

BREEDING BIOLOGY OF BROOD PARASITIC BROWN-HEADED COWBIRDS (*MOLOTHRUS ATER*) CHARACTERIZED BY PARENT–OFFSPRING AND SIBLING–GROUP RECONSTRUCTION

BILL M. STRAUSBERGER¹ AND MARY V. ASHLEY

Department of Biological Sciences (MC 066), University of Illinois at Chicago, 845 W. Taylor Street, Chicago, Illinois 60607, USA

ABSTRACT.—We characterized several equivocal aspects of the breeding biology of the brood parasitic Brown-headed Cowbird (*Molothrus ater*) at a study site in northeastern Illinois. A total of 175 offspring and a partial sample of parents were sampled and genotyped at six microsatellite loci. A combination of sibling-group and parentage assignment enabled us to characterize mating behavior and area used for reproduction by breeding adults, even for those not sampled. We assigned a mean of 4.7 (range 1–13) and 4.4 (range 1–16) offspring to 33 female and 32 male parents, respectively. Adults typically reproduced with a “primary partner” but up to three partners were common. Offspring females and males were spread over 9 and 12 ha, respectively. Half of the polygynous males produced offspring with an additional mate that parasitized nests near to or within areas overlapping those of their primary partner. That suggests that mate choice takes place at females’ egg-laying areas as opposed to more remote social areas. Multiple females frequently parasitized a single host nest and areas used for reproduction overlapped extensively for individuals of either sex. High frequencies of parasitism and superparasitism indicate a high cowbird density relative to that of hosts. Frequent promiscuity by cowbirds at our site contrasts with other studies reporting monogamy and may be due to higher densities of breeding cowbirds at our site. Received 15 March 2002, accepted 5 February 2003.

RESUMEN.—En este estudio caracterizamos varios aspectos equívocos sobre la biología reproductiva del parásito de nidos *Molothrus ater* en el noreste de Illinois. Se muestrearon un total de 175 crías y una muestra parcial de los padres para determinar sus genotipos con base en seis loci de microsatélites. Usando grupos de hermanos y asignación de parentela, caracterizamos la conducta de apareamiento y el área utilizada para la reproducción por los adultos, incluso para aquellos no muestreados. Asignamos un promedio de 4.7 (rango 1–13) y 4.4 (rango 1–16) crías a 33 hembras y 32 machos, respectivamente. Los adultos generalmente se aparearon con una “pareja principal”, pero comúnmente tuvieron hasta tres parejas. La progenie de hembras y machos se extendió sobre un área de 9 y 12 ha, respectivamente. La mitad de los machos polígnicos produjeron progenie con una pareja adicional que parasitó nidos ubicados cerca o dentro de áreas que se sobreponían a las áreas de la pareja principal. Esto sugiere que la elección de pareja se lleva a cabo en las áreas en donde las hembras ponen los huevos y no en áreas sociales más remotas. Frecuentemente, varias hembras parasitaron un mismo nido y las áreas utilizadas para la reproducción se sobrepusieron extensamente para individuos de ambos sexos. La alta frecuencia de parasitismo y súper-parasitismo indican una alta densidad de *M. ater* con relación a la de sus hospederos. La frecuente promiscuidad de *M. ater* en nuestro sitio de estudio contrasta con otros estudios que reportaron monogamia, lo que podría deberse a las altas densidades de individuos reproductivos en nuestro sitio de estudio.

OBLIGATE BROOD PARASITES lay their eggs in the nests of other species, leaving incubation and care of the parasitic young to surrogate hosts. Because they lack parental obligations found in ~99% of other bird species (Payne 1977), research of obligate brood parasites has the poten-

tial to contribute to our understanding of basic avian behavior, particularly regarding theories concerning mating and spacing patterns. The potential to increase fitness through parental care, despite the ensuing reduction of additional mating opportunities, is a critical component of avian mating system hypotheses (Orians 1969, Emlen and Oring 1977, Wittenberger and Tilson 1980). Monogamy is thus theoretically improbable for brood parasites that are free from constraints of parental care (Yokel 1986). Limiting

¹Present address: Zoology Department, Bird Division, The Field Museum, 1400 S. Lake Shore Dr., Chicago, Illinois 60605-2496, USA. E-mail: bstrauserger@fmnh.org

our understanding is the inherently furtive nature of parasitic reproduction. Observing parasitic behavior and reproduction is exceptionally difficult; there is no single nest to focus observations, ovipositioning is covert, and both parasitic parents are typically absent from nests they parasitize following egg laying (see Fitch and Shugart 1984, Fleischer 1985).

The Brown-headed Cowbird (*Molothrus ater*) is an obligate brood parasite known to parasitize 226 host species (Friedmann and Kiff 1985, Ortega 1998). Evidence that cowbirds are typically monogamous conflicts with theoretical predictions. Sometimes cowbirds are reported to engage in alternative mating strategies (reviewed in Barnard 1998). Those nonmonogamous strategies may be real and reflect varying environmental conditions experienced by cowbirds over their vast transcontinental range, or they may be an artifact of observer bias (Ankney and Scott 1982, Teather and Robertson 1986). In support of the latter, most mating-system studies have been based on behavioral observations that do not accurately reflect the genetic mating system making conclusions suspect (e.g. Avise 1996, Westneat and Webster 1994). Using microsatellites, Alderson et al. (1999a) concluded that cowbirds breeding at the Delta Marsh in Manitoba were, with few exceptions, monogamous. Clearly, genetic data from other sites are needed to identify species-wide mating patterns.

