

ABSTRACT

Title of Document: THE EFFECT OF RELATEDNESS ON
MATING BEHAVIOR IN THE SATIN
BOWERBIRD (*Ptilonorhynchus violaceus*)

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Behavior is a main component of sexual selection theory in which male competition and female mate choice influence the evolution of a species. Relatedness commonly affects behavioral interactions, but the potential for relatedness to influence sexual selection is often overlooked. Here I show that relatedness affects mating behaviors in satin bowerbirds.

Bowerbirds are a model species for non-resource based mating systems in which males provide only sperm to females, and females are free to mate with their preferred males, typically resulting in high skews in male mating success. Males build stick structures (bowers) on the ground to attract, and copulate with, females. Males compete, in part, by destroying neighboring males' bowers. Females search among multiple adjacent bowers and then select subsets of these males for courtship

and then copulation. Automated video monitoring of bowers allows identification of males that destroy bowers and females that visit bowers for courtship or copulation. Using microsatellite genetic markers to estimate relatedness, I show that paternity assignments based on observed copulations match the genetic sires of offspring, supporting the hypotheses that copulations occur only at bowers and that male reproductive success can be reliably estimated from observed copulations. Next, I report that competing males are less aggressive, in the form of bower destructions, towards relatives than non-relatives and that this restraining effect of relatedness on aggression favors the close spatial association of relatives' bowers. These results support the hypothesis that relatedness affects male competition and ability to maintain attractive displays for females. Lastly, I investigate the influence of relatedness on female mate choice. I show that females do not actively prefer or avoid relatives in mate choice. However, females bias the areas in which they search for mates to be inclusive of relatives and then mate randomly with respect to relatedness within their search areas, resulting in tendencies to mate with relatives in some years. This effect of relatedness on female mate searching may be due in part to the spatial association of related males, and highlights the influence of mate searching rather than active mate preferences on overall mate choice patterns.

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BOWERBIRD (*Ptilonorhynchus violaceus*)

By

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Preface

This dissertation contains three chapters. Each chapter contains an abstract, introduction, methods, results, and discussion section, as well as figures, tables and captions. The first chapter is presented in the format in which it was published (*The Auk*. 2007. 124, 857-867), the second chapter is presented in the format in which it was published (*Behavioral Ecology*. 2009. 20, 410-415), and the third chapter is presented in manuscript form. A single bibliography section is at the end for references cited throughout the dissertation.

Dedication

This dissertation is dedicated to my grandparents, John M. and Anne C. Reynolds,
and Col. William C. and Neville A. Holter, whose examples of dedication,
perseverance, creativity and intellectual curiosity inspired me.

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Introduction

Ever since the publication of Hamilton's (1964) kin selection theory, the potential for genetic relatedness to influence animal behavior has been of major interest in behavioral ecology. In particular, information on relatedness has revealed key insights into sexual selection and the evolution of mating systems (Birkhead and Moller 1992, Emlen 1995, Pusey and Wolf 1996, Hughes 1998, Kempenaers 2007). Sexual selection is the process by which male competition and female mate choice lead to variation in individual reproductive success (Darwin 1871). Kin selection theory recognizes that, in addition to individual reproductive success, one can pass copies of their genes on to the next generation by helping to increase the reproductive success of relatives (Hamilton 1964). In cooperatively breeding species, individuals forego their own reproduction to help raise the offspring of others, and recognition that helpers are often closely related to the breeders they help has greatly improved our understanding of the evolution of this mating system (Emlen 1995, Lehmann and Keller 2006, West et al. 2007; but see Cockburn 1998, Clutton-brock 2002). Additionally, the use of genetic tools, such as microsatellite markers, to assess relatedness in natural populations has revealed the true nature of other mating systems, particularly monogamous systems. Genetic paternity analyses have revealed widespread patterns of extra-pair paternity in monogamous species, especially among birds, causing researchers to re-examine the evolution of monogamy (Westneat 1987, Birkhead and Moller 1992, Hughes 1998, Hasslequist and Sherman 2001).

Relatedness can directly affect male competition and female mate choice. Commonly, one or both sexes will disperse to reduce the risk of relatives competing with each other for resources or mates (Greenwood 1980), however, this is not always the case. When related males compete for access to females, they may benefit from helping their relatives “win” over unrelated males rather than competing against their relatives. Brothers form coalitions to help each other gain access to females or breeding sites in African lions (Packer et al. 1991), acorn woodpeckers (Koenig et al. 2000), and brown jays (Williams and Rabenold 2005). In wild turkeys, groups of brothers perform cooperative displays for females though typically only one male gets to mate (Krakauer 2006). In lekking species, related males commonly reside on the same leks (Petrie et al. 1999, Hoglund et al. 1999, Shorey et al. 2000, Bouzat and Johnson 2004, Regnaut et al. 2006), prompting one hypothesis to argue that males join leks where relatives are the most attractive males to help increase the relatives’ mating success (Kokko and Lindstrom 1996). Another hypothesis suggests that males may receive less aggression from relatives at competing display sites (Saether et al. 2002), but neither of these hypotheses have been directly tested.

While it may benefit individuals to help their relatives reproduce, it is generally considered to be detrimental to reproduce with relatives (Keller and Waller 2002), and relatedness has been shown to have strong effects on female mate choice in typically outbreeding species (see Kempenaers 2007). Offspring of related parents commonly suffer reduced fitness, called inbreeding depression (Charlesworth and Charlesworth 1987), due to increased homozygosity which results in the expression of deleterious recessive alleles or loss of heterosis at genetic loci (Keller and Waller

2002). Many studies have shown that females prefer unrelated over related males both experimentally (see Pusey and Wolf 1996) and in natural mate choice (see Kempenaers 2007). A few studies have suggested that females seek extra-pair mates who are less related to them than their social mates (Masters et al. 2003, Tarvin et al. 2006, Oh and Badyaev 2006, Bishop et al. 2007).

However, it has been suggested that in some situations, it may be beneficial to mate with relatives despite the potential costs of inbreeding depression (Parker 1979, Smith 1979, Bateson 1983). Females in some species have been shown to prefer relatives over non-relatives both in experiments (Bateson 1982, Barnard and Fitzsimons 1988, Burley et al. 1990, Thunken et al. 2007, Schorring and Jager 2007) and in natural mate choice (Peacock and Smith 1997, Krokene and Lifjeld 2000, Duarte et al. 2003, Cohen and Dearborn 2004, Shutler et al. 2004, Ryder et al. 2010). Extra-pair mates were more related to females than social mates in two studies (Barber et al. 2005, Kleven et al. 2005). Thus, while it is clear that relatedness can have an important effect on natural mate choice, predicting the direction of this effect may be complicated.

Non-resource based (NRB) mating systems, including leks, are key models for sexual selection studies (Andersson 1994), yet most of the work on relatedness in sexual selection has focused on species with resource based mating systems. This discrepancy may be attributable to the difficulty of observing mate choice in NRB species because females do not co-reside with their mates on territories (e.g. Petrie and Kempenaers 1998). Nonetheless, it is important to understand how relatedness influences sexual selection in these model species. In NRB mating systems, males

provide nothing to females except sperm. Thus females do not consider material benefits they may receive from males in mate choice and appear to base their choices primarily on male sexual displays. Females typically prefer a few top males while many males do not mate, resulting in high skew in male mating success (Bradbury 1981) and strong sexual selection for male displays (Andersson 1994). Accordingly, males in NRB species typically have extravagant displays which include colorful plumage and elaborate dances and vocalizations (Hoglund and Alatalo 1995). To win the attention of females, males compete by vigorously displaying but also by fighting with each other and interrupting the courtship and copulation of neighboring males (Hoglund and Alatalo 1995, Westcott 1997). Thus, NRB species provide prime examples of both male competition and female mate choice.

Here, I investigate the effects of relatedness on sexual selection in satin bowerbirds (*Ptilonorhynchus violaceus*), a model NRB species. In satin bowerbirds, courtship and copulation take place at bowers, stick structures that males build on the ground and decorate. Males compete primarily by destroying each others' bowers and stealing bower decorations (Borgia 1985a). The unique dependence on a specific location, the bower, for reproduction allows detailed recording of mating behaviors by placing automated video cameras at each bower. This unparalleled comprehensive video record allows identification of color-banded females who visit individual males' bowers for courtship or copulation as well as identification of color-banded males who destroy or steal from bowers (Borgia 1995). Using microsatellite markers to estimate genetic relatedness between individuals, I perform a detailed analysis of

the effects of relatedness on male competition and female mate choice in a natural population of satin bowerbirds.

Critical to studies of any mating system is an accurate understanding of mating patterns and reproductive success. Estimates of reproductive skew may be far from precise if cryptic copulations are common (Lanctot et al. 1997, Wilmer et al. 1999, Lank et al. 2002) or if multiple mating by females complicates assessments of paternity (Jones et al. 2005, Whittingham and Dunn 2005). In NRB species, observed skews in mating success have led to the common assumption of strong sexual selection in these species (Andersson 1994, Hoglund and Alatalo 1995), but genetic testing is required to confirm that these observed skews reflect actual reproductive skews. In Chapter 1, I conduct a paternity analysis to evaluate the match between observed copulations and genetic paternity in satin bowerbirds. I show that for all 11 chicks sampled from 9 nests, the genetic sires were observed to copulate with the chicks' mothers at bowers. This finding is important on two levels. First, it confirms that off-bower copulations are rare if they occur at all, and that video observation at bowers accurately represents mating behavior in satin bowerbirds. Second, it shows that reproductive success can be reasonably inferred from observed copulations and that estimated rates of multiple mating by females are not affected by cryptic copulations. Thus, previous studies in satin bowerbirds testing hypotheses about the evolution of male display and female mate choice (e.g., Borgia 1985a,b, 1986, 1993; Hunter and Dwyer 1997; Uy et al. 2000; Patricelli et al. 2002; Coleman et al. 2004, Keagy et al. 2009) are further supported by the finding that male mating success translates into reproductive success.

In Chapter 2, I evaluate the role of relatedness in male competition. Satin bowerbirds have an exploded lek mating system (Gilliard 1969) in which male display sites (bowers) are more dispersed than in traditional leks. In several lekking species males establish their display sites near relatives (e.g., Petrie et al. 1999, Hoglund et al. 1999, Shorey et al. 2000, Bouzat and Johnson 2004, Regnaut et al. 2006), raising the question of whether kin selection is involved in the formation of leks (Kokko and Lindstrom 1996). I test the hypothesis that males are less aggressive (in the form of bower destruction) towards relatives than non-relatives (Saether 2002) and that this effect favors the close spatial association of relatives. I show that male satin bowerbirds direct fewer bower destructions towards relatives than equidistant non-relatives, and that relatives are among males' two nearest neighbors more often than expected by chance. I also show that males with more relatives nearby receive fewer bower destructions, indicating that males benefit from aggregating with relatives through reduced aggression. This allows males to maintain more attractive displays for females and demonstrates how kin selection can directly affect sexual selection.

In Chapter 3, I assess the role of relatedness in female mate searching and mate choice. While many authors argue that females should avoid mating with relatives due to the costs of inbreeding depression (see Keller and Waller 2002), two hypotheses predict that females prefer relatives in mate choice: the kin selection model of mate choice (Parker 1979, Smith 1979, Waser et al. 1986, Lehmann and Parrin 2003, Kokko and Ots 2006), and the optimal outbreeding model (Bateson 1983). Recent studies have supported these models, showing that females mate with

relatives more often than expected by chance (e.g., Krokene and Lifjeld 2000, Duarte et al. 2003, Cohen and Dearborn 2004, Ryder et al. 2010). In satin bowerbirds I test both predictions, that females should prefer or avoid relatives in mate choice. I show that females tended to mate with relatives more often than expected by chance in individual years, but that “lifetime” mate choice did not favor relatives. Females did not avoid mating with relatives in any year. I also show, using a uniquely detailed analysis of female mate searching, that females tended to search for mates in areas that contained their relatives’ bowers, but that within these areas, females did not favor relatives for courtship or copulation. I test several more specific aspect of mate choice including the choices to mate with more than one male, to reject top males, and to return to previous mates, and show that relatedness does not influence these decisions by females. The tendency to mate with relatives in some years, therefore, does not appear to be due to an active preference for relatives, nor does it appear to affect female lifetime reproduction. Rather females tend to search in areas of the exploded lek that include relatives, but then choose mates randomly with respect to relatedness within their search areas. These results suggest that there may be a spatial effect that causes females to search near their relatives, but that relatedness does not actively influence mate choice in satin bowerbirds.

Chapter 1: Behavioral paternity predicts genetic paternity in satin bowerbirds (*Ptilonorhynchus violaceus*), a species with a non-resource-based mating system

ABSTRACT

The potential for differences between genetic paternity and paternity inferred from behavioral observation has long been recognized. These differences are associated with the challenge for females seeking both genetic and material benefits; this challenge is less severe in species with polygynous, non-resource-based mating systems (such as leks) than in those with resource-based systems. We present the first study of paternity patterns in a non-resource-based species that does not form true leks. We compared paternity inferred from observed mating behavior to genetically assigned paternity in the Satin Bowerbird (*Ptilonorhynchus violaceus*) using eight microsatellite markers. Mating behavior was observed and recorded via automated video cameras positioned at all bowers (29-34 bowers each year) in the study site throughout each mating season. We obtained blood samples, and identified mothers for 11 chicks in nine nests. For all chicks, the most likely genetic father had been observed to mate with the mother in the year the chick was sampled. All most likely genetic fathers were assigned with high confidence and all were bower-holding males. These results demonstrate that genetic paternity can be inferred from observed mating behavior with reasonable confidence in Satin Bowerbirds. Observed male

mating success is therefore a reliable predictor of reproductive success, and this suggests that high skew in observed male mating success translates directly to high skew in reproductive success.

