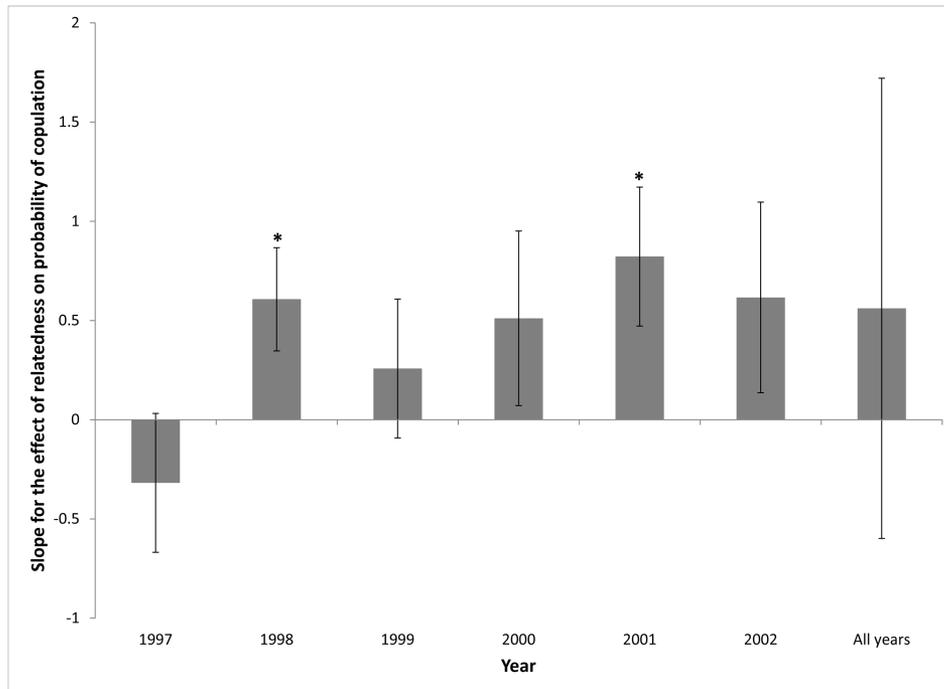


Figure 1

The logistic regression slopes (on the logit scale) for the overall effect of relatedness (\pm SE) on the probability of copulation among all male–female pairs in the population. Asterisks indicate significant slopes.

Relatedness and rejection of top males

Females who receive courtship from but then reject top males may do so to avoid mating with relatives. Relatedness did not affect whether females who were courted by the top 3 males mated with them or rejected them (chi-square tests: all $P \geq 0.231$; [Supplementary material 1](#)), nor whether females whose search areas included top males chose to receive courtship from them or not (chi-square tests: all $P \geq 0.143$; [Supplementary material 1](#)).

DISCUSSION

We investigated how relatedness influences mate choice in a natural population of satin bowerbirds, testing predictions of both the inbreeding avoidance and kin selection models. There was no consistent effect of relatedness on mate choice across years, although in 2 of 6 years, 1998 and 2001, relatedness was significantly positively associated with mate choice. In the remaining 4 years, results did not differ significantly from random expectation ([Table 2](#)). In a multiyear analysis, we found that relatedness did not significantly affect mate choice. These results suggest that female mate choice per se is not influenced by relatedness in satin bowerbirds.

Because females tended to mate with relatives more often than expected by chance in 2 of 6 years, and did not strongly avoid mating with relatives in any year, we investigated each stage of the mate search process to determine when the bias toward relatives might have occurred. Our results suggest that a bias in favor of relatives most likely occurs at the earliest stage, when females establish their search areas. In 2 years, 2001 and 2002, females' search areas included relatives more often than expected by chance ([Table 2](#)). Females did not choose relatives for courtship from among the males in their search areas more often than expected ([Table 2](#)). Nor did females tend to copulate with relatives from among the males who courted them ([Table 2](#)). These results suggest that females

tended to search for mates in areas populated by relatives in some years, but then chose randomly with respect to relatedness within those search areas. This is consistent with a previous finding that related males have bowers near each other ([Reynolds et al. 2009](#)), so that females who search in the area of one relative may also have other male relatives nearby.

The tendency for females to mate with relatives in 2 years appears to result from this spatial effect of relatedness on mate searching rather than from an active preference for relatives. If there were an active preference for relatives, we expected to observe it consistently across years and at each stage of mate searching, or at least at later stages when females make their final choice, but females did not significantly prefer relatives at the courtship or copulation stages of mate searching in any year. In 2001, females chose search areas that were biased toward relatives and this alone appears to have led to the bias in mate choice. In 1998, marginal biases toward relatives at both the search area and courtship stages of mate searching combined ([Table 2](#)) resulted in the tendency to mate with relatives. In 2002, there was also a bias toward relatives at the search area stage of mate searching, but this did not result in a bias in mate choice.