The furtive nature of cowbirds has also made it difficult to accurately characterize the reproductive areas used by female cowbirds and whether females can maintain exclusive use of host nests in such areas. The ability of brood parasites to defend host nests in a given area is likely influenced by both parasite and host density (Elliott 1980; Dufty 1981, 1982a; Teather and Robertson 1986) and size of the area. When brood parasites such as cowbirds are at a high density relative to that of hosts, defense of an area may be impractical, resulting in highly overlapping areas among individuals. Evidence for that relationship has been found for both parasitic cowbirds (Elliott 1980) and cuckoos (*Clamatos* spp.; Martinez et al. 1998). Based on visual observations, radiotelemetry data, or genetic analysis, reported areas that cowbirds use for reproduction vary in size from 3.3 to 68 ha. Teather and Robertson (1985) predicted a positive relationship between size of reproductive

area and extent of overlap; larger territories are more difficult to defend and thus overlap more extensively. Although intuitively appealing, that relationship is not supported by data: three studies reporting relatively large areas used for reproduction found minimal overlap (Dufty 1982a, Rothstein et al. 1984, Raim 2000); two other studies, including one using genetic analysis, report areas used for reproduction intermediate in size (approximately 8–10 ha) with extensive overlap (Teather and Robertson 1985, Hahn et al. 1999); and two studies, one of which was based on genetic analysis, that reported small (3.3–4.5 ha) areas of reproduction report both extensive overlap and near exclusive use of areas (Darley 1968, 1983, see also 1982; Alderson et al. 1999a).

We used microsatellite DNA to characterize reproductive patterns of individual cowbirds at The Morton Arboretum in northeastern Illinois. Our objective was to sample DNA from cowbird offspring (i.e. eggs and nestlings) and identify sibling relationships on the basis of allele sharing. Additionally, we obtained a partial sample of adult genotypes for use in parentage analysis. Microsatellite DNA has been widely used to infer parentage in natural populations, but reconstruction of sibling groups, without extensive sampling of parents, has been less common (but see Blouin et al. 1996, Emery et al. 2001, Thomas and Hill 2000, Smith et al. 2001). We used a combination of parentage assignment, sibling-group assignment, and reconstruction of unsampled parents to characterize two important aspects of cowbird breeding biology, specifically their mating system and spacing systems. Mating system was characterized by determining whether offspring of individual males and females were full siblings or half siblings. Information on mating and spacing patterns was obtained by mapping offspring sired by individual males. For example, if offspring of separate partners are found near one another, it is likely that mate choice takes place in the areas used for reproduction rather than at remote feeding areas (Rothstein et al. 1984). Finally, by mapping the location of maternally related offspring, we characterize areas that individual females use for reproduction. We compare our results to those of other cowbird studies that have used genetical markers for parentage assignment.

METHODS

Study site and sample collection.—In 1995, a total of 205 cowbird eggs and nestlings (hereafter combined as “offspring”) were located throughout the breeding season. We conducted systematic searches for bird nests at The Morton Arboretum in northeastern Illinois (41°50′N, 88°18′W; see Strausberger and Ashley 1997; Strausberger 1998a). The entire site consists of ~133 ha having diverse habitats. However, <100 ha provide habitat for breeding birds as a result of land management practices. Offspring’s DNA was obtained from blood, plucked blood feathers, or embryos (Strausberger and Ashley 2001). Freshly laid eggs were incubated artificially to promote tissue growth prior to sample collection.

We obtained DNA samples from adults ($n = 27$; 8 females and 19 males) by drawing 3–15 μ L of blood via veinipuncture. Adults were captured using baited Potter traps placed at three locations throughout the study site. We trapped adults between the hours of 0700 and 1400 on 21 days. Prior to their release, we banded adults with a unique color-combination of leg bands and released them. We genotyped additional mothers from residual DNA that remained on the surface of their eggshells (see Strausberger and Ashley 2001). All samples were placed into 1.5 mL microcentrifuge tubes and stored at -70°C for later DNA extraction.

Microsatellite analysis.—DNA was obtained using standard phenol–chloroform extraction (Sambrook et al. 1989). We used six microsatellite primer pairs for genotyping individuals. Three loci (Ma μ 10, Ma μ 25, and Ma μ 29) are described in Alderson et al. (1999b) whereas we developed Ma μ 101, Ma μ 102, and Ma μ 104 by screening *M. ater* genomic libraries. Library screening protocols, primer sequences, and reaction conditions for Ma μ 101 and Ma μ 104 are given in Strausberger and Ashley (2001). Locus Ma μ 102 is a (TG) $_n$ repeat having a forward primer sequence of 5′-TCGAGGGAATGGATGGGATGAG-3 and a reverse primer sequence of 5′-CAGGGG-TTGATCTGGGTTGTC-3′. Polymerase chain reaction (PCR) conditions for Ma μ 102 and Ma μ 101 are similar. The forward primer of each pair was fluorescently labeled and analyzed on a 373-A DNA Sequencer using GENESCAN software (Applied Biosystems, Foster City, California). We calculated expected heterozygosities, polymorphic information content (PIC), goodness-of-fit Hardy-Weinberg equilibrium tests, and average exclusion probabilities using CERVUS 1.0 (Marshall et al. 1998).