INTRODUCTION

Paternity assignments of offspring based on behavioral observations and those based on genetics and other methods may sometimes disagree (Bray et al. 1975, Westneat 1987, Morton et al. 1990, Gowaty 1996). Mating systems differ in the extent to which this discrepancy in paternity assignments may exist (e.g. Petrie and Kempenaers 1998). Differences between observed and genetic paternity may be most common when there are conflicts in the kinds of benefits females receive from different males (e.g., between genetic and material benefits). For example, it may profit females to gain material assistance for their offspring through socially monogamous pairings but seek enhanced genetic quality from extra-pair copulations (Borgia 1979, Birkhead and Møller 1992). Consistent with this hypothesis, the level of extra-pair paternity across passerine bird species is inversely related to the observed level of polygyny (Hasslequist and Sherman 2001). This “benefit conflict” hypothesis has received considerable support from paternity studies in mating systems in which males and females co-reside on territories (e.g., Hasselquist et al. 1996, Petrie and Kempenaer 1998, Petrie et al. 1998, Johnsen et al. 2000). Fewer studies have compared behavioral and genetic paternity in species in which males and females do not co-reside on territories.

In non-resource-based (NRB) mating systems, such as lekking systems, males are not involved in parental care and females appear to base mate choice on indicators of genetic benefits. Females are free to choose their mates, and multiple females are able to mate with top males. Females also appear to face less conflict and fewer constraints on mate choice than in resource-based mating systems because females are not competing for access to territories held by preferred males. Consistent with this view, behavioral studies of NRB mating systems typically reveal high skews in male mating success (Borgia 1985a, Alatalo and Lundberg 1986, Wiley 1991, Höglund and Alatalo 1995) and individual females typically mate with only one male (Westneat et al. 1990, Uy et al. 2001; but see Lanctot et al. 1997, Lank et al. 2002). Observed male mating skew in NRB systems has been important in the development of hypotheses regarding how sexual selection has influenced the evolution of NRB mating systems (e.g., Patricelli et al. 2002, Bro-Jorgensen and Durant 2003, Coleman et al. 2004, Ekblom et al. 2005). However, these hypotheses are contingent on the assumption that observed skew in male mating success reflects actual reproductive skew. The intensity of sexual selection may be higher or lower than expected if actual mating patterns differ from those observed (Jones et al. 2001, Whittingham and Dunn 2005). Important differences between observed and actual characteristics of a mating system could be revealed if genetic paternity does not match behaviorally observed paternity (e.g. Lanctot et al. 1997).

Behavioral assignment of paternity in NRB species is complicated by the fact that males have limited contact with females; thus more temporally specific observation is needed to identify mating pairs in NRB species than in species where

males and females co-reside on territories. Fortunately, in many NRB species, males and females mate at specific sites where copulations can be observed. Several factors can affect the accuracy of observational paternity assessment, including incomplete coverage of known breeding sites, unknown breeding sites, or cryptic liaisons away from typical breeding sites (e.g. Wilmer et al. 1999). Although many studies have obtained observations of mating in NRB species (see Höglund and Alatalo 1995), relatively few had effective coverage of most or all mating sites and assessed the accuracy of these observations using genetic techniques (e.g., Alatalo et al. 1996, Semple et al. 2001).

Satin Bowerbirds (*Ptilonorhynchus violaceus*) have been used as a model NRB mating system to test a wide variety of hypotheses about the evolution of male display and female choice (e.g. Borgia 1985a, 1986, 1993; Borgia and Collis 1989; Hunter and Dwyer 1997; Uy et al. 2000, 2001; Patricelli et al. 2002, 2003, 2004; Doucet and Montgomerie 2003; Coleman et al. 2004). Critical to our work on satin bowerbirds has been a video camera system that is activated by passive infrared detectors. The system records all the behavior that occurs at bowers during the mating season (Borgia 1995). We closely monitored the performance of cameras to assure their continuous operation. Each year, between 72% and 85% of females and all male bower-holders in our study population are color-marked for individual identification. Earlier behavioral studies have reported high skew in male mating success (Borgia 1985a), which is consistent with predictions from mating systems theory (Emlen and Oring 1977, Borgia 1979), and male mating success is strongly associated with bower

and display characteristics (Borgia 1985a, 1993; Patricelli et al. 2002, 2003; Coleman et al. 2004).

We are interested in determining how well genetic paternity of Satin Bowerbird chicks matches the observed mating patterns. Seventy-five percent of females mate with only one male and most of the remaining females copulate with two males (Uy et al. 2001), thus complicating behavioral paternity assignments. However, it is possible that other factors may affect our ability to use the observational data to predict genetic paternity. Females may form liaisons or be forced to copulate away from bowers (see Borgia 1995), though this has not been supported by any observations. Although we believe we have located all bowers in the study site each year, it is possible that females mate with males at undetected bowers. Finally, despite our efforts to record all copulations at monitored bowers, it is possible that we have missed some.

Here, we use microsatellite markers to determine the most likely (ML) genetic fathers of nestlings, and compare these to the identities of males observed to mate with the nestlings' mothers at bowers on our video camera system (hereafter "observed mating partners"). By comparing genetic with observed paternity, we test the hypotheses that (1) genetic sires of offspring matched the mothers' observed mating partners, (2) mating occurred only at bowers (e.g. Borgia 1993, 1995b; Uy et al. 2000), and (3) our camera system accurately recorded reproductive behavior in the study population.

METHODS

Study species

Satin Bowerbirds are endemic to the coastal ranges of eastern Australia (Cooper and Forshaw 1977). They have a non-resource-based, exploded lek mating system in which males court females at bowers that the males build on the ground and decorate. Females and juveniles have green and yellow mottled plumage and adult males acquire iridescent blue/black plumage in their seventh year. Females visit and observe displays at several bowers before building their nests, and then return to a subset of bowers to select a mate (Uy et al. 2001). The bower, bower decorations, ectoparasite levels and male behavioral display elements affect female mate choice (Borgia 1985a, Borgia and Collis 1989, Coleman et al. 2004, Loffredo and Borgia 1986, Patricelli et al. 2002, 2003). Females have one nest per season containing one or two eggs, and 46% of nests produce at least one fledgling (Donaghey 1981).

Field methods

The present study focused on a population of Satin Bowerbirds located at Wallaby Creek (Tooloom National Park, 28°28'S, 152°26'E), New South Wales, Australia (see Borgia 1985a) during the mating seasons, 1996-1998. Since 1977, individual birds in this population have been trapped and banded with a unique 3-color band combination repeated on each leg. Blood was collected from wing vein punctures and stored in lysis buffer (Longmire et al. 1997). Blood samples were stored in the field for ≤ 4 months at 0° C and subsequently stored at -20° C.

Following an exhaustive search for bowers on the study site before the mating season, infrared-sensored Hi-8 video cameras were positioned at each bower throughout the mating season to record all courtships and copulations (Borgia 1995). We monitored 29, 34, and 32 bowers in the years 1996, 1997 and 1998, respectively. Cameras were visited twice daily and tapes and batteries were changed as necessary to ensure recording of all visits to bowers. Cameras were framed on bowers so that the plastic leg bands of birds visiting bowers could be identified from videotapes. Tapes were reviewed in the lab, and time and date of all copulations, as well as the identities of males and females involved, were recorded.

Satin Bowerbird nests at Wallaby Creek were located by visually following females in flight. Most nests were found along the creek. Nests were typically >20m high in trees, often on thin, brittle branches, so collection of blood samples of chicks was difficult. A professional arborist was hired to climb trees to gain access to chicks in the nests. Chicks were lowered to the ground in a sack where blood was taken from a wing vein puncture, and then returned to the nest (except one chick that died in hand). Females observed after this procedure continued to care for the nestlings. Mothers were assigned behaviorally to each nest on the basis of the band combination of the female observed tending the nest. The observed mating partner assigned to each chick was defined as the male (or males) observed on video to have copulated with the chick's mother at his bower, as in past observational studies (e.g., Borgia 1985a, Uy et al. 2000, Patricelli et al. 2003, Coleman et al. 2004).

Molecular methods

Genomic DNA was isolated from blood samples by phenol:chloroform extraction (Sambrook et al. 1989). Individuals were genotyped at eight microsatellite loci developed for Satin Bowerbirds (Table 1). Five dinucleotide (SBC40, SBC46, SBC49, SBC188, SBC193) and one hexanucleotide (SBC44) loci were developed following the methods of Glenn et al. (1997, 1998) using the library enrichment protocol of Ostrander et al. (1992). Two other dinucleotide loci (SB11, SB16.2) were developed following the method described by Degan et al. (1999).

We carried out polymerase chain reaction (PCR) amplification in 25 μ l reactions with final concentrations of 1x Promega Buffer, 1.5 mM MgCl₂, 0.8-1.2 mM dNTPs, 0.2 - 0.3 μ M of each primer (one primer was fluorescently labeled), 0.04 units/ μ l Promega Taq polymerase, and 1.6-4 ng/ μ l template DNA. Reactions for SB16.2 also contained 0.8 mg/ml BSA. We carried out the PCR reactions for six loci as follows, on a Perkin Elmer 480 thermocycler (Perkin Elmer, Boston, Massachusetts): initial 4 min denaturation at 94°C, followed by 35 cycles of 45 s each at 95°C, the locus-specific annealing temperature (Table 1), and 72°C, and a final extension at 72°C for 30 min. Microsatellite fragments for these loci were sized on 6% polyacrylamide gels on an ABI 373XL sequencer (Applied Biosystems, Foster City, California) for 6 h. Reactions for SB11 and SB16.2 were carried out as follows, on an MJ Research PTC-225 thermocycler (MJ Research, Waltham, Massachusetts): initial denaturation of 3 min at 94°C, five cycles of 94°C for 30 s, 60°C for 30 s with a 1°C drop each cycle, 72°C for 30 s, then 25 cycles of 30 s each at 93°C, 55°C, and

72°C, and a final extension of 5 min at 72°C. Fragments of SB11 and SB16.2 were sized on an ABI 3100 capillary sequencer.

Fragment profiles for all loci were visualized using GENESCAN, version 3.1 (Applied Biosystems), and genotypes were scored independently by at least two researchers for all individuals. Allele frequencies and deviations from Hardy-Weinberg equilibrium were determined using CERVUS (Marshall et al. 1998). Linkage disequilibrium was assessed using GENEPOP, version 3.1 (Raymond and Rousset 1995). No microsatellite locus deviated significantly from Hardy-Weinberg equilibrium and no loci showed significant linkage disequilibrium, each after Bonferroni corrections for multiple comparisons. Characteristics of individual loci are given in Table 1. The mean expected heterozygosity for all eight loci was 0.752 and the total exclusionary power was 0.982 for the first parent and 0.999 for the second parent.

Parentage analysis

Allele frequencies for the study population were estimated from 171 genotyped birds (92 females, 69 mature males, and 10 juvenile males), not including chicks. We sampled 69 candidate fathers (approximately 90% of all mature blue-plumaged males observed) including 35 of the 36 bower-holders observed during 1996-1998. The other 34 sampled males were blue-plumaged non-bower-holding males that were present at the study site. Including these males in the paternity analysis allowed us to detect potential copulations with non-bower-holding males that might have occurred away from bowers or through marauding visits to bowers while

resident males were courting females. Previous observations suggested that females do not mate with males outside the study site (Uy et al. 2001). We included all 69 males as candidate fathers in the paternity test for each chick because, by maximizing the number of candidate fathers, we increased the possibility that an unexpected, unobserved male may be assigned as the ML genetic father. This was critical for testing the hypothesis that all copulations were observed and for exposing the possibility that the mating system does not operate exactly as observations suggest.

Maternity assignments were confirmed genetically by determining whether mothers and chicks shared an allele at each microsatellite locus. Genetic paternity was determined using the program CERVUS (Marshall et al. 1998). The program determined the maternal alleles for each chick, and then compared the paternal alleles to each candidate male and calculated a log-likelihood ratio (LOD) score for each candidate. The LOD score was calculated as the log-likelihood of a given male being the father compared with a random male. The candidate male with the highest LOD score is assigned as the ML genetic father for the chick and CERVUS reports a confidence level for each assignment.

Confidence in assignments is determined by the statistic delta (Δ), which is the difference between LOD scores of the two most likely candidate fathers. Critical Δ values associated with each confidence level were drawn from a distribution of Δ scores determined by simulation of 1000 offspring genotypes, each of which was compared to a pool of randomized candidate parent genotypes, one being the paternal genotype used to derive the offspring genotype. Confidence levels were defined as the percentage of Δ scores in the simulated distribution above the critical Δ value for

which the ML genetic father was correctly assigned (Marshall et al. 1998). In determining the confidence level (CL) in each assignment, CERVUS allowed user modification of simulation parameters to more accurately simulate the characteristics of the study system. Our simulation parameters were as follows: 1,000 cycles, 77 candidate fathers, 90% of which were genotyped, 99% of loci typed, and a typing error rate of 1%.

