



BEHAVIORAL PATERNITY PREDICTS GENETIC PATERNITY IN SATIN BOWERBIRDS (*PTILONORHYNCHUS VIOLACEUS*), A SPECIES WITH A NON-RESOURCE-BASED MATING SYSTEM

SHEILA M. REYNOLDS,^{1,3,5} KATIE DRYER,^{2,3} JONATHAN BOLLECK,^{2,3,6}
J. ALBERT C. UY,^{2,7} GAIL L. PATRICELLI,^{2,8} TIMOTHY ROBSON,⁴ GERALD BORGIA,^{1,2}
AND MICHAEL J. BRAUN^{1,3}

¹Behavior, Ecology, Evolution and Systematics Program, Biology–Psychology Building, University of Maryland, College Park, Maryland 20742, USA;

²Department of Biology, Biology–Psychology Building, University of Maryland, College Park, Maryland 20742, USA;

³Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Suitland, Maryland 20746, USA; and

⁴School of Integrative Biology, University of Queensland, St. Lucia, Qld 4072 Australia

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 of 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 9 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. Received 11 July 2005, accepted 16 July 2006.

Key words: mating success, microsatellites, non-resource-based mating system, paternity, *Ptilonorhynchus violaceus*, reproductive success, Satin Bowerbird.

La Paternidad Comportamental Predice la Paternidad Genética en *Ptilonorhynchus violaceus*, una Especie con un Sistema de Apareamiento que No Está Basado en los Recursos

RESUMEN.—Las diferencias potenciales entre la paternidad genética y la paternidad inferida a partir de observaciones de comportamiento han sido reconocidas desde hace mucho tiempo. Estas diferencias están asociadas con el desafío de las hembras

⁵E-mail: sheilar@umd.edu

⁶Present address: Bioinformatics Centre, University of Copenhagen, 2100 Copenhagen Ø, Denmark.

⁷Present address: Department of Biology, Syracuse University, 108 College Place, Syracuse, New York 13244, USA.

⁸Present address: University of California, Section of Evolution and Ecology, One Shields Avenue, Davis, California 95616, USA.

de buscar beneficios genéticos y materiales. Este desafío es menos severo en las especies con sistemas de apareamiento poligínicos que no están basados en los recursos (como las asambleas de cortejo) que en aquellas con sistemas basados en los recursos. Presentamos el primer estudio de patrones de paternidad en una especie cuyo apareamiento no está basado en los recursos y que no forma asambleas de cortejo verdaderas. Comparamos la paternidad inferida a partir de observaciones de comportamientos de apareamiento con la paternidad asignada genéticamente en *Ptilonorhynchus violaceus* usando ocho marcadores micro-satelitales. El comportamiento de apareamiento fue observado y registrado por medio de cámaras de video automáticas ubicadas en todos los sitios de despliegue (29–34 sitios de despliegue cada año) en el área de estudio a lo largo de cada estación reproductiva. Obtuvimos muestras de sangre e identificamos las madres de 11 pichones en 9 nidos. Para todos los pichones, el padre genético más probable fue observado apareándose con la madre en el año en que el pichón fue muestreado. Todos los padres genéticos más probables fueron determinados con alta seguridad y todos fueron machos que ocuparon un sitio de despliegue. Estos resultados demuestran que la paternidad genética puede ser inferida a partir de la observación del comportamiento de apareamiento con un grado razonable de certeza en *P. violaceus*. Por lo tanto, el éxito de apareamiento observado de los machos predice el éxito reproductivo de modo confiable, y esto sugiere que los sesgos marcados en el éxito de apareamiento observados en los machos se traducen directamente en sesgos marcados en el éxito reproductivo.