Parentage analysis and sibling assignment.—Perhaps the most direct way to determine mating patterns and fecundity based on genetic analysis is to compare genotypes of both sampled offspring and parents; however, complete sampling is often impossible. Because we were unable to completely sample parents, we

used a combination of sibling group and parentage assignment to identify or infer unsampled parents. For example, for the latter, with one parent identified by a parentage analysis of DNA, we knew the number of offspring sampled but not the number of unsampled mates. By inferring relationships (full or half siblings) among offspring, we inferred the minimum number. In practice, if 10 offspring of a single parent are assigned to two full-sibling groups, then we concluded that that parent produced 10 offspring with two different mates.

Sibling relatedness among offspring was examined using KINSHIP 1.2 (Goodnight and Queller 1999). KINSHIP is a program that tests hypotheses of relatedness, based on Queller and Goodnight’s r (Queller and Goodnight 1989), between pairs of individuals. KINSHIP calculates the ratio of a primary hypothesis (in our case full-sibling relatedness between offspring) to the null hypothesis (in our case no relation between offspring). Significance of the ratio is determined by simulation routine. The simulation generates offspring pairs, based on population allele frequencies, and finds the ratio necessary to reject the null hypothesis. In our case, rejection indicates the offspring being tested are full siblings.

To accept a hypothesized relationship, KINSHIP gives three levels of significance: ≥ 0.95 , 0.99, and 0.999. We considered offspring to be full-siblings when each of the pair-wise comparisons for all members of the putative sibling group had a ≥ 0.99 chance of being full-siblings. By choosing such a high level of confidence, we may have excluded some true full-siblings; however, we chose to be conservative and avoid false assignments that may be especially likely to occur among half-siblings (Blouin et al. 1996). Often times, half-sibling groups fell out beside their full-sibling group in the KINSHIP matrix output, making interpretation of half-siblingship between groups relatively straightforward. All putative full and half-sibling groups were further explored in a spreadsheet by visually examining the genotypes of all members of a putative sibling group. When a maximum of four alleles was found across all loci, full-siblingship was assigned to the group. In the absence of mistyping, a full-sibling group can have no more than four alleles (two from each parent) per locus.

We used the likelihood approach implemented in CERVUS 1.0 (Marshall et al. 1998) to assign parentage of sampled adults to cowbird offspring. In the past, parentage studies have been exclusionary such that mismatches of alleles between potential parents and offspring could potentially exclude the true parent. With mismatches between parent and newborn arising from mutation, null alleles, and human error, a more reasonable method of assigning parentage is through a likelihood analysis. The likelihood-based method in CERVUS provides a level of statistical significance that the candidate parent is the true parent.

An additional advantage of using CERVUS is that it takes into account the incomplete sampling typical of large-scale studies (Marshall et al. 1998). Likelihood is determined on the basis of simulation routine using allele frequencies of a study population. Thus, the ability of CERVUS to determine parental relationships depends largely on the genetic variability of the loci used and their resulting power to exclude potential parents. For each offspring, we first tested for maternal and then paternally related adults. CERVUS parameters are listed in Table 1.

For offspring within a full-sibling group, CERVUS did not always assign the same level of confidence to all candidate parent-offspring pairs of the full-sibling group. That is expected due to varying genotypes of the offspring and differences in allele frequencies among individuals. For example, when an offspring and parent share rare alleles, confidence in parentage is higher than when sharing common alleles. Thus, in cases where CERVUS identified the parent of a newborn at the <95% level, we inspected the results of KINSHIP. All offspring from full-sibling groups having parents with a <95% probability were manually examined. If all offspring in the sibling group contained the parent's alleles at all loci, then parentage for that sibling group was accepted for that parent. In that way, we minimized the occurrence of type I error (i.e. failure to assign true parents to offspring). When assigning a parent to an offspring who lacked any full-siblings, we required a $\geq 95\%$ probability from CERVUS that the most likely candidate parent be the true parent of the offspring.

In some cases we were able to infer half-sibling relations by reconstructing the parental genotype from two closely related sibling groups having one known parent. When a parent of a full-sibling group was identified, the unshared alleles present in the full-siblings, but not in the parent, were assigned to the unsampled parent. To be parsimonious in the use of that reconstruction method, we used it only in

cases where we genotyped a parent of one or more half-sibling groups consisting of at least four individuals. Sibling groups were considered half-siblings to one another only when the reconstructed parental genotype was consistent with being the parent of both half-sibling groups.