Close relatives of the true parent in the candidate pool can reduce the accuracy and confidence in paternity assignments (Marshall et al. 1998). We estimated pairwise relatedness coefficients (Queller and Goodnight 1989) using SPAGeDi (Hardy and Vekemans 2002) and found that, on average, each adult male in our sample has approximately two adult male relatives at the full-sibling level in our sample (because of the variance in relatedness coefficient estimates, we considered pairs with relatedness coefficient estimates >0.37 as being related at the full-sibling level; S. M. Reynolds, G. Borgia, M. J. Braun unpubl. data). Therefore, we included two full siblings of the correct candidate father in the simulation candidate pool.

To compare observed and genetic paternity, we considered each chick to be a unique data point, even in the case of nest-mates, because of the possibility of multiple paternity. To estimate the likely proportion of copulations that are unobserved (e.g., off-bower, at an undetected bower, or undetected at a monitored bower) we calculated the probability of obtaining our results by chance assuming various proportions of unobserved copulations using the following equation: probability of our results = $(1-x)^m * x^n$, where x is the assumed proportion of all copulations that are unobserved, m is the number of chicks for which we observed

their genetic parents copulating, and n is the number of chicks for whom we did not observe their genetic parents copulating.

RESULTS

We identified mothers and their observed mating partners for 11 chicks from 9 nests in the three-year sampling period (Table 2). Two of the nests belonged to the same mother (RLL) in consecutive years. Two nests contained two chicks and all other nests held one chick. Six mothers shared an allele at each locus with all of the chicks in their nests. One mother (GOR) was not genotyped (but see below) and another mother (REY) mismatched her single nestling at one locus. For seven chicks (six nests), a single male was observed copulating with the mother on video, and for three chicks (two nests) there were two observed copulation partners (Table 2).

For one chick (NTP), the mother was not unambiguously observed mating on video. Thus, we could not identify specific observed mating partners. This chick was sampled in 1996, the last year that lower-resolution VHS video cameras were used. It is possible that this mother (WRL) was observed mating on video but the complete band sequence could not be discerned. In 1996, copulations were recorded with four different males in which the female visitor's leg bands were partially discernable and were consistent with WRL; for example, the first band was white (W), or the last band was light blue (L), but we could not discern the other band colors. Because we could not conclusively discern which of these copulations involved WRL, we included all four of these males as observed mating partners for this chick.

For every chick, the ML genetic father was a bower-holding male and was among the observed mating partners of the mother (Table 2). For the seven chicks whose mothers had one observed mating partner, that male was assigned as the ML genetic father in each case. For the three chicks whose mothers had two observed mating partners, the ML genetic father was one of those two males. For the chick (NTP) whose mother's band combination (WRL) could not be unambiguously resolved on video, the ML genetic father was among the four possible males based on video observation. There was a second male with a matching genotype for chick NTP, but he had a lower LOD score, was not among the observed mating partners of the mother, and may be related to the ML genetic father (relatedness coefficient estimate, $r=0.30$). Four ML genetic fathers were assigned with 99% CL, one with 98% CL, four with 96% CL, one with 92% CL, and one with 85% CL. For one chick (NTH), the mother was not genotyped, but her single observed mating partner was still assigned as the ML genetic father with 98% CL. For the two nests that each contained two chicks, the same male was assigned as the ML genetic father to both chicks in the nest. Among all 11 offspring-mother-father trios, there was a 1.1% mismatch rate (2 out of 176 offspring alleles), which is consistent with the expected possibility of mutation, null alleles, or typing error (Marshall et al. 1998).

Our results show no evidence for unobserved copulations. However, because only 11 chicks were sampled, we cannot rule out the possibility that unobserved copulations occur in this population. To estimate our ability to detect unobserved copulations, we calculated the probability of obtaining our results by chance, assuming a range of values for the possible proportion of unobserved copulations.

Figure 1 shows, for example, that if 6% of copulations were unobserved, there was a 50% chance of observing the parents copulating for all 11 chicks.

DISCUSSION

The present study compared paternity assignments based on genetic data with paternity inferred from behavioral observation for 11 Satin Bowerbird chicks and found that all genetic assignments of paternity were consistent with the observational data. No females had chicks with unobserved mating partners. All of the ML genetic fathers were bower-holding males, and all had been observed mating with the mothers. The high exclusionary power of the microsatellite loci and the high confidence in the genetic paternity assignments indicate that the genetic fathers of the chicks have been identified. It is unlikely that one of the few unsampled adult males in the population would also match these chicks genetically. In the one case where two males had genotypes consistent with fatherhood for one chick, the male with the highest LOD score was an observed mating partner of the mother, whereas the other male appeared to be related to that male at the half-sib level ($r=0.30$). These results support the hypothesis that genetic paternity can be inferred with reasonable confidence from behavioral observation of matings at bowers in Satin Bowerbirds.

Studies of other polygynous species that have employed genetic techniques to assign paternity (e.g., Gibbs et al. 1990, Wilmer et al. 1999) have often found that behaviorally observed mating success (i.e., the number of different females observed to mate with each male) differs significantly from actual mating success. This discrepancy may be attributable to differences between the assumed patterns of

mating behavior and the actual, perhaps cryptic, behavior of the organisms. We directed our observational efforts only at bowers, leaving open the possibility of unobserved off-bower copulations. The fact that the genetic paternity of every chick in our sample was consistent with the video data supports the hypothesis that all or most copulations occur at bowers and that our video data provides an accurate record of copulations that occur at bowers. Given the limitations of small sample size, we cannot rule out the possibility of some level of unobserved copulations. However, the perfect match obtained between observed copulations and genetic paternity indicates that the vast majority of copulations were observed (see Fig. 1).

A discrepancy between observed and actual mating success in polygynous populations may lead to an error in estimates of male mating skew (Lanctot et al. 1997). An accurate characterization of mating skew is important for understanding the evolution of male display behaviors and female mate choice strategies (Whittingham and Dunn 2005). Intensive monitoring of all known breeding sites is a critical first step toward this end. For example, the discrepancy between observed mating skew and genetically determined reproductive skew in lekking male Buff-breasted Sandpipers (*Tryngites subruficollis*) may have been attributable to females mating at known, but less intensively monitored, solitary display sites or neighboring leks (Lanctot et al. 1997). Here, we report that observed mating behavior is a good predictor of genetic paternity in a Satin Bowerbird population when all or most relevant mating sites are monitored. This supports conclusions drawn from earlier intensive observational studies that continuously recorded behaviors at all known bowers and suggested an extreme mating skew in Satin Bowerbirds (e.g., Borgia

1985a, Uy et al. 2001). (The sample of observed mating partners we discuss here does not directly reflect this skew because of its small size and because it is biased toward males that mate often.) Additionally, the perfect match between observed and genetic paternity in the present study suggests that automated observation methods similar to our video camera system may allow for more complete monitoring of mating behavior than traditional human observations in species with predictable copulation sites.

The only differences between observed matings and genetic paternity in our analysis occurred when females mated with more than one male in a season. Two of the 10 observed mating partners did not sire offspring with particular females because those females mated with multiple males. With a larger sample, Uy et al. (2001) observed that 25% of females mate with more than one male, and our genetic results provide no evidence to refute this figure. Our results show that for females observed copulating with multiple males, the sires were among the observed mating partners, and not some other unobserved males. Additionally, females observed to copulate with only one male were not found genetically to have mated cryptically with additional males. Because most females mate singly, and few copulations were unobserved in this population, observed male mating success is a reasonably good predictor of male reproductive success (assuming hatching and fledging successes are equal among sires). Because Satin Bowerbird clutches often contain only one egg (two at most), multiple paternity would have minimal impact on overall estimates of male reproductive skew. However, the consequences of multiple mating by females may be substantial for individual males because they would sire either all or none of

the female's offspring. Given that multiple mating by females is not rare, observational estimates of male reproductive success can be improved by weighting each female's contribution to a male's reproductive success by the total number of males with whom females mated.

In NRB species, there is no conflict for females between genetic and material benefits they receive from their mates. Therefore, frequent multiple mating by females is not expected in NRB species because they are relatively unconstrained from mating with males of the highest genetic quality. As a result, a skew in male mating success is expected to arise because some males will be generally preferred by females. The match between observed and genetic paternity in this Satin Bowerbird population supports these predictions for NRB species in two ways. First, our results confirm the high mating skew reported for this model NRB species by showing that few, if any, cryptic copulations occur which might reduce this skew. Second, our paternity test confirms the observation that most females copulate with only one male. The occurrence of multiple mating by some Satin Bowerbird females is unexpected by this "benefit conflict" hypothesis, but it is not inexplicable. For example, females-- particularly inexperienced females-- may copulate with multiple males if they make mistakes in their assessments of potential mates.

In conclusion, we found that paternity inferred from behavioral observation at bowers closely matches genetic paternity in Satin Bowerbirds. This result is consistent with the hypothesis that most or all copulations in this species occur on bowers. The result also shows that video observation at bowers (Borgia 1985a, Uy et

al. 2000, Patricelli et al. 2003, Coleman et al. 2004) provides an accurate record of mating activity in the population.

Table 1. Primer sequences, number of alleles, annealing temperature (T_A) and expected (H_E) and observed (H_O) heterozygosities for Satin Bowerbird microsatellite loci.

Locus	Primer Sequences ^a	Alleles	$T_A(^{\circ}C)$	H_E	H_O
SB11	AGCCATGTCCTTGTTTTTCATCC* GAATACCTGAGCAAAGTATAA	7	60-55 ^b	0.783	0.800
SB16.2	CTTTTCAGGTCCTGTCATGGCT* GTCGTTTCGCACAGATTTCTTAG	16	60-50 ^b	0.894	0.870
SBC40	ACGGGGCAAATCAGAAGAAGTAG CCGTTGGGAGCTGATGATGTC*	7	49	0.625	0.623
SBC44	ACACGTGAGAGACAATGTGTA* ATCACTGGAAGAAATGTCTGT	12	58	0.749	0.760
SBC46	CATTCCTGCTGAGTGACTG* CATAAAGCCTCACTTCAGACT	5	58	0.690	0.710
SBC49	GGGGTGTCTGCGATTTCT TGGATGTTGGTACGCAGTGTAAG*	8	49	0.743	0.765
SBC188	CAGGGAGGATGGGAACAG* TCAGGATACCATGGGGAG	5	58	0.697	0.641
SBC193	ACTCCGCTGTTCGTTTGC GAGGTTATTTGGGGGCTG*	11	50	0.840	0.860

^a fluorescently labeled primers indicated by an asterisk (*).

^b step-down PCR (see text for details).

Table 2. Paternity assignment. For each chick, the most likely (ML) genetic father (bold and underlined) was among the observed mating partners of the chick's mother. Log-likelihood ratio (LOD) scores, delta scores (Δ), and confidence levels (CL) for the most likely genetic fathers were determined using CERVUS from a sample of 69 candidate males.

Chick	Year	Mother	Observed mating partners	LOD	Δ	CL	Number of perfectly matched males ^a	Number of allelic mis-matches ^b
NTG	1996	GLE	<u>WWE</u>	5.53	2.43	96%	0	1
NTP ^c	1996	WRL	OEK, OWW, <u>RKW</u> , YLW	6.95	1.09	92%	2	0
NTH	1997	GOR ^d	<u>OMR</u>	4.93	2.61	98%	1	0
NTK	1997	REY	<u>WGR</u>	6.31	2.55	96%	1	1
NTL	1997	RLL	KKK, <u>OEK</u>	4.49	0.46	85%	1	0
NTM	1997	RLL	KKK, <u>OEK</u>	5.79	4.91	99%	1	0
NTO	1997	RWG	<u>WGR</u>	5.07	3.76	96%	1	0
NTE	1998	EWV	<u>KKK</u> , WWE	7.22	5.36	99%	1	0
NTI	1998	KWW	<u>KRD</u>	7.66	6.78	99%	1	0
NTJ	1998	KWW	<u>KRD</u>	5.89	2.53	96%	1	0
NTN	1998	RLL	<u>OEK</u>	8.44	6.91	99%	1	0

^a Number of candidate males (including the ML genetic father) who had zero mismatching loci with the chick.

^b Number of allelic mismatches between the chick, mother, and ML genetic father.

^c See text for details on observed mating partner assignment for this chick.

^d This mother was not genotyped.

FIGURE CAPTION

Figure 1. Probability of observing the parents copulating for 11 out of 11 chicks by chance, assuming a range of theoretical proportions of all copulations that are unobserved. The probability was calculated as $(1-x)^{11}$ where x is the theoretical proportion of copulations that were unobserved. The calculation shows that we had a 50% chance of obtaining our result if 6% of copulations were unobserved. Similarly, we had only a 5% chance of obtaining our result if 24% of copulations were unobserved.

