MATING SYSTEM AND HOST CHOICE OF THE BROWN-HEADED COWBIRD

MOLECULAR GENETIC ANALYSIS OF THE MATING SYSTEM AND HOST CHOICE OF AN OBLIGATE BROOD PARASITIC BIRD, THE BROWN-HEADED COWBIRD (*MOLOTHRUS ATER*)

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GERALD WAYNE ALDERSON, B.Sc

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AUTHOR: Gerald Wayne Alderson, B.Sc. (McMaster University)

SUPERVISOR: Dr. H. Lisle Gibbs

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CHAPTER 1 - GENERAL INTRODUCTION

Parasitism can be defined as a biological interaction in which one party benefits at the expense of another (Keeton and Gould 1986). However unlike predation, the parasite does not kill its host. In the case of avian brood parasitism, one bird lays its eggs in the nest of a member of its own or another species and then abandons its offspring to the care of its new foster parents. These foster parents or 'hosts' then raise the parasitic young at the expense of their own brood. Brood parasitic birds have reproductive advantages over those which provide parental care for their young because: 1) parental care provided by several fosterers allows a female to produce more successful offspring than she is capable of rearing herself in one season, and 2) parasite offspring are usually distributed among many host nests thereby increasing the probability that at least some offspring will escape predation (Payne 1977).

Obligate interspecific brood parasitism, where females only parasitize the nests of other species, is a reproductive strategy adopted by approximately 1% of all bird species and is practiced by members of five families (Anatidae, Cuculidae, Indicatoridae, Icteridae, and Ploceidae). The degree to which interspecific brood parasites reduce host nesting success varies with the reproductive tactics of the parasite. For example, Common Cuckoo chicks (*Cuculus canorus*) eject eggs and young nest mates from the host nest with the help of an instinctive urge to push out of the nest anything that touches the sensitive

shallow depression in the parasitic nestling's back (Lack 1968). Young African Greater Honey-guides (*Indicator indicator*) stab host nestlings to death with special mandibular hooks that drop off after two weeks of age (Friedmann 1955). In contrast, black-headed duck hatchlings seek only protection and warmth for 1-2 days post hatching and then leave the nest with no further cost to their host (Weller 1968). Finally, Brown-headed Cowbird nestlings intermediately affect host reproductive success by diverting parental resources such as food away from the host's young (Payne 1977). This loss of host fitness results in selective pressure for host defenses against parasitism such as egg ejection (Neudorf and Sealy 1992), clutch abandonment (Burgham and Picman 1989), or increased nest defense early in the nesting cycle (Burgham and Picman 1989, Briskie and Sealy 1989, Neudorf and Sealy 1992). To circumvent these host responses, adaptation in brood parasites has resulted in selective pressure for egg mimicry (Rothstein 1990), egg removal, or shorter incubation periods (Briskie and Sealy 1990, Payne 1977).

The Brown-headed Cowbird is the most abundant and widely distributed obligate interspecific brood parasite in North America. Although the breeding behaviour of this bird has been widely studied, most findings are contradictory. The mating system of the Brown-headed cowbird has been described as ranging from monogamous (Laskey 1950, Dufty 1982a, 1982b, Yokel 1986), to promiscuous (Elliot 1980). Most studies also suggest that cowbirds parasitize multiple host species (Friedmann 1929, p 177-188, Jones 1941, McGeen & McGeen 1968, Elliot 1977, Fleischer 1985). However, a few suggest that some individuals may be host specialists (Walkinshaw 1949, McGeen & McGeen

1968). Few of these studies have used genetic techniques to determine the actual mating patterns and to investigate the breeding biology of males and females in a single marked population. The main objective of this study was to use molecular genetic DNA markers as well as behavioural observation to study the mating system and host specificity of a Brown-headed Cowbird population at Delta Marsh, Manitoba. More specifically, my objectives were to: 1) determine whether DNA microsatellite markers are useful for determining parentage in Brown-headed Cowbird populations 2) document the genetic mating system and the patterns of host use by individual females in a population of resident cowbirds.