PATERNITY ASSIGNMENTS OF offspring based on behavioral observations and those based on genetics and other methods 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 to seek enhanced genetic quality from extrapair copulations (Borgia 1979, Birkhead and Møller 1992). Consistent with this hypothesis, the level of extrapair paternity across passerine bird species is inversely related to the observed level of polygyny (Hasselquist 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 Kempenaers 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 can 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 1985, 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-Jørgensen 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 1985, 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 1995a). We closely monitored the performance of the video-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 1985), which is consistent with predictions from mating-system theory (Emlen and Oring 1977, Borgia 1979), and male mating-success is strongly associated with bower and display characteristics (Borgia 1985, 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 1995a), though this is not supported by any observations. Although we believe that 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 used microsatellite markers to determine the most-likely (ML) genetic fathers of nestlings and compared these with the identities of males observed mating with the nestlings' mothers at bowers on our video-camera system (hereafter "observed mating partners"). By comparing genetic with observed paternity, we tested 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 1985; Loffredo and Borgia 1986; Borgia and Collis 1989; Patricelli et al. 2002, 2003; Coleman et al. 2004). Females have one nest per season containing one or two eggs, and 46% of nests produce at least one fledgling (Donaghy 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 1985), during the mating seasons, 1996–1998. Since 1977, individual birds in this population have been trapped and tagged with a unique three-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 1995a). 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 >20 m 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 1985, 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, and 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 (SBB11 and

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 (°C)	H_E	H_O
SBB11	AGCCATGTCCTTGTTTTTCATCC* GAATACCTGAGCAAACCTGATAA	7	60–55 ^b	0.783	0.800
SBB16.2	CTTTTCAGGTCCTGTCATGGCT* GTCGTTCCGACAGATTCTTAG	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	CATTCCTGCTGAGTGAAGT* CATAAAGCCTCACTTCAGACT	5	58	0.690	0.710
SBC49	GGGGTGTCTGCGATTCT TGGATGTTGGTACGCAGTGTAAAG*	8	49	0.743	0.765
SBC188	CAGGGAGGATGGGAACAG* TCAGGATACCATGGGGAG	5	58	0.697	0.641
SBC193	ACTCCGCTGTTTCGTTTGC GAGGTTATTTGGGGGCTG*	11	50	0.840	0.860

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

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

SBB16.2) were developed following the method described by Degnan et al. (1999).

We carried out polymerase chain reaction (PCR) amplification in 25- μ L reactions with final concentrations of 1 \times Promega Buffer, 1.5 mM MgCl₂, 0.2–0.3 mM each dNTP, 0.2–0.3 μ M of each primer (one primer was fluorescently labeled), 0.04 units μ L⁻¹ Promega Taq polymerase, and 1.6–4 ng μ L⁻¹ template DNA. Reactions for SBB16.2 also contained 0.8 mg mL⁻¹ 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 SBB11 and SBB16.2 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 SBB11 and SBB16.2 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 (~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 CERVUS (Marshall et al. 1998). The program determined the maternal alleles for each chick and then compared the paternal alleles with 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 was assigned as the ML genetic father for the chick, and CERVUS reported a confidence level for each assignment.

Confidence in assignments was determined by the statistic delta (Δ), which is the difference between LOD scores of the two most likely candidate fathers. Critical delta values associated with each confidence level were drawn from a distribution of delta scores determined by simulation of 1,000 offspring genotypes, each of which was compared with 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 delta scores in the simulated distribution above the critical delta 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 = $(1 - x)^m \times x^n$, 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 which 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 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).

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 partners	LOD	Δ	CL	Number of perfectly matched males ^a	Number of allelic mismatches ^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.

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 discernible 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 of 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 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

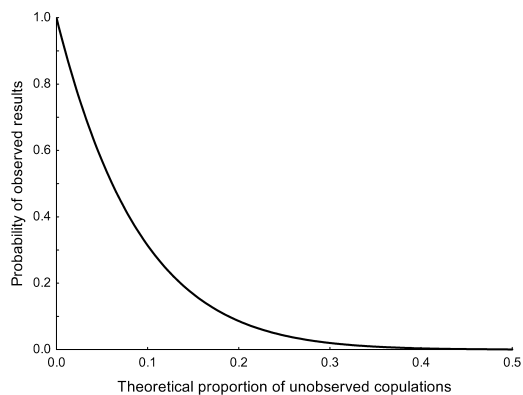


FIG. 1. Probability of observing the parents copulating for 11 out of 11 chicks by chance, assuming a range of theoretical proportions of all copulations that are unobserved. The probability was calculated as $(1 - x)^{11}$ where x is the theoretical proportion of copulations that were unobserved. The 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.