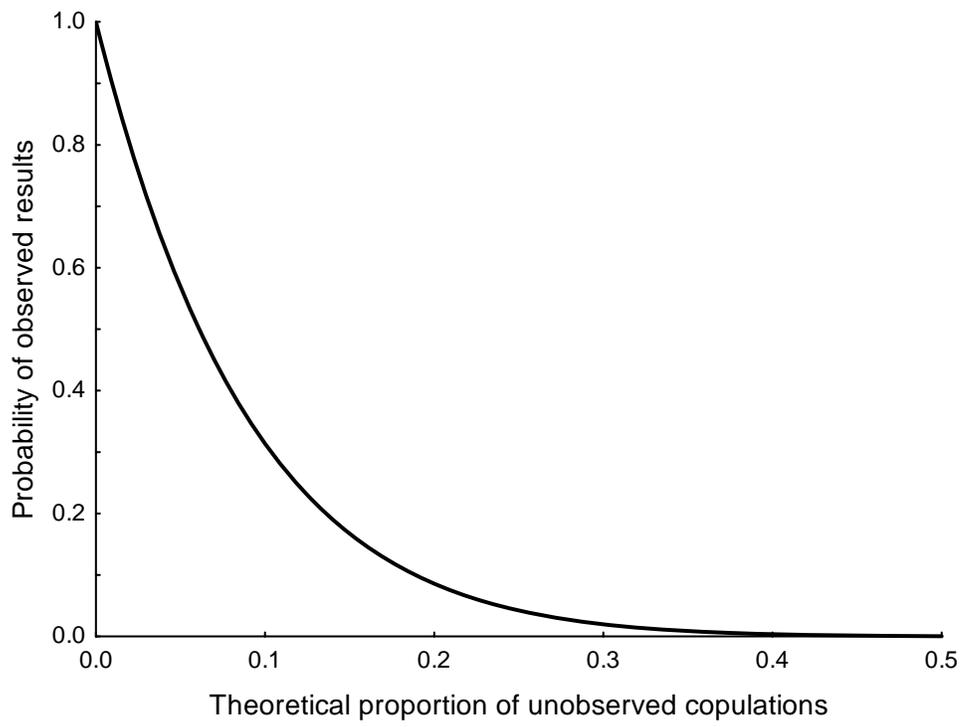


Figure 1

Chapter 2: Lekking satin bowerbird males aggregate with relatives to mitigate aggression

ABSTRACT

Males in several lekking species aggregate with their relatives to display for females, suggesting that kin selection can affect sexual selection. Several hypotheses have been proposed to explain this behavior, but no general explanation has emerged. In most species with lek mating systems, neighboring males have intense aggressive interactions that can affect the quality of their sexual displays. Here we test the hypothesis that the presence of related neighbors mitigates the negative consequences of this aggression. Male bowerbirds build stick display structures (bowers) that are used by females in mate assessment and are commonly destroyed by males' two nearest neighbors. We show that kin aggregate as first or second nearest neighbors, and males direct fewer bower destructions towards kin than equidistant non-kin. Males with more relatives nearby receive fewer bower destructions. These results suggest that the restraining effect of relatedness on aggression favors the close spatial association of related males' display sites. An alternative hypothesis, that related males aggregate to gain copulations from females attracted to successful relatives was not supported.

INTRODUCTION

Non-resource-based (NRB) mating systems, including leks, occur in approximately 6% of bird species (Gill 1995) but are of special interest because they offer the opportunity to study mate choice where material contributions by males are not involved in mate choice (Borgia 1979, Bradbury 1981). Several recent studies have suggested a role for relatedness in affecting the spatial organization of males at breeding leks (e.g. Petrie et al. 1999, Shorey et al. 2000, Regnaut et al. 2006). These studies are noteworthy because, unlike social species where individuals remain in their family group their whole life (e.g. Emlen and Wrege 1988, Komdeur 1994), males in NRB species do not co-occupy territories with their relatives after fledging. Thus, spatial associations of close kin on display arenas are not likely to be by-products of lifelong social associations (but see Krakauer 2005); rather, they appear to result from an active process of locating kin and choosing to display near them. Because males at neighboring display sites frequently interact (Höglund and Alatalo 1995) relatedness effects on these interactions may have important consequences for males' ability to compete with each other or to attract mates.

Several hypotheses have been proposed to explain how relatedness may affect male display site location (e.g., McDonald and Potts 1994, Kokko and Lindstrom 1996, Saether 2002), but most have not been rigorously tested. Krakauer (2005) showed that in cooperatively displaying wild turkeys, display partners are close relatives, and subordinate partners benefit from cooperation through inclusive fitness by helping their relatives mate. However, this explanation has limited application because males in most NRB species do not engage in highly cooperative displays nor

do they form social groups with kin across their lifetime. Also, two other studies of cooperatively displaying species did not find that display partners were related (McDonald and Potts 1994, Loiselle et al. 2006).

In species without cooperative display, it is unclear how males might benefit from preferentially positioning themselves near relatives. However, spatial associations of relatives have now been documented in several such species (peacocks, Petrie et al. 1999; black grouse, Höglund et al. 1999; white bearded manakins, Shorey et al. 2000; lesser prairie chickens, Bouzat and Jonson 2004; and capercaillies, Regnaut et al. 2006) but not in others (e.g. sage grouse, Gibson et al. 2005; white-crowned and blue-crowned manakins, Loiselle et al. 2006; spotted bowerbirds, Madden et al. 2004; and great bustards, Martin et al. 2002). Kokko and Lindstrom (1996) proposed that these associations should occur when females prefer larger leks because, when new males have little potential to mate themselves, they should join leks where a relative is the top male, thereby making that relative more attractive and accruing inclusive fitness benefits for themselves. This hypothesis may explain associations of relatives when they occur at the level of one lek versus another, but it is difficult to differentiate this model from philopatry (Höglund et al. 1999), and it does not explain associations among display sites within leks (see Shorey et al. 2000).

A second hypothesis is that males may be less aggressive towards relatives than other nearby males (Hamilton 1964), and spatial associations among relatives can result if males attack related neighbors less often than unrelated ones (Saether 2002). Males at display arenas are competing with each other, so reduced aggression

is predicted only if individuals have the opportunity to discriminate in favor of kin at the expense of non-kin (Griffin and West 2002). Therefore, reduced aggression due to kinship depends on the presence of individuals of differing relatedness (including both close kin and non-kin) at sites likely to attract aggression. Because aggressive interactions are often important in affecting the quality of sexual display and the location of male's display sites in NRB species (Höglund and Alatalo 1995, Westcott 1997), this hypothesis has potential to be widely applicable across NRB species.

NRB species differ in the degree of aggregation of display sites from tightly clustered leks to widely dispersed display sites (Höglund and Alatalo 1995). All species in which spatial associations among relatives have been observed have traditional lek mating systems with highly aggregated display sites. In at least one species with widely dispersed display sites, the spotted bowerbird, relatives were not found to associate (Madden et al. 2004). Differences in dispersion may explain some of the observed variation in the tendency of relatives to cluster because greater dispersion may decrease the level of interaction among males, reducing the opportunity for social interactions favoring kin at display sites.

Here we study the effects of relatedness on display site location and aggression in satin bowerbirds (*Ptilonorhynchus violaceus*), an NRB species with an exploded lek mating system (Gilliard 1969) in which male display sites are not tightly aggregated but in which males are known to commonly destroy the bowers of males at adjacent display sites (Marshall 1954, Vellenga 1970, Borgia 1985b, Hunter and Dwyer 1997). We test the hypotheses that 1) related males aggregate, 2) there is

reduced aggression (bower destruction) among these closely associated relatives, and 3) males benefit from associating with their relatives.

Satin bowerbirds are a valuable model for studying male aggressive interactions because of the importance of aggression to male mating success and our ability to reliably monitor these behaviors. Adult males build bowers on display sites located at least 100 m apart, and neighboring males interact by destroying each others' bowers. Bower destructions are common and have been shown to lower bower quality (Borgia 1985b), which is an important influence in female mate choice (Borgia 1985a). Bower destructions are inversely correlated with female return rates for additional courtships, and two experimental studies have shown that destructions reduce male mating success (G. Borgia, unpublished data). Individual females visit on average 2.64 (\pm 0.18 s.d.) adjacent bowers (Uy et al. 2001), and the vast majority of destructions are directed at males' two nearest neighbors (Borgia 1985b). Thus, bower destructions reduce the display quality and attractiveness of neighboring males, who are each other's main sexual competitors (Borgia 1985b, Pruett-Jones & Pruett-Jones 1994). Selection may favor the aggregation of relatives in this species if related neighbors destroy each others' bowers less often, allowing them to maintain more attractive displays.

METHODS

Field methods

We continuously monitored 32 contiguously distributed adult male bowers throughout the mating season of 1997 (November 9 to December 20) at Tooloom

National Park, New South Wales, Australia (28°28'S, 152°26'E). Birds were trapped and banded with unique color leg band combinations (Borgia 1995). Blood samples were taken from wing vein punctures and stored in DNA extraction buffer. Behaviors at bowers were recorded using Hi8 video cameras controlled by infrared sensors, positioned at each bower on the study site, and birds were identified on video by their leg bands (Borgia 1995). Inter-bower distances were calculated from GPS coordinates that were taken in 2001.

Relatedness estimation and classification

DNA was extracted from blood samples (Reynolds et al. 2007) and genotyped at 16 microsatellite loci; 14 loci were developed for satin bowerbirds (Reynolds et al. 2007, Bardeleben et al. 2005), 1 for manakins (Shorey et al. 2000) and 1 for indigobirds (Sefc et al. 2001) (Table 3). Genotypes at eight loci were analyzed as described previously (Reynolds et al. 2007); newer loci were analyzed using an ABI3130 capillary sequencer (Applied Biosystems, Foster City, CA) and Genemapper software (Applied Biosystems). Population allele frequencies were estimated from a total of 248 birds sampled within the study area. Using GENEPOP 3.1 (Raymond and Rousset 1995), all loci were found to be in Hardy-Weinberg equilibrium and unlinked after Bonferroni corrections. Relatedness coefficients (r) were estimated using SPAGeDi 1.2 (Hardy and Vekemans 2002) following the method of Queller and Goodnight (1989).

We expected only close relatives (half-siblings or closer [see Petrie et al. 1999, Komdeur 1994]) to modulate their destruction behavior because inclusive

fitness benefits decrease rapidly with more distant relationship (Hamilton 1964). Therefore, we used a cutoff value of r to identify pairs of males whose genetic similarity was consistent with that of close relatives. To pick an appropriate cutoff value of r , we simulated genotypes for 1000 pairs each of four relationship types (parent-offspring, full-sibling, half-sibling, and unrelated) using the observed allele frequencies and evaluated the distributions of r estimates for each pair type (Figure 2). All relationship types had r distributions centered on their theoretical values (0 for unrelated pairs, 0.25 for half-siblings, and 0.5 for full-sibling and parent-offspring pairs), and all types had equal variances except for parent-offspring pairs. Our simulations showed that a cutoff value of 0.13, the point of intersection between the distributions of unrelated and half-sibling pairs, simultaneously minimizes both Type I error- the proportion of unrelated pairs misclassified as related- and Type II error- the proportion of related pairs misclassified as unrelated (Blouin et al. 1996). So we defined close relatives as pairs having $r \geq 0.13$; otherwise pairs were considered unrelated.

Data analyses

All statistical analyses were performed in Statistica 6.0 (Statsoft Inc., Tulsa, OK) except for the permutation test of bower locations that was written in SAS 9.1 (SAS Institute, Cary, NC). To compare destructions directed toward related versus unrelated neighbors, we included every male in the study site that 1) had his nearest close relative within 600 m of his own bower, and 2) had a nonrelative the same distance away as the nearest close relative (± 35 m). If two nonrelatives satisfied

criterion 2, then we chose the one whose distance from the focal male was most similar to that of the close relative. We limited neighbor distances from the focal male to 600 m because 95% of all destructions performed by focal males occurred within 600 m of their bowers. Among males who destroyed bowers of their second nearest neighbors, the numbers of destructions directed at first and second nearest neighbors did not differ (2-tailed paired t test: $t_{14} = 0.19$, $p = 0.86$) and the mean difference in distance from the focal male between these two neighbors was 68 m, suggesting that a distance difference of 35 m or less should have no effect on the expected number of destructions. We used bower destructions as an indicator of male aggression because they could be most reliably scored from videotapes. Males also steal bower decorations (Marshall 1954, Vellenga 1970, Hunter and Dwyer 1997), and a previous study tracking movement of marked bower decorations showed that bower destructions and decoration stealing are highly correlated and commonly occur on the same visits by males to victims' bowers (Borgia and Gore 1986). All tests for which we had *a priori* directional predictions were 1-tailed (Gibbons and Pratt 1975).

To test for spatial association of relatives, we ranked each male's neighbors based on distance. Neighbor ranks are not reciprocal, therefore each male's set of ranked nearest neighbors was considered independently. We then permuted male assignments to the existing bower locations and recalculated the number of close relatives among focal males' two nearest neighbors. We compared the observed number of relatives with the null distribution generated from 1000 permutations.

We used Gamma statistics to assess the relationship between the number of destructions given or received and the number of close relatives nearby because

destructions were not normally distributed, the relationships are not necessarily linear, and Gamma tests are the most appropriate when there are many ties in the variable rankings (Siegel and Castellan 1988). We excluded two males from these analyses and from Figure 3; one because the number of destructions he received is not comparable to other males because he only retained his bower for a small fraction of the mating season. The other excluded male was an outlier who established his bower at a new site in 1996 and received four times the number of destructions (71) received by the second most destroyed male in 1997. Ninety-four percent of these destructions were received from his two nearest neighbors, who were the closest set of neighbors to any of the eight males who established new bowers the previous year. The excluded male abandoned his bower site after this onslaught of destructions at the end of the 1997 season. Given the tendency of males not to tolerate neighbors that are closer than a couple hundred meters to their bowers (see Borgia 1985b), we felt that his choice of bower location drove the high observed rate of destruction and justified his exclusion from our analysis.