We found no evidence that females avoided inbreeding through mate choice discrimination. Females did not mate with relatives less often than expected by chance in any year ([Table 2](#)). Females who mated with relatives were not more likely to mate with a second male in the same season or to switch mates the following season ([Supplementary material 1](#)). Also, females who rejected the most attractive males were not more likely to be related to them ([Supplementary material 1](#)). Sex-biased dispersal is a common inbreeding avoidance strategy ([Pusey and Wolf 1996](#)), especially in passerines ([Clarke et al. 1997](#)), but we did not detect a sex bias in satin bowerbird dispersal. Although the lack of a sex bias does not rule out the possibility that dispersal effectively separates male and

female relatives, it appears to yield incomplete separation at best in satin bowerbirds because 15% of all male–female pairs were related at or above the half-sibling level and each female had on average 4 related males present in the population. The absence of inbreeding avoidance in satin bowerbirds suggests that either inbreeding depression is not sufficiently costly in this species to drive the evolution of inbreeding avoidance behaviors (or the costs of inbreeding depression may be compensated for by inclusive fitness benefits) or females do not encounter close male relatives sufficiently often in mate searching for inbreeding avoidance to evolve (Jaimeson et al. 2009; Alho et al. 2012). Also, females may not be able to detect relatedness to their potential mates, although this argument is contradicted by evidence that male satin bowerbirds can identify relatives (Reynolds et al. 2009) and recent studies suggesting that bird olfaction is more sophisticated than previously thought and may allow for kin recognition (Krause et al. 2012; Leclaire et al. 2012; Strandh et al. 2012).

Alternatively, anecdotal evidence suggests that females may avoid mating with their fathers by searching for mates away from their fathers' bowers but do not avoid more distant relatives' bowers. Of the 6 possible father–daughter pairs we identified, the females tended to search in areas that did not include their fathers' bowers. Only one female, in 1 of her 5 years of mate searching, chose a search area that included her father's bower. If females hatch in nests near their fathers' bowers, then this avoidance of close relatives may be accomplished with a simple strategy of mate searching away from the natal nest. Such a strategy might also protect females from mating with their full-sibling brothers because related males tend to have bowers near each other (Reynolds et al. 2009). In fact, the relatives we observed within females' search areas were on the order of half-siblings (mean $r = 0.19$). This idea is illustrated in the one case of a putative family group that we identified genetically, consisting of a father, mother, 2 sons, and 1 daughter, all of which were full siblings (all offspring matched both parents at all loci, except the daughter who mismatched each parent at one locus; all sibling pairs had $r \geq 0.42$). One son took over his father's bower site and the other son took over a nearby bower site. The mother's nest was near the father's bower, however the daughter's mate searching areas (in 5 years) included bowers of 3 half-siblings' but not her father or full-sibling brothers (Figure 2).

Overall, our findings in satin bowerbirds suggest that females do not actively prefer or avoid relatives in mate choice. There was no consistent effect of relatedness on mate choice across years or across mate choice stages. We tested several additional predictions of the inbreeding avoidance model but found no evidence that female mating behaviors reduced inbreeding. However, females may avoid close inbreeding with their fathers (and possibly full-sibling brothers) by searching for mates away from their natal nest, though we did not have enough data to test this hypothesis. Additionally, the fact that we only identified possible fathers for 6 of 119 (5%) females suggests that females may typically disperse from their natal populations, though not significantly more so than males (an alternative explanation for the lack of fathers is that they died or left the population). If females immigrated to our study population, then they must have dispersed along with some of their relatives because each female had on average 4 male relatives in the study population. Dispersal did not prevent females from encountering their relatives during mate choice, and, interestingly, females tended to search among their intermediate relatives' bowers in some years. This resulted in a tendency to mate with these intermediate

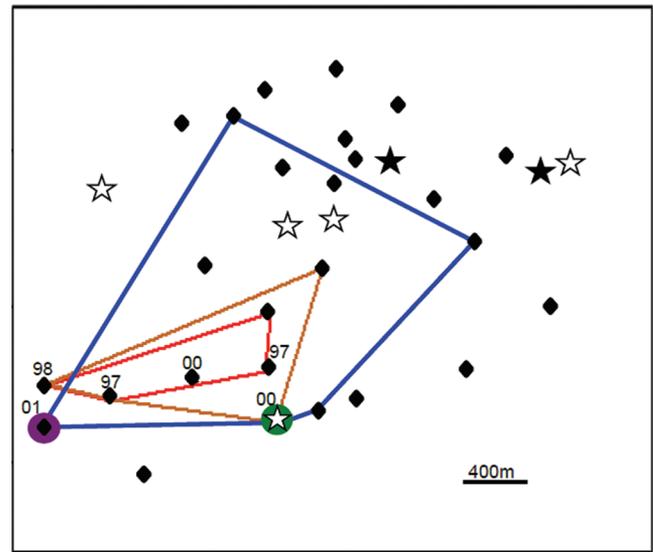


Figure 2

Mate searching areas and locations of relatives of 1 female. The black diamonds indicate bower locations. The polygons and large dots represent her search areas for 1997 (red), 1998 (brown), 1999 (green dot, only one bower), 2000 (blue), and 2001 (purple dot, only one bower). The filled stars indicate the bower locations of her first-order relatives (left filled star: father's bower in 1996–1998 then brother's bower in 1999; right filled star: a different brother's bower in 1999). The open stars indicate the bower locations of her second-order relatives (e.g., half-siblings). The numbers represent the year in which she mated at the indicated bower.

relatives despite choosing randomly with respect to relatedness at later stages of mate searching. These results do not provide strong support for either the inbreeding avoidance or kin selection models of mate choice in satin bowerbirds but do not entirely rule them out either.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>

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