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., 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 provide 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 the 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 1985, 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 copulating 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 that 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 effect 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 the males of 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 that 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 1985, Uy et al. 2000, Patricelli et al. 2003, Coleman et al. 2004) provides an accurate record of mating activity in the population.

ACKNOWLEDGMENTS

We thank S. W. Coleman, T. Glenn, C. Huddleston, D. McDonald, J.-F. Savard, T. Yuri, P. Zwiers, and three anonymous reviewers for providing valuable comments and other forms of assistance. A. Goldizen helped with access to microsatellite markers. This work would not have been possible without the contributions of the many assistants who helped with the field work and video analysis. We also wish to thank the Kennedy, Mulkay, Tener, and Bell families in Wallaby Creek for their hospitality and assistance. For assisting our work in Australia, we thank the Australian Bird and Bat Banding

Scheme, the New South Wales National Parks Services, the Queensland Parks and Wildlife Service, and the Departments of Biology at James Cook and Wollongong universities. The work was done with the approval of the University of Maryland animal-welfare committee. This work was funded by National Science Foundation (NSF) grants to G.B. (Animal Behavior Program IBN 0518844), NSF-Research Training Grant Biology of Small Population and Eugenie Clark awards to G.L.P. and J.A.C.U., an Australian Research Council grant to A. Goldizen, an Australian postgraduate award to T.R., and University of Maryland Behavior, Ecology, Evolution, and Systematics summer fellowships to G.L.P., J.A.C.U., and S.M.R. Additional funding for the lab work was provided by the Smithsonian Institution, Washington, D.C.

LITERATURE CITED

- ALATALO, R. V., T. BURKE, J. DANN, O. HANOTTE, J. HÖGLUND, A. LUNDBERG, R. MOSS, AND P. T. RINTAMÄKI. 1996. Paternity, copulation disturbance and female choice in lekking Black Grouse. *Animal Behaviour* 52:861–873.
- ALATALO, R. V., AND A. LUNDBERG. 1986. The sexy son hypothesis: Data from the Pied Flycatcher *Ficedula hypoleuca*. *Animal Behaviour* 34:1454–1462.
- BIRKHEAD, T. R., AND A. P. MØLLER. 1992. *Sperm Competition in Birds: Evolutionary Causes and Consequences*. Academic Press, London.
- BORGIA, G. 1979. Sexual selection and the evolution of mating systems. Pages 19–80 in *Sexual Selection and Reproductive Competition in Insects* (M. S. Blum and N. A. Blum, Eds.). Academic Press, New York.
- BORGIA, G. 1985. Bower quality, number of decorations and mating success of male Satin Bowerbirds (*Ptilonorhynchus violaceus*): An experimental analysis. *Animal Behaviour* 33:266–271.
- BORGIA, G. 1986. Satin Bowerbird parasites: A test of the bright male hypothesis. *Behavioral Ecology and Sociobiology* 19:355–358.
- BORGIA, G. 1993. The cost of display in the non-resource-based mating system of the Satin Bowerbird. *American Naturalist* 141:729–743.
- BORGIA, G. 1995a. Complex male display and female choice in the Spotted Bowerbird: Specialized functions for different bower decorations. *Animal Behaviour* 49:1291–1301.