RESULTS

We evaluated relatedness among displaying male satin bowerbirds to understand the effect of kinship on bower destruction and display site location. The mean nearest neighbor distance was $310 \text{ m} \pm 141$ (s.d.) and, because these distances were not normally distributed, the median nearest neighbor distance was 255 m (range: 135 to 750 m). Individual bowers were destroyed on average 7.22 ± 13 (s.d.) times during the mating season and 77% of bower destructions were directed at

males' two nearest neighbors (Figure 3). The mean relatedness among all males was -0.005 ± 0.13 (s.d.) which was not different from zero ($n = 496$, $t = -0.86$, $P = 0.39$). Of the 496 pairwise relationships among the 32 bower-holding males, 73 (15%) pairs were closely related ($r \geq 0.13$) and there was a mean of 4.6 ± 2.9 (s.d.) close relatives per focal male. Given the high skew in male mating success of this species (Borgia 1985a, Reynolds et al. 2007), this low level of relatedness among resident males in the population suggests that natal dispersal is sufficient to prevent a high concentration of relatives within populations. This low proportion of relatives among competing males leads us to predict that competition among relatives is not likely to erase the benefits of kin selection (West et al. 2002), and that males should be less aggressive toward (give fewer bower destructions to) relatives than non-relatives.

To test this prediction, we compared the number of destructions executed by focal males on closely related versus unrelated neighbors' bowers. To control for the effect of distance on bower destruction (Borgia 1985b) (Figure 3), we limited this comparison to those males who had both a closely related and an unrelated neighbor within 600 m and at equivalent distances (± 35 m). Focal males destroyed the bowers of their closely related neighbors significantly less than those of their unrelated neighbors (Wilcoxon matched pairs test: $T = 4.00$, $n = 8$, $P = 0.045$; Figure 4). The modal number of destructions given to relatives was zero, suggesting that males commonly avoid destroying relatives' bowers. In one exceptional case, the focal male gave five destructions to his related neighbor, but these males had the lowest r among related pairs in this analysis ($r = 0.142$), and because they were very close to the cutoff of 0.13, they were the most likely pair among those classed as relatives to

be misclassified (see Figure 2). Excluding this pair from our analysis showed an even stronger effect of relatedness on bower destruction (Wilcoxon matched pairs test: $T = 0.00$, $n = 7$, $P = 0.014$). This result supports our prediction that males are less aggressive towards their relatives when we control for interbower distance. Consistent with this result was a significant negative correlation in which males with more relatives nearby (among their two nearest neighbors) performed fewer total destructions (Gamma correlation: $Z = -1.73$, $n = 30$, $P = 0.041$).

If males aggregate with relatives because of this reduced aggression, then close relatives should be found disproportionately among males most likely to give destructions. Because the vast majority of bower destructions are directed toward focal males' first and second nearest neighbors (Figure 3), we made the *a priori* prediction that close relatives would be overrepresented among males' two nearest neighbors. The mean relatedness among first and second nearest neighbors was not significantly different from zero (mean \pm s.d. = 0.007 ± 0.14 , $t_{63} = 0.05$, $P = 0.48$). Because the proportion of relatives in the population was low, we assessed whether these relatives were randomly distributed among display sites. We compared the number of close relatives observed among the two nearest neighbors of each male to a null model in which males were randomly assigned to the existing display sites. We found that close relatives were among focal males' two nearest neighbors significantly more often than expected by chance (1000 permutations, $n = 64$ neighbors, $P = 0.001$). The observed number of close relatives among focal males' two nearest neighbors (18) was nearly twice the expected number (9.5). The finding that close relatives disproportionately occupy nearby bowers, where destructions are

most likely to originate, suggests that relatives associate to mitigate the effects of these bower destructions.

If males benefit from displaying near their close relatives, then males with more close relatives nearby should receive fewer total bower destructions than males with fewer close relatives nearby. As predicted, we found a significant negative correlation between the total number of destructions males receive and the number of close relatives among their two nearest neighbors (Gamma correlation: $Z = -2.22$, $n = 30$, $P = 0.013$; Figure 5).

DISCUSSION

These results show that relatedness is important in modulating aggressive interactions among aggregated male satin bowerbirds. Males directed fewer bower destructions toward related than equidistant unrelated neighbors, and relatives were overrepresented among males' two nearest neighbors, the positions from which bower destructions were most likely to originate. Furthermore, males with more relatives nearby received fewer destructions overall, indicating that aggregating with relatives benefits males by allowing them to maintain more attractive displays.

Bower destruction can have important evolutionary consequences since this behavior directly affects males' ability to attract mates (Borgia 1985a,b). Males may benefit from destroying their neighbors' bowers in two ways. First, the destroyer's own display becomes relatively more attractive than the victim's, causing females to prefer the destroying male rather than the victim (Borgia 1985b, Pruett-Jones and Pruett-Jones 1994). Second, if the destroyer's relatives are nearby, then females

deflected from destroyed bowers may mate with these relatives, providing the destroyer an inclusive fitness benefit. Males should avoid targeting their related neighbors for destruction unless there is a high probability that the deflected females will mate with the destroyer, because such destructions will lower the inclusive fitness benefits from that relative. These benefits to destroyers also suggest that nearby relatives of the destroyer may benefit from bower destruction behavior that is modulated by relatedness, through reduced destruction of their own bowers and increased mating opportunities. Together, these effects appear to favor the spatial association of relatives.

Another study has tested for a spatial association of relatives in a bowerbird species, the spotted bowerbird, and did not find aggregation of relatives (Madden et al. 2004). Spotted bowerbirds have much lower levels of bower destruction, one tenth that observed in satin bowerbirds (Borgia and Mueller 1992, Madden et al. 2004), which may be due to their much more widely dispersed display sites (>1 km apart on average). With very low levels of destruction, spotted bowerbirds would gain little from reduced destructions by having relatives as nearest neighbors and thus we would not predict aggregation of relatives in this species.

An alternative explanation for why kin associate is that males gain more mating opportunities from being near their highly successful relatives (Saether 2002). This could occur if a highly successful male has more receptive female visitors than he can accommodate, such that females spill over to the related neighbors who resemble the preferred male. This hypothesis predicts that clustering of kin should be focused around highly successful males. In our study, the distribution of relatives

near (within the two nearest neighbors) the top three males (54, 25, 22 copulations, respectively, 47% of all copulations) did not differ from the distribution of relatives near all other males ($\chi^2_1 = 0.02$, $P = 0.90$), and this result did not change when we considered the top six males (13, 12, 11 copulations, respectively for the fourth through sixth males, 64% of all copulations; $\chi^2_1 = 0.01$, $P = 0.92$), nor when we considered the four nearest neighbors of each top male (top three males: $\chi^2_1 = 0.23$, $P = 0.63$; top six males: $\chi^2_1 = 0.12$, $P = 0.73$). These results do not support the alternative hypothesis that the observed spatial association of relatives is due to a tendency to associate with highly successful relatives.

In this study we assessed the spatial association of related males by identifying closely related pairs using a cutoff r value, and then determining whether these relatives were near neighbors rather than randomly positioned among display sites. Our approach differs from the more often used approach of comparing the mean relatedness of neighboring males to the mean relatedness of the general population. Our approach is more sensitive to the association of relatives when the proportion of close relatives in the population is not high, as may often be true if there is not a high level of philopatry. When we assessed mean relatedness of nearest neighbors, our results were similar to several other studies of species with NRB mating systems (McDonald and Potts 1994, Martin et al. 2002, Madden et al. 2004, Gibson et al. 2005, Loiselle et al. 2006, DuVal 2007) that did not find a significant difference from the population mean. However, mean relatedness fails to capture an overrepresentation of relatives among nearest neighbors when the proportion of relatives in the population is low (15% in this study), because the large number of

unrelated pairs contributes to a lower mean relatedness. In the present study, a high proportion of first and second nearest neighbors ($46 / 64 = 71\%$) were unrelated resulting in a low mean relatedness, obscuring the critical finding that relatives were significantly overrepresented in this group.

How satin bowerbirds recognize their kin is not understood, but it may be achieved through Major Histocompatibility Complex (MHC)-based self-referent phenotype matching (Manning et al. 1992). Males apply a paint to their bower walls, composed of saliva and masticated plant material, which both males and females appear to taste when they visit a bower (Bravery et al. 2006). It is possible that MHC signals can be detected from the saliva in this paint to allow kin recognition.

We have shown that male satin bowerbirds benefit from displaying near relatives through reduced bower destruction by these relatives. As predicted, relatives were overrepresented among males' two nearest neighbors, the positions from which the preponderance of bower destructions originated. This supports the hypothesis that relatives aggregate to benefit from nepotistic restraint on bower destruction and suggests an important way in which kin selection influences the operation of sexual selection in NRB species. This effect of kinship on male aggressive behavior is striking because there is no evidence that satin bowerbirds form associations with kin until they become established on display sites, unlike cooperatively breeding species where individuals maintain lifetime associations with kin (Stacey and Bock 1978). Although bower destruction is unique to bowerbirds, other kinds of aggressive interactions that affect male display quality and mating success, such as threat, physical fights, or courtship interruptions, are common among

neighboring males in other species with aggregated display sites (Höglund and Alatalo 1995, Westcott 1997). Our results suggest that spatial associations of relatives in these species may also be driven by the mitigating effects of relatedness on aggression.

Table 3: Characteristics of microsatellite loci.

Locus	No. of alleles	Allele size range	H_e^a	H_o^a	Amplification Protocol^b
SB11	7	230-244	0.782	0.797	A
SB16.2	17	227-263	0.891	0.923	A
SBC40	7	183-195	0.631	0.641	A
SBC44	12	126-212	0.743	0.743	A
SBC46	5	183-197	0.683	0.706	A
SBC49	8	154-172	0.744	0.794	A
SBC188	5	180-188	0.698	0.655	A
SBC193	11	202-222	0.839	0.851	A
Ind38	9	155-193	0.597	0.602	B
Man7	2	159-163	0.189	0.188	C
AAGG-129	33	191-268	0.942	0.962	D
AAGG-130	16	234-316	0.847	0.735	D
AAGG-167	27	105-224	0.912	0.927	D
AAGG-187	37	105-468	0.941	0.95	D
AAGG-197	13	161-209	0.879	0.904	D
AAGG-209	11	96-138	0.863	0.877	D

^a Expected (H_e) and observed (H_o) heterozygosities calculated in Genepop (Raymond and Rousset 1995).

^b Amplification protocols were as follows: A) as reported in Reynolds et al. (2007).

B) Microsatellite amplification was carried out on an MJ Research PTC-225 (MJ

Research, Waltham, MA) thermocycler in a final reaction volume of 25 μ l containing 1x buffer solution, 2 mM $MgCl_2$, 0.8 mg/ml BSA, 0.2 mM each dNTP, 0.2 μ M each primer (Sefc et al. 2001), 0.05 units/ μ l Taq polymerase, and 0.8 ng/ μ l template DNA. Thermocycling profile consisted of 94° C for 4 min, 35 cycles of 94° C for 20 s, 65° C for 20 s, 72° C for 30 s, and a final extension time at 72° C for 10 min. C) Microsatellite amplification was carried out on an MJ Research PTC-225 (MJ Research) thermocycler in a final reaction volume of 25 μ l containing 1x buffer solution, 1.5 mM $MgCl_2$, 0.25 mM each dNTP, 0.25 μ M each primer (Shorey et al. 2000), 0.04 units/ μ l Taq polymerase, and 1.6 ng/ μ l template DNA. Thermocycling profile consisted of 94° C for 3 min, 35 cycles of 94° C for 30 s, 50° C for 30 s, 72° C for 30 s, and a final extension time at 72° C for 7 min. D) Microsatellite amplification was carried out on a Parallax 350 (Brooks Automation, Chelmsford, MA) thermocycler in a final reaction volume of 4 μ l containing 1x buffer solution, 1.5 mM $MgCl_2$, 0.4 mM each dNTP, 0.4 μ M each primer (Bardeleben et al. 2005), 0.025 units/ μ l Taq polymerase, and 0.75 ng/ μ l template DNA. Thermocycling profile consisted of 94° C for 10 s, 28 cycles of 94° C for 0 s, 52° C for 0 s, 72° C for 15 s, and a final extension time at 72° C for 60 s.

FIGURE CAPTIONS

Figure 2: Distributions of relatedness coefficient (r) estimates for 1000 simulated pairs of each of four relationship types: unrelated (solid line), half-siblings (dashed line), full-siblings (hatched line), parent-offspring (dotted line). Pairs with $r \geq 0.13$ (vertical line) were considered close relatives and pairs with $r < 0.13$ were considered unrelated.

Figure 3: Destructions given to ranked neighbors. Mean (\pm s.e.m.) number of bower destructions directed by focal males toward neighbors ranked by distance. No destructions were given beyond the 12th nearest neighbor of a focal male.

Figure 4: Destructions given by focal males to equidistant neighbors. Closely related neighbors are shown with hatched bars and unrelated neighbors are shown with filled bars. Focal males are ordered by the relatedness coefficient (r) between themselves and their related neighbor (leftmost focal male had the highest r to his relative and rightmost focal male had the lowest r to his relative).

Figure 5: Relationship between total bower destructions received by males and the number of close relatives among their two nearest neighbors.