Areas used for reproduction.—We characterized the "areas used for reproduction" by adults having three or more dispersed offspring. We defined those areas as the size of area over which we found offspring of a given parent. When cowbirds are monogamous, those areas are equal in size and location. For polygynous males, we identified whether offspring from additional mates were found within the area of reproduction used by the male's primary mate. Additionally, we determined the increase in the size of that area by polygynous compared to monogamous males. Locations of offspring were mapped onto a U.S. Geological Service aerial photograph. Areas used by individuals for reproduction were depicted and the area calculated using animal movement extension and the minimum convex polygon function in ARCVIEW GIS 3.2.

We used a Student's *t*-test to determine whether reproduction areas were similar for male and female cowbirds. We used linear regression analysis to test for correlations between number of offspring, size of reproduction area, and number of mates.

RESULTS

In 1995, we found a total of 373 nests of 29 altricial species. Of those, 126 (33.8%) nests from 13 species were parasitized (Strausberger and Ashley 1997). Cowbirds laid eggs from 28 April (week 1) to 18 July (see Strausberger 1998a). We successfully genotyped 175 (85%) of 205 cowbird offspring. We genotyped 27 adults from blood samples and seven additional mothers from eggshells.

Loci had 7–27 alleles each (Table 2). Expected heterozygosities ranged from 53–92% and combined expected heterozygosity across all six loci was 81.5% (Table 2). Combined, those loci have the parental exclusion probabilities of 0.99399 and 0.99954 for the first and second parent, respectively (Table 2). None of the six loci deviated significantly from Hardy-Weinberg expectations, indicating a low probability of null alleles.

A total of 141 (80.6% of the 175 genotyped) offspring were assigned to a parent ($n = 76$ offspring) or were placed into a full-sibling group ($n = 65$ offspring; Table 3). We were unable to assign 34 offspring to either a parent or full-sibling group. Those offspring were likely those of unsampled adults and without any full-siblings.

TABLE 1. Parameters used in simulation of maternity and paternity inference with the CERVUS 1.0 program (Marshall et al. 1998). Proportion of loci typed and the error rate are averages for all six loci.

Parameter	Value
Number of candidate females ^a	40
Number of candidate males ^a	60
Proportion of loci typed	0.97
Rate of typing error	0.01
Number of tests	10,000
Relaxed confidence level	70%
Strict confidence level	95%

^aNumbers of candidate females and males were estimated from the frequencies of observations of banded and unbanded individuals at the site.

Table 2. Summary statistics^a for the microsatellite loci used in this study.

Locus	Number sampled ^b	Number of alleles	Range of allele sizes (bp)	H_{exp} ^c	PIC ^c	P_{Eif} ^d	P_{Em} ^e
Maμ 10 ^f	203	8	161–175	0.74	0.715	0.363	0.544
Maμ 25 ^f	206	27	127–185	0.92	0.912	0.72	0.836
Maμ 29 ^f	209	25	126–178	0.918	0.91	0.714	0.832
Maμ 101	210	20	147–189	0.876	0.862	0.601	0.751
Maμ 102	204	7	173–185	0.53	0.481	0.15	0.301
Maμ 104	208	24	137–185	0.92	0.885	0.72	0.836
Summary	210	111	126–189	0.815	0.794	0.99399	0.99954

^a Calculated with CERVUS 1.0 (Marshall et al. 1998).

^b Number of individuals sampled includes both adults and juveniles from which DNA samples were obtained from either embryos, blood, blood feathers, or egg shells.

^c H_{exp} is the expected heterozygosity (Nei 1987) and polymorphic content (PIC) measures informativeness related to expected heterozygosity across all loci (Hearne et al. 1992).

^d P_{Eif} is the exclusion probability in the absence of genetic information on the first parent (Marshall et al. 1998).

^e P_{Em} is the exclusion probability given genetic information on the second parent (Chakravarti and Li 1993).

^f Loci from Alderson et al. (1999b).

We identified a total of 31 full-sibling groups (Table 4).

One or both parents were identified for 14 full-sibling groups (full-sibling groups 1–8, 10, 12, 14–17; Table 3). An additional four paternal genotypes (5112AZ-REC, 6Z-REC, 7Z-REC, 9Z-REC) could be sufficiently reconstructed for informative use. Reconstructed genotypes that were compatible with a single male, in combination with high relatedness values between offspring of different full-sibling groups (determined from KINSHIP), allowed us to confidently determine that full-sibling groups 1, 2, 3 were maternally related half-siblings (Table 3). Groups 3 and 4, 8 and 9, and 10 and 11 were paternally related half-siblings. Full-sibling groups 12 and 13 and offspring 753B were paternally related half-siblings through the reconstructed male 9Z-REC (Table 3).

We identified 12 mothers directly (parentage analysis) from DNA samples (obtained from blood or eggshells), 7 from a combination of parentage analysis and sibling-assignment, and 14 were inferred from offspring's full-sibling relationships alone, or in combination with, paternal genotypes. Combining those methods, we identified a maximum total of 33 mothers (Table 3). Those mothers produced 1–13 (4.7 ± 2.83) offspring each (Table 3). We identified a maximum of 32 fathers; three fathers directly from their DNA (obtained from blood) and 29 indirectly (from offspring's full-sibling relationships, in combination with a known maternal genotype, or both) by reconstructing their genotype. Fathers had 1–16 (4.4 ± 3.42) offspring each. The above

values for the potential number of mothers and fathers are maximums because two full-sibling groups lacking a known parent could share a parent. Without one parent genotyped, we were unable to confidently infer half-sibling relationships among offspring. In summary, a maximum of 33 mothers and 32 fathers produced 141 offspring.