- BORGIA, G. 1995b. Why do bowerbirds build bowers? *American Scientist* 83:542–547.
- BORGIA, G., AND K. COLLIS. 1989. Female choice for parasite-free male Satin Bowerbirds and the evolution of bright male plumage. *Behavioral Ecology and Sociobiology* 25:445–453.
- BRAY, O. E., J. J. KENNELLY, AND J. L. GUARINO. 1975. Fertility of eggs produced on territories of vasectomized Red-winged Blackbirds. *Wilson Bulletin* 87:187–195.
- BRO-JØRGENSEN, J., AND S. M. DURANT. 2003. Mating strategies of topi bulls: Getting in the centre of attention. *Animal Behaviour* 65:585–594.
- COLEMAN, S. W., G. L. PATRICELLI, AND G. BORGIA. 2004. Variable female preferences drive complex male displays. *Nature* 428:742–745.
- COOPER, W. T., AND J. M. FORSHAW. 1977. *The Birds-of-Paradise and Bowerbirds*. Collins, Sydney, Australia.
- DEGNAN, S. M., B. C. ROBERTSON, S. M. CLEGG, AND C. C. MORITZ. 1999. Microsatellite primers for studies of gene flow and mating systems in white-eyes (*Zosterops*). *Molecular Ecology* 8:157–168.
- DONAGHEY, R. H. 1981. The ecology and evolution of bowerbird mating systems. Ph.D. dissertation, Monash University, Clayton, Australia.
- DOUCET, S. M., AND R. MONTGOMERIE. 2003. Bower location and orientation in Satin Bowerbirds: Optimizing the conspicuousness of male display? *Emu* 103:105–109.
- EKBLOM, R., S. A. SÆTHER, D. HASSLEQUIST, D. HANNERSJÖ, P. FISKE, J. A. KÅLÅS, AND J. HÖGLUND. 2005. Female choice and male humoral immune response in the lekking Great Snipe (*Gallinago media*). *Behavioral Ecology* 16:346–351.
- EMLEN, S. T., AND L. W. ORING. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* 197:215–223.
- GIBBS, H. L., P. J. WEATHERHEAD, P. T. BOAG, B. N. WHITE, L. M. TABAK, AND D. J. HOYSACK. 1990. Realized reproductive success of polygynous Red-winged Blackbirds revealed by DNA markers. *Science* 250:1394–1397.
- GLENN, T. C., H. C. DESSAUER, AND M. J. BRAUN. 1998. Characterization of microsatellite DNA loci in American alligators. *Copeia* 3:591–601.
- GLENN, T. C., R. S. OJERIO, W. STEPHAN, AND M. J. BRAUN. 1997. Microsatellite DNA loci for genetic studies of cranes. Pages 36–45 in *Proceedings of the 7th North American Crane Workshop* (R. P. Urbanek and D. W. Stahlecker, Eds.). North American Crane Working Group.
- GOWATY, P. A. 1996. Battle of the sexes and origins of monogamy. Pages 21–52 in *Partnerships in Birds* (J. M. Black, Ed.). Oxford University Press, Oxford, United Kingdom.
- HARDY, O. J., AND X. VEKEMANS. 2002. SPAGeDi: A versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2:618–620.
- HASSELQUIST, D., S. BENSCH, AND T. VON SCHANTZ. 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the Great Reed Warbler. *Nature* 381:229–232.
- HASSELQUIST, D., AND P. W. SHERMAN. 2001. Social mating systems and extrapair fertilizations in passerine birds. *Behavioral Ecology* 12:457–466.
- HÖGLUND, J., AND R. V. ALATALO. 1995. *Leks*. Princeton University Press, Princeton, New Jersey.
- HUNTER, C. P., AND P. D. DWYER. 1997. The value of objects to Satin Bowerbirds *Ptilonorhynchus violaceus*. *Emu* 97:200–206.
- JOHNSON, A., V. ANDERSEN, C. SUNDING, AND J. T. LIFJELD. 2000. Female Bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature* 406:296–299.
- JONES, A. G., D. WALKER, C. KVARNEMO, K. LINDSTROM, AND J. C. AVISE. 2001. How cuckoldry can decrease the opportunity for sexual selection: Data and theory from a genetic parentage analysis of the Sand Goby, *Pomatoschistus minutus*. *Proceedings of the National Academy of Sciences USA* 98:9151–9156.
- LANTOT, R. B., K. T. SCRIBNER, B. KEMPENAERS, AND P. J. WEATHERLAND. 1997. Lekking without a paradox in the Buff-breasted Sandpiper. *American Naturalist* 149:1051–1070.
- LANK, D. B., C. M. SMITH, O. HANOTTE, A. OHTONEN, S. BAILEY, AND T. BURKE. 2002. High frequency of polyandry in a lek mating system. *Behavioral Ecology* 13:209–215.
- LOFFREDO, C. A., AND G. BORGIA. 1986. Male courtship vocalizations as cues for mate choice in the Satin Bowerbird (*Ptilonorhynchus violaceus*). *Auk* 103:189–195.