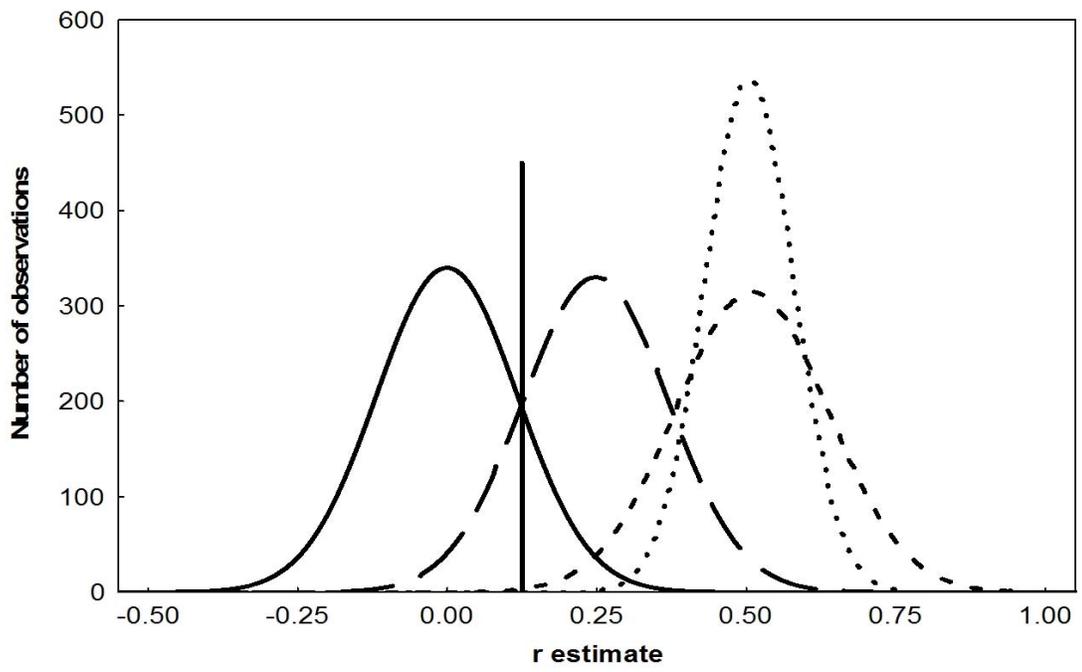


Figure 2

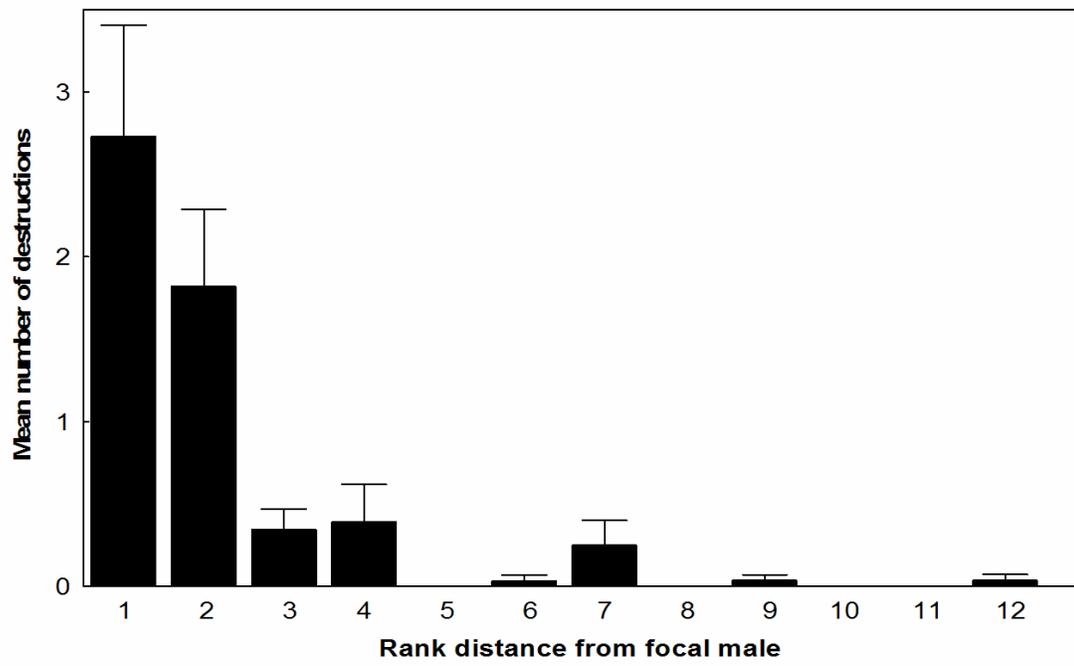


Figure 3

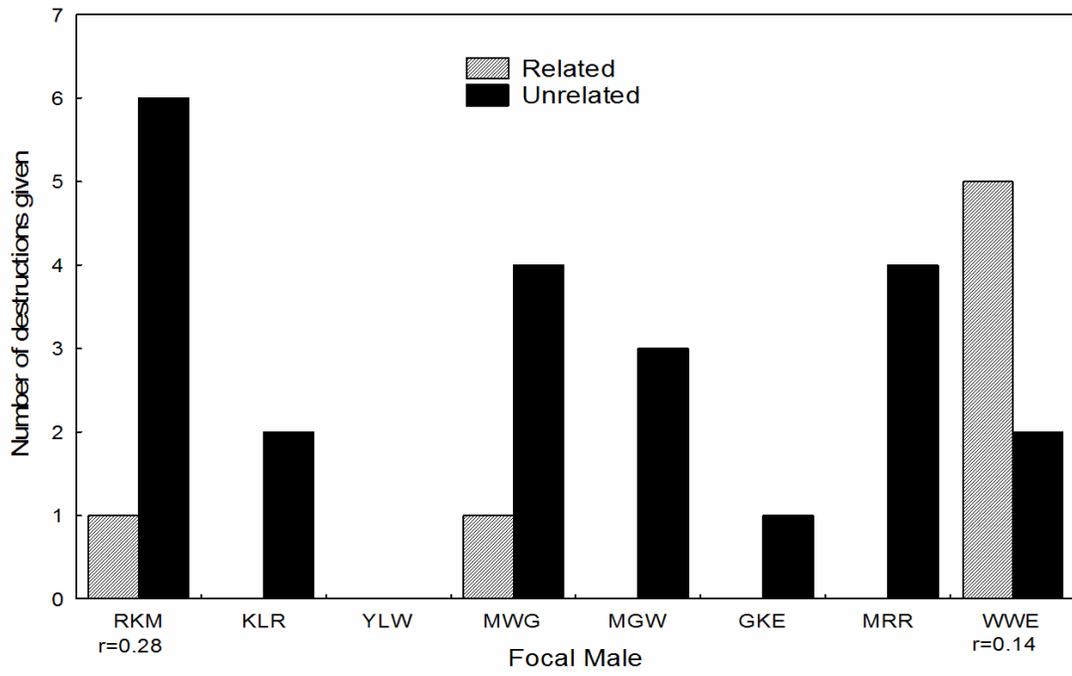


Figure 4

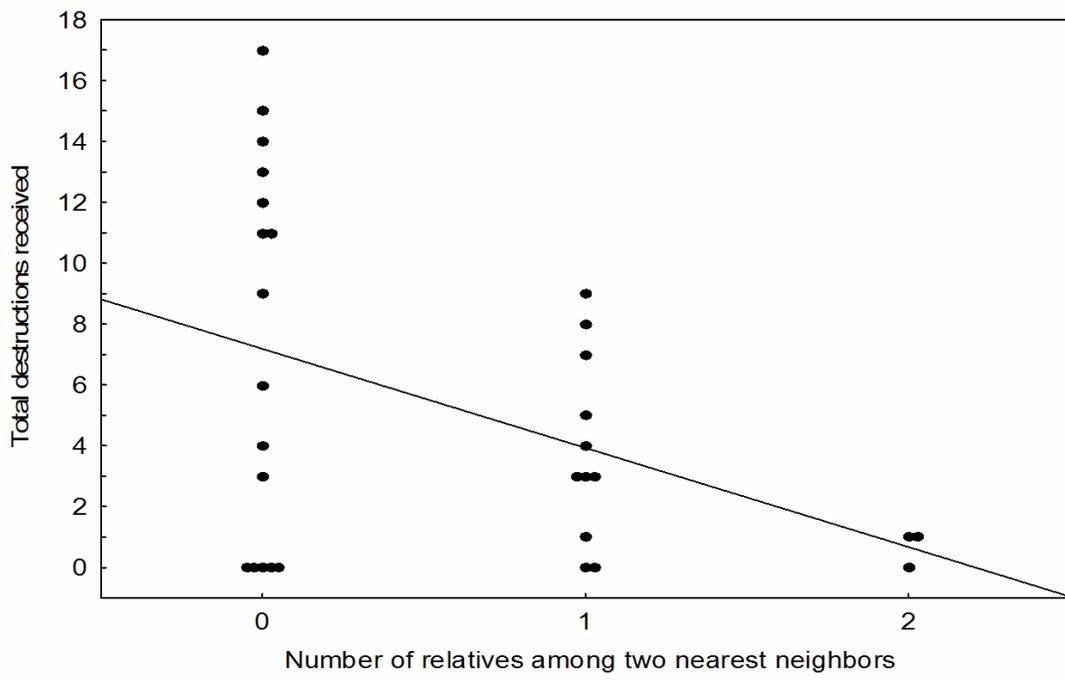


Figure 5

Chapter 3: Relatedness and mate choice in satin bowerbirds: is there a preference for relatives?

ABSTRACT

In typically outbreeding species, relatedness may be a key influence on mate choice because females can avoid a reduction in offspring fitness by choosing unrelated sires. However, two models of mate choice suggest that it may be adaptive to mate with relatives under some conditions, and examples are accumulating in which females commonly mate with relatives. We tested the hypotheses that females actively preferred or avoided relatives in mate choice in satin bowerbirds, a species with a non-resource based mating system in which females choose mates without regard to material resources provided by males. Females have a complex mate choice process in which they search among multiple males' bowers in limited areas, then visit a subset of those males for courtship, and then typically visit one male for copulation. Our results suggest that, within individual years, females copulate with relatives ($r \geq 0.13$) more often than expected by chance, although "lifetime" mate choice, with respect to the six year study, is not affected by relatedness. Females appeared to search for mates preferentially in areas that included their relatives' bowers in two years and in a combined analysis of annual results, but chose males for courtship and copulation randomly with respect to relatedness within these areas. This suggests that matings with relatives are a consequence of this spatial effect of relatedness on mate searching rather than an active preference for relatives.

Relatedness did not influence additional aspects of female choice such as mating with more than one male, rejecting top males, or returning to previous mates. There was no evidence for inbreeding avoidance through mate choice. Our results suggest that relatedness does not play an active role in mate choice, but that a tendency for females to search in the areas of their relatives' bowers results in a tendency to mate with relatives in some years.

INTRODUCTION

The factors that affect female mate choice are of major interest in sexual selection (Andersson 1994, Jennions and Petrie 1997). Relatedness to potential mates may play a key role in mate choice because, for most outbreeding species, females can circumvent the reduction in offspring fitness associated with inbreeding depression by avoiding mating with relatives (Charlesworth and Charlesworth 1987, Pusey and Wolf 1996, Keller and Waller 2002). Conversely, other models suggest that it may be adaptive to actively choose relatives over non-relatives in mate choice. The optimal outbreeding model (Bateson 1983) argues that breeding with individuals that are too distantly related can be detrimental to fitness due to the break up of co-adapted gene complexes (Tregenza and Wedell 2000), and predicts preferences for mates of intermediate relatedness. The kin selection model (Parker 1979, Smith 1979) proposes that, under certain conditions, females increase their inclusive fitness by mating with relatives and that the costs of inbreeding depression must be fairly high to override these benefits (Parker 1979, Smith 1979, Waser et al. 1986, Lehmann and Perrin 2003, Kokko and Ots 2006). The predictions of these models oppose those

from the inbreeding avoidance hypothesis and suggest that relatedness may play a complex role in mate choice.

Preferences for relatives in mate choice have now been reported in several species. In mate choice tests siblings were preferred over non-relatives in cichlid fish (Thunken et al. 2007), and cestodes (Schorring and Jager 2007), and cousins were preferred over both siblings and non-relatives in Japanese quail (Bateson 1982), mice (Barnard and Fitzsimons 1988), and zebra finches (Burley et al 1990). Observations of natural mate choice found that relatedness between females and their mates was higher than expected by chance in pikas (Peacock and Smith 1997), blue tits (Krokene and Lifjeld 2000), greater white-toothed shrews (Duarte et al. 2003), great frigate birds (Cohen and Dearborn 2004), tree swallows (Shutler et al. 2004), and wire-tailed manakins (Ryder et al. 2010). Kleven et al. (2005) showed that female barn swallows were more related to their extra-pair mates than to their social mates or to random males, and Barber et al. (2005) found that female tree swallows paired with more genetically similar mates had lower proportions of extra-pair young in their nests. Additionally, human females have shown preferences for males with similar major histo-compatibility complex (MHC) alleles to themselves via odor and facial cues (Jacob et al. 2002, Roberts et al. 2005; but see Wedekind et al. 1995).

While the hypothesis that females actively prefer relatives in mate choice can explain observations of elevated relatedness among mates, detailed information on the individual decisions faced by mate searching females is needed to differentiate this from alternative hypotheses. For example, tendencies to mate with relatives more often than expected may result from limited natal dispersal which increases the

encounter rates of relatives in mate choice (Jaimeson et al. 2009). In this case, mate choice may be random with respect to relatedness, but because available mates are biased towards relatives, females mate with relatives at a high rate (Bohank 1999, Duarte et al. 2003, Francisco et al. 2007). Information on the relatedness of females to their potential mates can help determine whether females actively reject non-relatives in favor of relatives. Also, knowledge of the consistency of female mate choice in favor of relatives, both within and between mating seasons, can show whether tendencies to mate with relatives are due to active preferences or if they are spurious events. To date, no study has examined the role of relatedness in mate choice in this level of detail.