Full-sibling groups ($n = 31$) had 2–12 (4.5 ± 2.54) offspring each (Table 3), indicating frequent repeat mating among pairs. However, 14 full-sibling groups (left side of Table 3) shared only a single parent, indicating that pairs are frequently polygynous. Both sexes sometimes reproduced with multiple partners. Data from the 11 genotyped mothers who had multiple (2–13) offspring indicates that 5 (46%) mated polyandrously with 2–3 (2.2 ± 0.45) males each. However, those females produced 51 of their 60 (85%) offspring with their primary partner, indicating a comparatively high degree of mate fidelity. All fathers that we identified directly from genotyping ($n = 2$) or genotype reconstruction ($n = 4$), sired multiple (2–16) offspring from 2–3 (2.17 ± 0.41) females each, indicating that all six were polygynous. But like females, a total of 38 of 51 (75%) of males' offspring were produced with their primary partner, indicating a high degree of mate fidelity. Inspection of Table 3 confirms that cowbirds having multiple partners did not fit a pattern of "sequential monogamy" (e.g. female 5910BW2 produced young with two different males in week 8). Using only genotyped and reconstructed genotypes of adults, for both sexes the number of mates increased with the number of offspring genotyped (Fig. 1).

TABLE 3. Summary of Brown-headed Cowbird relationships determined from genetic analyses^a.

Offspring with at least one identified parent ^b					Offspring without any identified parents ^b				
Full sibling group ^c	Mother's ID	Father's ID	Offspring's ID ^d	Week ^e	Full sibling group ^c	Mother's ID	Father's ID	Offspring's ID ^d	Week ^e
1	5910BW2	Unknown 1Z	5263B	3	18	Unknown 8W	Unknown 15Z	5171C	2
			763B	8				677A	5
2		Unknown 2Z	672C	5				677B	5
			6262A	8				6813A	2
3		5112AZ-REC	5910B	1				6918D	5
			5101B	1				6192A	7
4	5112AW		51715B	2				6202A	6
			5101C	1				6282A	8
			5112A	1				711A	8
			51714A	2				771A	9
			51714B	2	19	Unknown 9W	Unknown 16Z	651A	5
			5259A	3				661A	5
			5263A	3				661B	5
			6191C	4				672A	5
5	5111AW	Unknown 3Z	5111A	1				6153A	6
			596B	1				6191A	4
6	75W	Unknown 4Z	5125A	1				6191B	4
			688A	5				6193A	3
			688B	5				6201B	3
			754C	6				711B	8
7	593CW	Unknown 5Z	5125A	1				7141A	10
			688A	5				20	Unknown 10W
			751A	9					
			8	6293W				6Z-REC	5124A
5166A	2	21			Unknown 11W	Unknown 18Z	599B		1
				6261A			8		
		762A		8					
9	Unknown 1W		763A	8					
			51711B	2					
			51711C	2					
			754B	6					
10	594AW	7Z-REC	594A	1					
			594B	1	22	Unknown 12W	Unknown 19Z		
			626B	4					
			6222A	2					
11	Unknown 2W		593A	1					
			593B	1	23	Unknown 13W	Unknown 20Z		
N/A	766W	Unknown 8Z	52610B	3					
			599A	1					
12		9Z-REC	52610A	3				24	Unknown 14W
			5312B	4					
			694A	5					
			6113A	3					
			6115A	5					
			6131C	6	25	Unknown 15W	Unknown 22Z		
6171A	6							6152C	6

TABLE 3. Continued.

Offspring with at least one identified parent ^b					Offspring without any identified parents ^b				
Full sibling group ^c	Mother's ID	Father's ID	Offspring's ID ^d	Week ^e	Full sibling group ^c	Mother's ID	Father's ID	Offspring's ID ^d	Week ^e
12			761A	5	25	Unknown 15W	Unknown 22Z	6284A	6
			775A	5					
			7115A	7	26	Unknown 16W	Unknown 23Z	5179A	2
			7183B	7				5231A	2
13	Unknown 3W		673A	3				5245A	3
			673B	3				52610D	3
			6112C	6				682A	5
N/A	Unknown 4W		753B	8				753A	8
14	5910BW1	Unknown 10Z	5191A	2	27	Unknown 17W	Unknown 24Z	51711A	2
			5192A	1				754A	6
			5198A	2					
			5269A	3	28	Unknown 18W	Unknown 25Z	5259B	3
N/A		DEADZ	6206A	3				678B	5
								6201A	3
N/A	Unknown 5W	7118Z	6113B	3					
15	5910AW1		5910A	1	29	Unknown 19W	Unknown 26Z	51715C	2
			5168A	1				6217A	4
			7182A	9					
			7182C	9	30	Unknown 20W	Unknown 27Z	684A	5
			6131A	6				6131B	6
N/A		Unknown 11Z	7183A	7				752A	6
								753C	8
16	Unknown 6W	B49Z	5254B	3					
			5312A	4	31	Unknown 21W	Unknown 28Z	5173A	2
			6912A	5				5173B	2
			6213B	6					
			6221A	4					
N/A	Unknown 7W		6222B	2					
17	5233AW	Unknown 12Z	5233A	3					
			615A	4					
		Unknown 13Z	678A	5					
N/A	594CW	Unknown 14Z	594C	1					