- LONGMIRE, J. L., M. MALTBIE, AND R. J. BAKER. 1997. Use of "lysis buffer" in DNA isolation and its implication for museum collections. Occasional Papers, The Museum of Texas Tech University 163:1-3.
- MARSHALL, T. C., J. SLATE, L. E. B. KRUK, AND J. M. PEMBERTON. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7: 639-655.
- MORTON E. S., L. FORMAN, AND M. BRAUN. 1990. Extrapair fertilizations and the evolution of colonial breeding in Purple Martins. *Auk* 107:275-283.
- OSTRANDER, E. A., P. M. JONG, J. RINE, AND G. DUYK. 1992. Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. *Proceedings of the National Academy of Sciences USA* 89:3419-3423.
- PATRICELLI, G. L., J. A. C. UY, AND G. BORGIA. 2003. Multiple male traits interact: Attractive bower decorations facilitate attractive behavioural displays in Satin Bowerbirds. *Proceedings of the Royal Society of London, Series B* 270:2389-2395.
- PATRICELLI, G. L., J. A. C. UY, AND G. BORGIA. 2004. Female signals enhance the efficiency of mate assessment in Satin Bowerbirds (*Ptilonorhynchus violaceus*). *Behavioral Ecology* 15:297-304.
- PATRICELLI, G. L., J. A. C. UY, G. WALSH, AND G. BORGIA. 2002. Male displays adjusted to female's response. *Nature* 415:279-280.
- PETRIE, M., C. DOUMS, AND A. P. MØLLER. 1998. The degree of extra-pair paternity increases with genetic variability. *Proceedings of the National Academy of Sciences USA* 95: 9390-9395.
- PETRIE, M., AND B. KEMPENAERS. 1998. Extra-pair paternity in birds: Explaining variation between species and populations. *Trends in Ecology and Evolution* 13:52-58.
- QUELLER, D. C., AND K. F. GOODNIGHT. 1989. Estimating relatedness using genetic markers. *Evolution* 43:258-275.
- RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2): Population-genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248-249.
- SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, New York.
- SEMPLE, K., R. K. WAYNE, AND R. M. GIBSON. 2001. Microsatellite analysis of female mating behaviour in lek-breeding Sage Grouse. *Molecular Ecology* 10:2043-2048.
- UY, J. A. C., G. L. PATRICELLI, AND G. BORGIA. 2000. Dynamic mate-searching tactic allows female Satin Bowerbirds *Ptilonorhynchus violaceus* to reduce searching. *Proceedings of the Royal Society of London, Series B* 267: 251-256.
- UY, J. A. C., G. L. PATRICELLI, AND G. BORGIA. 2001. Complex mate searching in the Satin Bowerbird *Ptilonorhynchus violaceus*. *American Naturalist* 158:530-542.
- WESTNEAT, D. F. 1987. Extra-pair fertilizations in a predominantly monogamous bird: Genetic evidence. *Animal Behaviour* 35:877-886.
- WESTNEAT, D. F., P. W. SHERMAN, AND M. L. MORTON. 1990. The ecology and evolution of extra-pair copulations in birds. Pages 331-369 in *Current Ornithology*, vol. 7 (D. M. Power, Ed.). Plenum Press, New York.
- WHITTINGHAM, L. A., AND P. O. DUNN. 2005. Effects of extra-air and within-pair reproductive success on the opportunity for selection in birds. *Behavioral Ecology* 16:138-144.
- WILEY, R. H. 1991. Lekking in birds and mammals: Behavioral and evolutionary issues. *Advances in the Study of Behavior* 20: 201-291.
- WILMER, J. W., P. J. ALLEN, P. P. POMEROY, S. D. TWISS, AND W. AMOS. 1999. Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (*Halichoerus grypus*). *Molecular Ecology* 8: 1417-1429.

Associate Editor: D. B. McDonald