Satin bowerbirds (*Ptilonorhynchus violaceus*) provide a unique opportunity to study the role of relatedness in mate choice in great detail. Satin bowerbird courtship and copulation occur at bowers on the ground which can be monitored with automated video cameras. This allows for an unparalleled comprehensive record of mate searching and mate choice (Borgia 1995), including identification of specific males that are sampled and rejected by females. Satin bowerbirds have a non-resource-based mating system in which females receive nothing from mates except sperm and there is no paternal care. Males are thus able to copulate with many females and females are free to choose mates without regard to material benefits from males (Borgia 1979). Satin bowerbird mating system and life history suggest that there are many opportunities to mate with relatives. Females are reproductive in their first or second year while males retain their bowers 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 are searching. Also, high skewness in male mating success (Borgia 1985a) and small clutches (Donaghey 1981) 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 multi-step mate searching process (Uy et al. 2001) which allows us to assess the effect of relatedness at different stages of mate searching. Females search among adjacent bowers in limited areas of the larger display arena (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 preference.

The inbreeding avoidance hypothesis predicts that females should mate with relatives less often than expected by chance and may explain additional aspects of female mating behavior such as multiple mating (Stockley et al. 1993, Bensch et al. 1994, Petrie and Kempenaers 1998, Kempenaers 2007). Theoretically female satin bowerbirds are expected to copulate with only one male because they are unconstrained from choosing their preferred male (Borgia 1979). However eighteen percent of females copulate with more than one male (G.B. unpublished results) and it is unclear why this occurs. Females who mate with relatives may experience reduced fertilization success (Gage et al. 2006) that would cause them to mate again. In some socially monogamous species, females are more likely to seek extra-pair

copulations when paired with a relative (e.g., Freeman-Gallant et al. 2006, but see Kleven et al. 2005). Additionally, inbreeding avoidance may explain why some females reject highly attractive males. The top three males at our study site typically mate with 50% of females, but some females receive courtship from and then reject these males. Furthermore, females who mate with relatives may be less likely to return to those mates in subsequent years if they experience inbreeding depression through reduced hatching success or fledging success (e.g., Bensch et al. 1994, Kempenaers et al. 1998, Van de Castele et al. 2003).

To assess the role of relatedness in satin bowerbird mate choice, we tested both the active preference and inbreeding avoidance hypotheses. First we assessed whether females copulated with relatives more or less often than expected by chance. We tested this in each of six years and across female “lifetimes,” with respect to the six year study. We also determined at which stage of mate searching females’ choices were biased by relatedness. We tested the additional predictions that females who mated with multiple males were more related to their first mates than females who mated with one male, and that multiply mating females were more related to their first mates than to their second mates. Also, we tested the hypothesis that females rejected top males because they were related to them. Lastly, we tested whether females who mated with relatives returned to those mates the following year more or less often than females who mated with non-relatives.

METHODS

Field methods

We continuously monitored male displays and female mate searching throughout the mating seasons (November-December) from 1997 to 2002 at Tooloom National Park, New South Wales, Australia (28°28'S, 152°26'E). 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). 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 sixteen 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. Additionally, we identified possible parent-offspring (PO) pairs by the patterns of allele sharing between individuals. Parent-offspring 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 one 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.

Data analyses

We conducted chi-square tests in each year to determine if the proportion of copulating pairs that were related was significantly different from the proportion of all pairs present that year that were related. Females who mated with more than one male in a year were assigned their last mate as their observed mate that year to control for pseudo-replication of females. To summarize the results from multiple years, we used a Fisher's combined probability (FCP) test. We also assessed female "lifetime" mate choice, with respect to the six year study, by testing whether females mated with relatives more or less than expected across all years of the study, using a Monte Carlo randomization test (Manly 1997). We summed across all females the number of relatives that they mated with throughout the six year study period. This test included all mates of females who mated multiply in any year. We then randomized female mate choice within years, from among the bower-holders present each year, and recalculated the test statistic. The result of this test was qualitatively the same whether we preserved the observed skew in male mating success in the randomization or not and we report only the latter.

We used Monte Carlo randomization tests (Manly 1997) to determine at what stages of mate searching relatedness played a role. Mate searching was divided into three 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' choice of search area, 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 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 "owned" any search area 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 "owned" which 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 females tended to receive courtship from relatives, 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 one relative and at least one non-relative, and who did not receive courtship from every male in their search area. We counted the number of relatives across all females that were chosen for courtship as our test statistic. In the third stage of mate searching, females typically choose one male for copulation from among the males that courted them.

The samples for this test included all females who mated after receiving courtship from at least one relative and at least one non-relative, and who did not mate with every male that courted her. Similar to the previous analysis, we randomized which of the courting males females chose for copulation and we counted the number of relatives across all females that were chosen for copulation as our test statistic.

To assess the effect of relatedness on multiple mating by females, we identified all females who were observed to copulate with more than one 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. Among multiply mating females, we also used sign tests to compare the relatedness of females to their first mates with relatedness to their second mates. For rejection of top males, we tested whether females who copulated with top 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. The top three males were identified by ranking all bower-holding males by the total number of copulations they received that year. We also tested whether the tendency to mate with relatives is influenced by male quality. For each year, we compared the mate ranks of females who mated with relatives with females who did not mate with relatives using Mann-Whitney U tests. Lastly, we tested whether females who were related to their mates were more or less likely to return to those mates the next 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.

All statistical analyses were performed in Statistica 6.0 (Statsoft Inc., Tulsa, OK), except for the logistic regression which was conducted using SAS 9.1 (SAS Institute, Cary, NC) and the randomization tests which were written as Excel macros (Microsoft).

Tests of each of the hypotheses described above (except lifetime mate choice) were conducted multiple times with data from multiple years, and we were interested in deriving a summary result from the annual results. We conducted Fisher's combined probability (FCP) tests (Fisher 1954) to assess the overall result for each hypothesis (Sokal and Rohlf 1995). This is an imperfect approach because we violate the assumption of FCP test that all tests of a hypothesis are independent- we test hypotheses in multiple years for the same population of bowerbirds, with many individuals repeated across years. However, females are free to make different mate choices in different years. We do not correct for multiple testing across the different hypotheses because all hypotheses were planned *a priori*.

RESULTS

We genotyped 119 females and 48 males (5,712 total pairs) that were observed at bowers from 1997 to 2002. The annual sample sizes, numbers of copulating pairs and relatedness distributions are given in Table 4. On average 15.7% of all male-female pairs and 22.2% of copulating pairs were related at or above the

half-sibling level ($r \geq 0.13$) each year. There were 15 male-female PO pairs (0.26%) observed (mean $r = 0.48 \pm 0.09$ s.d.) among all individuals. The year in which there was the highest potential for PO pairs to mate was 1999, when both the male and female were present for 8 PO pairs. Across all years, we observed only one PO pair for which the male was in the female's search area. She received courtship from but did not mate with him.

In 5 of 6 years, the proportion of copulating pairs that were related was numerically higher than the proportion of all possible pairs that were related (Table 4), indicating a tendency for females to copulate with relatives. In the only year in which the proportion of copulating pairs that were related was lower than the proportion of all pairs that were related, 1997, the proportions differed by only one percent. Chi-square tests were conducted for each year, using only females' last mates to control for pseudoreplication of females in cases where individual females mated with more than one male. These tests returned p-values ≤ 0.05 in two years, 1998 and 2001 (1998: $n = 74$, $\chi^2 = 3.84$, $P = 0.050$; 2001: $n = 42$, $\chi^2 = 8.40$, $P = 0.004$; Table 5, Figure 6), when 24% and 29%, respectively, of copulating pairs were related, compared to 15% and 16%, respectively, of all pairs. In 1998 and 2001, 25% and 30%, respectively, of females mated with relatives (including all mates of females who mated multiply). Combining results from each year, we found that in annual analyses, females mated with relatives more often than expected by chance (FCP test: $\chi^2 = 23.1$, $df=12$, $P = 0.027$, Table 5). However, our analysis of "lifetime" mate choice, that is, all males females mated with across the six year study, showed that

females did not mate with relatives more or less often than expected by chance (randomization test: 1000 permutations, $n = 108$ females, $P = 0.536$).

Given that on an annual basis, females mated with relatives more often than expected, and did not strongly avoid mating with relatives in any year, we investigated when this bias in favor of relatives occurred during mate searching. We used one-tailed tests because we had a directional prediction based on the findings that females preferred relatives. Randomization tests of the prediction that females' search areas included more relatives than expected by chance returned p -values ≤ 0.05 in two years, 2001 and 2002 (2001: $n = 67$, $P = 0.034$; 2002: $n = 51$, $P = 0.050$; Table 5). Combining results across years, females' search areas included relatives more often than expected (FCP test: $\chi^2 = 25.8$, $df=12$, $P = 0.012$, Table 5). The relatives within females' search areas were mostly related at the half sibling level (mean r among these relatives was 0.19 ± 0.07 s.d.) and they included only one possible father across all years. Females did not choose relatives for courtship from among the males in their search areas more often than expected (FCP test: $\chi^2 = 13.7$, $df=12$, $P = 0.321$, Table 5), although in one year, 1998, there was a marginal trend in that direction ($n = 29$, $P = 0.055$, Table 5). Females did not choose relatives for copulation from among the males that courted them more often than expected (FCP test: $\chi^2 = 16.2$, $df=12$, $P = 0.181$, Table 5), though it should be noted that there were small sample sizes for this test in some years which may have reduced our power to detect a preference for relatives.

In addition to overall mate choice and mate searching, we assessed whether other aspects of female choice were influenced by relatedness including multiple

mating, rejection of top males, and return rates to previous mates. Relatedness to females' first mates did not explain why some females chose to mate with additional males (FCP test: $\chi^2 = 10.6$, $df=12$, $P = 0.561$, Table 5). Females who mated with two males were not more or less related to their first mate than to their second mate (FCP test: $\chi^2 = 11.1$, $df=12$, $P = 0.522$, Table 5). Relatedness did not affect whether females who were courted by the top three males mated with them or rejected them (FCP test: $\chi^2 = 8.43$, $df=12$, $P = 0.751$, Table 5), nor whether females whose search areas included top males chose to receive courtship from them or not (FCP test: $\chi^2 = 13.1$, $df=12$, $P = 0.364$, Table 5). Females who mated with relatives did not tend to mate with more successful males (FCP test: $\chi^2 = 16.5$, $df=12$, $P = 0.167$, Table 5). Lastly, relatedness to her mate did not predict whether a female returned to the same male the following year (FCP test: $\chi^2 = 4.65$, $df=10$, $P = 0.913$, Table 5).

DISCUSSION

We investigated how relatedness influences mate choice in a natural population of satin bowerbirds. We found that rather than avoiding relatives as mates females tended to mate with relatives more often than expected by chance (Table 4), and this difference was significant in two years and in a combined analysis of annual results (Table 5). Since females often return to the same mate year after year (Uy et al. 2000) we investigated whether the similar results in 1998 and 2001 could have been driven by the same females choosing the same relatives. Of the 74 females who mated in 1998 (Table 5), only 33 of them mated in 2001 and only 4 mated with the same relative consistently between those years. Since these four females represented

only 24% and 29% of the females mating with relatives in 1998 and 2001 respectively, it suggests that the 2001 result was not driven largely by the same females as the 1998 result. Additionally, the intervening two years in which females did not prefer relatives suggest that significant changes occurred in the identities of females and their mates between 1998 and 2001 to render results from those years reasonably independent.

In our analysis of “lifetime” mate choice, with respect to the six year study, females did not tend to mate with relatives. This result may appear contradictory to the result from the FCP analysis of annual mate choice patterns, but the two analyses test different things. The annual tests looked at the mating patterns within single years, and the FCP test represents a summary result of annual mate choice patterns. The FCP test indicated that females tended to prefer relatives as mates within single years. The “lifetime” analysis included each female only once and incorporated all mate choices made by individual females across the six year study. The “lifetime” analysis shows that even though females favored related mates in individual years, this effect did not carry through across the six year study. Individual females can mate with different males in different years, and the accumulation of mates across six years appears to even out the effects of relatedness on mate choice in individual years. Thus the annual trends in mate choice likely do not affect female lifetime reproductive success. Furthermore, we draw only tentative conclusions from the FCP analyses we report here because the data from individual years are not independent of each other, with many individuals repeated across years. Nonetheless, the annual results are suggestive of an effect of relatedness on mate choice, so we further

investigated if this bias toward relatives occurred at particular stages of mate searching.

The bias in favor of relatives occurred early in mate searching, at the stage when females established their search areas. In two years, 2001 and 2002, and in the combined analysis across years, females' search areas included relatives significantly more often than expected by chance (Table 5). Females did not choose relatives for courtship from among the males in their search areas more often than expected, although in 1998 there was a marginally significant trend (Table 5). Females did not tend to copulate with relatives from among the males who courted them, although the power of this analysis may have been limited by small sample sizes, especially in 2001 and 2002 ($n = 9$ and 8 , respectively). These results suggest that females may have preferentially searched in areas populated by relatives, but then chose randomly with respect to relatedness within those areas. Since related males have bowers near each other (Reynolds et al. 2009), females who search in the area of one relative may search among other relatives as well. Thus the tendency to mate with relatives in 1998 and 2001 may have resulted from this bias in mate searching rather than from an active preference for relatives.