^a We first placed offspring into full sibling groups using KINSHIP 1.2 (Goodnight and Queller 1999) and then assigned parentage using CERVUS 1.0 (Marshall et al. 1998). Parents ending in "-REC" were assigned based on genotypes reconstructed from full-sibling groups with one known parent genotyped (see text).

^b Offspring lacking assigned parents were placed into full sibling groups using Kinship 1.2 (Goodnight and Queller 1999). Vertical rule identifies offspring of the last parent listed.

^c Offspring having a full sibling group identified with N/A do not belong to any full sibling group as they have no full siblings.

^d Offspring in **bold-type** had nonmaternally related nestmates indicating competition among female cowbirds.

^e Week one begins April 28 (see Strausberger 1998a).

That correlation, combined with the incomplete sample of offspring, indicates that the actual number of mates per individual may be larger than the values we documented.

The size of the areas that female cowbirds ($n = 20$) used for reproduction were 9.10 ± 8.31 ha (Table 4). Areas containing offspring from different females overlapped so extensively (up to

six females sharing a given area over the course of the season) that it prohibited informative depiction. Nests frequently contained offspring from multiple mothers (bold-face font in Table 3). A total of 30 out of 76 (39.5%) offspring from genotyped mothers were found sharing a nest with offspring from a different mother. Considering that the study site consisted of

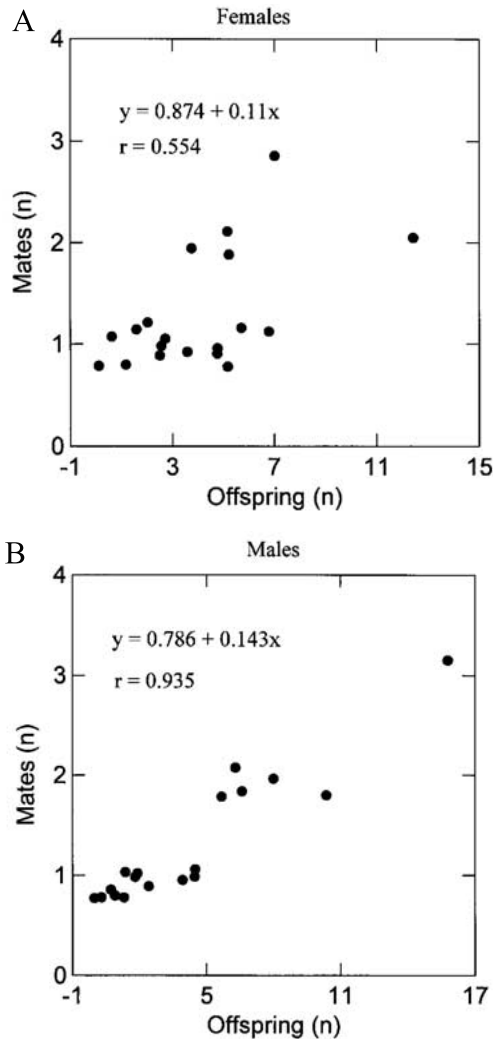


FIG. 1. Number of mates that identified (A) female ($P = 0.01$, $n = 19$), or (B) male ($P = 0.001$, $n = 18$) cowbirds had increased with number of offspring sampled.

~133 ha, and the sizes of areas used for reproduction by the mothers listed in Table 4 have a combined reproductive area of 182 ha, the areas used by females for reproduction overlapped by a minimum of 37% [(182–133)/133].

Interpretation of the “area used for reproduction” by male cowbirds is not as straightforward; it represents the parasitism area of females who had mated with a particular male. For males, the size of that area was 12.38 ± 10.68 (Table 4); not significantly different from that of females ($t = -1.08$, $df = 36$, $P = 0.29$). As with females, those areas for males overlapped extensively. Males listed in Table 4 reproduced

over a combined area of 223 ha, representing a minimum overlap of 68%. The area of reproduction for males is not much larger than that of females, and for the six polygynous males, the area where we found their “extrapair” offspring increased by a mean of only $7.22 (\pm 6.59)$ ha each. Those observations are consistent with the possibility that polygynous males typically mate with females that parasitize nests in overlapping or nearby areas. Indeed, three of six (50%) polygynous males produced half-sibling offspring within the egg laying area used by their primary mate.

Using data from all full-sibling groups, there was a significant positive relationship between home-range size and number of offspring sampled for each sex (Fig. 2). That correlation indicates a possible sampling effect that may result in an underestimation of the true size of reproductive areas.

DISCUSSION

Mating system.—Our goal was to provide a detailed characterization of the breeding behavior of brood parasitic Brown-Headed Cowbirds at our study site by using field collections and reconstruction of familial relationships using genetic markers. The extremely surreptitious nature of parasitizing others inherently prohibits direct methods of characterization. In consideration of the indirect nature of our data, we required that, for a given familial relationship to be accepted as true, there be an exceptionally high probability of it being so. Although conservative, we are confident that our findings reliably characterize cowbird breeding behavior. In the analyses presented here, we focus on several equivocal aspects of cowbird reproduction, including mating and spacing systems.

Similar to Great Spotted Cuckoos (*Clamator glandarius*), whose inferred mating relationships can be considered monogamous 70% of the time (Martinez et al. 1998), cowbirds at The Arboretum did not fall neatly into traditional mating system categories (e.g. monogamous, polyandrous, polygynous, or promiscuous). Instead, they exhibited varying degrees of each category. In general, individuals reproduced most frequently with a “primary partner,” and less often with additional individuals. Frequently occurring promiscuity by cowbirds at The Morton Arboretum contrasts with results

TABLE 4. Sizes of areas that Brown-headed Cowbirds used for reproduction were determined from offspring found in three or more host nests.

Mother's ID	Area (ha) ^a	Father's ID	Area (ha) ^a
5910BW2	7.38	5112AZ-REC	8.6
5112AW	3.47	Unknown 4Z	0.58
75W	0.58	Unknown 5Z	18.94
539CW	18.94	6Z-REC	5.3
6293W	4.75	7Z-REC	2.68
594AW	2.41	9Z-REC	38.33
766AW	24.99	Unknown 10Z	6.99
5910BW1	1.05	Unknown 7118Z	26.47
5910AW1	16.83	Unknown B49Z	19.78
Unknown 6W	4.18	Unknown 15Z	27.04
5233AW	1.47	Unknown 16Z	11.55
Unknown 8W	27.04	Unknown 18Z	19.53
Unknown 9W	11.55	Unknown 20Z	13.19
Unknown 11W	19.53	Unknown 21Z	7.17
Unknown 13W	13.19	Unknown 22Z	0.43
Unknown 14W	7.17	Unknown 23Z	5.86
Unknown 15W	0.43	Unknown 25Z	8.59
Unknown 16W	5.86	Unknown 27Z	2.6
Unknown 18W	8.59		
Unknown 20W	2.6		
Mean ± SD	9.1 ± 8.31	Mean ± SD	12.4 ± 10.72

^a Area determined from the size of the minimum convex polygon function of ArcView, GIS 3.2.

from the Delta Marsh, Manitoba, where cowbirds are, with few exceptions, monogamous (Alderson et al. 1999a). Differences in mating systems between that and other sites may be actual or artificial; the latter possibly resulting from inadequate sample sizes. In our study, number of mates increased with number of offspring assigned to an individual; thus, incomplete sampling of offspring may result in an underestimation of the number of sexual partners. Consistent with that possibility, the mean number of genotyped offspring that we obtained (4.7) from females was larger than that (2.8) obtained by Alderson et al. 1999b. Nevertheless, the near absence of polygamy in cowbirds at the Delta Marsh, and its frequent occurrence at our site, suggests that mating system differences between sites may indeed be real. The causes of those differences are unclear but may correspond to differing cowbird densities. Other studies report high degrees of polygamy occurring under high cowbird densities (Elliott 1980, Dufty 1982a, Yokel 1989), indicating that prevalence of monogamy is reduced as cowbird density increases. Both the frequency and intensity of cowbird parasitism is greater at The Arboretum than at the Delta Marsh, indicating a higher cowbird density.

Cowbirds at our site typically reproduced repeatedly with the same partner, as was evidenced by the large numbers of full-siblings and full-sibling groups. That predicts that factors other than the provisioning of parental care selects for degree of mate fidelity that we observed among cowbirds. Some researchers have applied traditional sexual selection theories, including female choice or male-male competition, to cowbirds to explain the occurrence of monogamy (Darley 1982; Dufty 1982a, b; see also Wittenberger and Tilson 1980; Yokel and Rothstein 1991). Here we propose an alternative explanation; males may assist females with their parasitic duties. Such assistance would be analogous to males of nesting species assisting females in parental duties. Thus, male cowbirds' assistance in parasitism could select for monogamy if, by acting together, the pair can produce more successful offspring than when apart.