The absence of an active preference for relatives is further suggested by the fact that relatedness had no other effects on mate choice. Females did not tend to choose relatives for copulation from among the males that courted them. Relatedness did not explain females' tendencies to mate multiply, to reject top males, or to return to previous mates. Furthermore, if females actively prefer relatives, then this pattern should be consistent across females and across years. In the two of six years in which

females tended to mate with relatives, a minority of females ($\leq 30\%$) mated with relatives. Also, across female “lifetimes,” females did not mate with relatives more often than expected.

Our analyses also indicate that females did not practice inbreeding avoidance through mate choice discrimination. Females did not mate with relatives less often than expected in any year. More specifically, the inbreeding avoidance hypothesis did not explain why some females mate with multiple males, reject top males, or switch mates from one year to the next. The costs of inbreeding depression in satin bowerbirds are unknown, and may not be sufficient to drive the evolution of mate choice discrimination. Additionally, these costs are usually highest among first-order relatives and we observed few of these pairs. This suggests that mating with first-order relatives may be avoided through dispersal (Pusey and Wolf 1996), while mating with second-order relatives is not avoided. Dispersal rates in satin bowerbirds are unknown, but at least some dispersal occurs (Nicholls et al. 2004) and it is not strongly sex-biased (S.M.R. in prep). It is also possible that females cannot recognize kin to discriminate against them, but this seems unlikely because males appear to have this ability (Reynolds et al. 2009).

The finding that females’ search areas tend to overlap with their relatives’ bowers may indicate that females are philopatric and search near their natal territory. However, this would suggest that fathers of females were in their search areas, because females are reproductive in their first or second year and males retain their bowers and relative mating success for multiple years. Of the 15 PO pairs we observed, only six were possible father-daughter pairs because the female was banded

in a year after the male's first bower. Thus we identified possible fathers of only five percent of the females in our study. If most females were philopatric, where were their fathers? It is possible that our sample of genotyped females was biased toward daughters of former bower-holders who died or lost their bowers before our study began in 1997. Fifty-nine percent of the females genotyped for this study were banded prior to 1996 (and 16% prior to 1987) so it may be that the fathers of many of these females were not still holding bowers in 1997. If females search among bowers near their natal site, and males attain bowers near their relatives (Reynolds et al. 2009) including their fathers, then females would tend to search among their half-siblings. Or females may search within their natal population but away from their natal site to reduce the chance of mating with their fathers when they are present. This idea is consistent with the observation that of the six females for whom we identified possible fathers only one included the possible father's bower in her search area, and that was in only one of five years of mate searching. Also, we genetically identified a single group of individuals which appeared to be a family, consisting of a father, mother, two sons and one daughter, all of which were full-siblings. 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 five years) included bowers of three half-siblings' but not her father or full-sibling brothers (Figure 7).

Alternatively, females may not be philopatric and disperse to the same populations as their male relatives (see Matthysen et al. 2005). This would result in females searching for mates in the same population as their male half-siblings, but

their parents would reside in a different population. If this scenario is correct, then it remains to be explained why females search preferentially among their relatives' bowers in their non-natal population. One possibility is that females may be drawn to the same areas as their relatives if they prefer the same microhabitats (Petrie et al. 1999).

Across species, the role of active preferences for relatives in natural mate choice is not well supported. Of seven studies that reported significant tendencies of females to mate with relatives in natural populations, three determined that a spatial association of relatives followed by random mating explained their findings (Peacock and Smith 1997, Duarte et al. 2003, this study), and two acknowledged the possibility of a spatial effect due to philopatry (Krokene and Lifjeld 2000, and Ryder et al. 2010). Only two studies ruled out a spatial effect and favored active preference to explain their findings (Cohen and Dearborn 2004, Kleven et al. 2005). Knowledge of how relatedness affects female choice at multiple stages of mate searching, and whether preferences for relatives are consistent throughout mate searching, can help assess the nature and consistency of these preferences. The current study was the only one to assess mating preferences for relatives at this level of detail. In species for which information on female mate searching is unavailable, it would be informative to assess mate choice in multiple years, in addition to pooling data across years, to see whether females' tendencies to mate with relatives are consistent across years which would support the active choice hypothesis. Furthermore, while experimental choice tests suggest a role for active preference (Bateson 1982, Barnard and Fitzsimons

1988, Burley et al 1990, Thunken et al. 2007, Schorring and Jager 2007), how these laboratory results relate to natural mate choice is unknown.

In summary, we found that females mated with relatives ($r \geq 0.13$) more often than expected by chance in two of six years, and in a combined analysis of annual mate choice patterns. However, “lifetime” mate choice was not affected by relatedness, presumably because individual females can mate with different males each year. We also found that females preferentially searched for mates in areas that included their relatives’ bowers in two years, and in a combined analysis across years, but we found no indication that females favored relatives at later stages of mate searching. Relatedness did not appear to influence other aspects of mate choice including the choice to mate with more than one male, to reject top males, or to return to previous mates. These results suggest that the tendency to mate with relatives in individual years may result from a spatial effect of relatedness on mate searching rather than an active preference for relatives. Additionally, we found no evidence that females discriminated against relatives in mate choice as predicted by inbreeding avoidance models, further suggesting that mate choice is not actively influenced by relatedness.

Table 4. Sample sizes and numbers of related pairs in each year. This table includes all mates of multiply mating females.

Year	No. genotyped birds (No. copulating birds)		No. unique pairs	No. copulating pairs	No. (%) related		No. possible PO pairs
	females	males			all pairs	copulating 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

Table 5: P-values (sample sizes) for each mate choice analysis. Significant results are in bold. Observed versus expected values for overall mate choice and mate searching tests are given below the p-values. All tests are two-tailed except for the three mate searching tests which were one-tailed. (TEBC = than expected by chance)

Hypothesis	Test	1997	1998	1999	2000	2001	2002	FCP^a
<i>Overall mate choice:</i>								
Females mate with relatives more or less often TEBC	χ^2 test ^b	0.280 (63) 6 / 9	0.050 (74) 17 / 11	1.00 (51) 9 / 9	0.624 (30) 6 / 5	0.004 (42) 14 / 7	0.271 (23) 6 / 4	0.027
<i>Mate searching:</i>								
Females' search areas include more relatives TEBC	Rand. ^c	0.828 (86) 38 / 43	0.107 (84) 45 / 38	0.072 (72) 41 / 33	0.235 (67) 64 / 59	0.034 (67) 27 / 19	0.050 (51) 28 / 21	0.012
Females choose relatives for courtship more often TEBC	Rand. ^d	0.39 (21) 27 / 26	0.055 (29) 41 / 37	0.249 (24) 34 / 32	0.903 (26) 26 / 30	0.59 (11) 21 / 21	0.374 (14) 17 / 16	0.321
Females choose relatives for copulation more often TEBC	Rand. ^e	0.609 (12) 11 / 11	0.264 (27) 22 / 20	0.339 (18) 12 / 11	0.163 (17) 8 / 6	0.156 (9) 14 / 12	0.217 (8) 7 / 5	0.181
<i>Multiple mating:</i>								
Multiply mating females are more or less related to their first mates than singly mating females	χ^2 test	0.548 (64)	0.450 (75)	0.445 (52)	0.460 (30)	0.131 (42)	0.746 (22)	0.561
Females are more or less related to first mates than second mates	Sign test	0.383 (21)	0.803 (16)	0.752 (10)	0.371 (5)	0.074 (5)	0.617 (4)	0.522

Table 5 continued

Hypothesis	Test	1997	1998	1999	2000	2001	2002	FCP^a
<i>Top male rejection:</i>								
Females who reject top males for copulation are related to them more or less often TEBC	χ^2 test	0.682 (60)	0.721 (35)	0.550 (44)	0.231 (34)	0.807 (24)	0.293 (25)	0.751
Females who reject top males for courtship are related to them more or less often TEBC	χ^2 test	0.377 (65)	0.302 (38)	0.143 (53)	0.360 (61)	0.623 (27)	0.399 (32)	0.364
Females who mate with relatives mate with males of higher/lower quality TEBC	Mann-Whitney U test	0.181 (63)	0.178 (75)	0.94 (51)	0.105 (31)	0.115 (46)	0.697 (23)	0.167
<i>Return to previous mates:</i>								
Females who mate with relatives are more/less likely to return to those mates the following year	Logistic regression	0.726 (48)	0.672 (53)	0.238 (20)	0.956 (19)	0.881 (18)	na	0.913

^a FCP = Fisher's combined probability test, combining the results from each year. The p-values are given.

^b Observed and expected values represent the number of females that mated with relatives. For the χ^2 test, expected values were calculated from the observed proportions of all pairs that were related (see Table 4).

^c Observed 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.

^d Observed 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.

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

FIGURE CAPTIONS

Figure 6. Relatedness coefficient (r) distributions for mated pairs and all pairs in 2001.

Figure 7. Mate searching areas and locations of relatives of one female (KRE). 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 black arrows indicate the bower locations of her first-order relatives (left arrow: father's bower 1996-1998 then brother's bower in 1999; right arrow: brother's bower in 1999). The white arrows indicate the bower locations of her second-order relatives. The numbers represent the year in which she mated at the indicated bower.

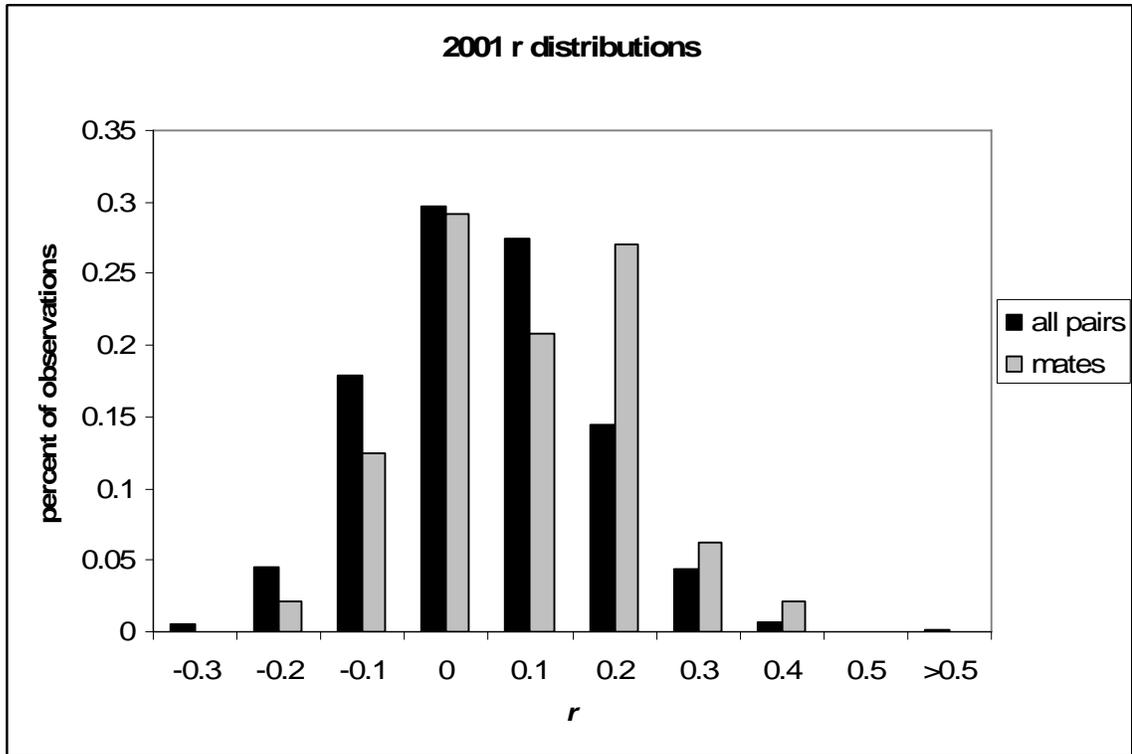


Figure 6

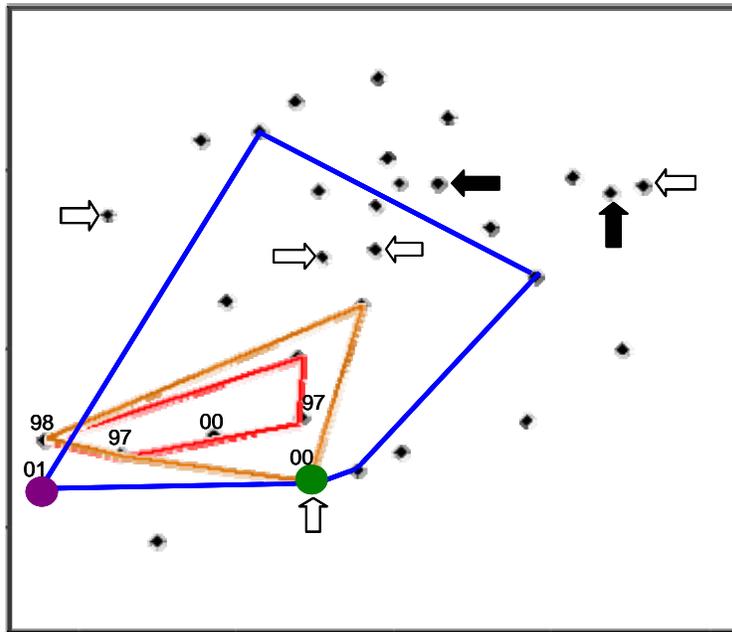


Figure 7

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