Consistent with the possibility that male cowbirds assist females in parasitic duties, some host species recognize and respond aggressively towards males (Strausberger and Horning 1998, B. M. Strausberger unpubl. data, see also Robertson and Norman 1976). Additionally, we made several observations at

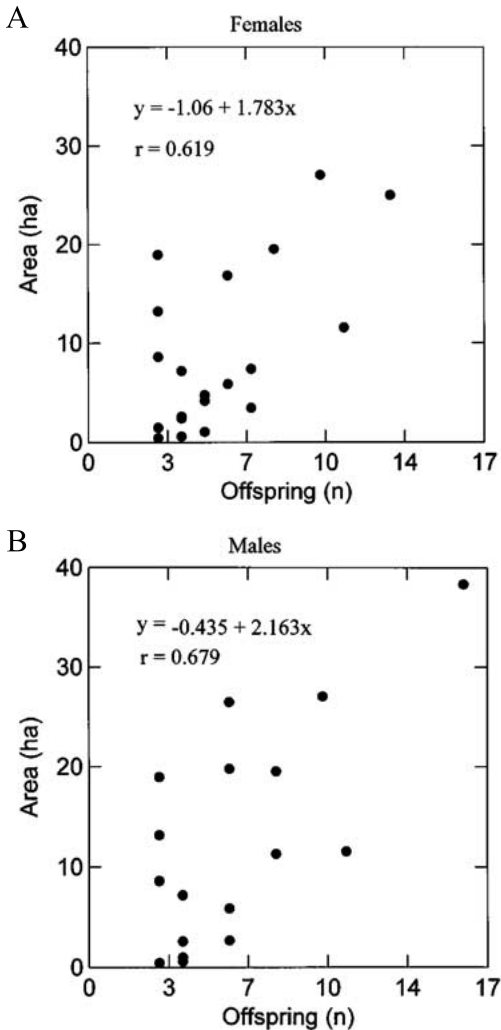


FIG. 2. Positive relationship between number of offspring sampled and size of area used for reproduction by (A) female ($P = 0.004$, $n = 20$), and (B) male ($P = 0.002$, $n = 18$) Brown-headed Cowbirds.

The Morton Arboretum that allude to the possibility. First, we observed a male cowbird make a close pass and then land within 1 m of two Song Sparrows (*Melospiza melodia*) that were aggressively attacking a female cowbird model that we placed at their nest for an unrelated experiment. Upon his landing, the sparrows temporarily postponed their attack, resuming it when the male departed. That observation indicates that the male's presence may have temporarily distracted the sparrow from their attack of the model female cowbird. We observed

male cowbirds attending females while females searched for host nests (Strausberger 1998b). On one occasion, a call from the male seemingly warned the apparently oblivious female cowbird of the approaching host (see Strausberger 1998b). Additionally, two males attended a female cowbird, from a distance of ~15 m, on a nest visit where she ejected a cowbird egg from a Northern Cardinal's (*Cardinalis cardinalis*) nest. Combined, those observations indicate that, similar to males of other parasitic species including the Giant Cowbird (*Scaphidura orizivora*), male Brown-headed Cowbirds occasionally attend females at host nests and possibly distract hosts or warn females of approaching and potentially dangerous hosts (Arias-de-Reyna 1998, Barnard 1998, Rothstein and Robinson 1998).

Areas of reproduction.—Reproductive areas used by females were of intermediate size and overlapped extensively, demonstrating that females could not maintain exclusive use of nests; territorial defense is apparently ineffective. Nests were frequently parasitized, and often by multiple females indicating a high density of cowbirds compared to that of available hosts. Local cowbird densities may be more important than the size of egg-laying area in explaining extent of overlap of those areas. Superparasitism of nests decreases offspring fitness—and indirectly parental reproductive fitness—through intraspecific competition among cowbird nestlings (Nice 1937, Nolan 1978). The apparently high density of cowbirds breeding at The Morton Arboretum is likely to result in reduced reproductive fitness among individuals who compete for nests or interfere with one another's reproduction such as by removing conspecifics' eggs from nests as we once observed.

Extensive overlap of reproductive areas may also contribute to the frequency of promiscuous matings. The finding that offspring of different females sired by an individual male were found in close proximity suggests that sexual interactions take place at, or near, the same areas where eggs are deposited. Half of the males that reproduced polygynously had offspring from extrapair matings that fell within the area used by their primary females for reproduction. Partially overlapping breeding areas contributes to males generally reproducing over larger areas than females but not significantly larger. As a consequence, our data is consistent with the possibility that mate-choice occurs at, or near, the

same areas where offspring are deposited and not at other areas, including afternoon feeding sites as has been raised (see Rothstein et al. 1984, Ortega 1998).

Highly variable genetic markers have provided an important advance to studying mating systems and reproductive patterns in natural populations. To date, most studies have been concerned with parentage assignment, which requires thorough sampling of candidate parents. In many study systems, such as ours, it is more feasible to extensively sample the offspring cohort than the parental generation (Valenzuela 2000, Emery et al. 2001), or to obtain samples of only one parent (DeWoody 2000, Feldheim et al. 2001). In such cases, we have demonstrated that it is possible to characterize reproductive patterns of unsampled adults by inferring sibling relationships among the offspring. We used a novel application of microsatellite genotyping to accomplish that goal using both sibling and parentage assignment. We assigned 81% of genotyped offspring to either a parent or a full-sibling group. That provided us with a comprehensive assessment of the mating strategy and spacing patterns used by cowbirds reproducing at our site. We found that by identifying gender and genotype of one or more parents, we can facilitate characterization of the genetic mating system of the entire population. Because few studies can sample all possible parents, this method should have wide application to other systems, particularly when offspring comprise sibling groups and highly informative microsatellite markers are used.

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