My findings provide the first evidence that microsatellites are useful for high resolution parentage analyses in brood parasitic bird species where there is no a priori information available on male or female parentage. In addition, they are the first to directly quantify the mating system of a Brown-headed Cowbird population and to suggest that individual females are best described as host generalists but may be showing some preference for host nests in one habitat over another.

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CHAPTER 2

Usefulness of DNA Microsatellite Markers For Parentage and Kinship Studies in an Obligate Brood Parasitic Bird, the Brown-headed Cowbird (*Molothrus ater*)

ABSTRACT

Recent studies suggest that single-locus microsatellite DNA markers have the potential to unambiguously resolve parentage among individuals in natural populations where maternity is known. However, their power for determining parentage when neither parent is known is unclear. Here, I investigate the usefulness of microsatellite DNA markers to determine parentage in a brood parasitic bird, the Brown-headed Cowbird (*Molothrus ater*) where, for a given offspring, no a priori knowledge of either parent is available. Seven polymorphic microsatellite DNA markers isolated from Brown-headed Cowbirds and Yellow Warblers (*Dendroica petechia*) were used to characterize genetically an individually marked breeding population of male and female cowbirds at Delta Marsh, Manitoba. Forty-four males, 21 females and 61 cowbird chicks were genotyped at seven loci using DNA amplified from blood and tissue samples. The mean exclusion probabilities pooled across all seven loci were 0.9964 for males and 0.9948 for females. Two null (nonamplifying) alleles at one locus were discovered and accounted for

by constructing alternate non-overlapping primer sets. Exclusion analyses performed using all individuals determined both paternity and maternity for 43 chicks and paternity only for four chicks. An additional microsatellite locus was then used in limited exclusion analyses to determine paternity for three additional chicks. Relatedness analyses placed 12 of the 18 remaining chicks not assigned both maternity and paternity into four unique full sibling groups. Overall, 90.16 % (55 of 61) of all offspring examined were placed into distinct parent/sibling groups demonstrating that this marker set is extremely useful for parentage studies in this species.

INTRODUCTION

The use of DNA-based genetic markers has revolutionized the study of avian mating systems (Burke and Bruford 1987, Wetton et al. 1987). Such markers provide powerful tools for identifying differences between the 'genetic' and 'social' mating systems within a species (Quinn et al. 1987, Burke et al. 1989, Gyllensten et al. 1990, Gibbs et al. 1994) and for establishing reproductive success in birds with high levels of extra-pair fertilizations (Gibbs et al. 1990, Morton et al. 1990, Lifjeld et al. 1993, Westneat 1993, Primmer et al. 1995).

To date, most studies have used multi-locus DNA fingerprinting for genetic analysis (Burke and Bruford 1987, Wetton et al 1987) and many have focused on socially monogamous species (e.g. Westneat 1990, Lifjeld et al. 1993). The low level of intraspecific brood parasitism detected in such species (Westneat 1990, Lifjeld et al. 1993, Primmer et al. 1995, but see Quinn et al. 1987) has meant that most studies are largely concerned with identifying paternity because the female who is identified as frequenting a nest is almost always the genetic mother of offspring in that nest. Less frequent have been studies which attempted parentage analyses for species which are not behaviourally monogamous and where social parentage is not as easily defined (but see Hann and Fleischer 1995).

Brood parasitic birds present a special challenge to researchers studying parentage in avian species because no a priori information is available on the potential parents of a

particular offspring. Social parentage is uncertain because these species lay their eggs in the nests of either their own or other avian species and do not care for their offspring upon hatching (Payne 1977, Rothstein 1990). This requires the identification of parents from large sets of males and females breeding in areas where chicks are sampled. To date, studies of this type have not been attempted because multi-locus fingerprinting patterns are difficult to quantify and use for a large-scale exclusion analysis involving multiple comparisons of large numbers of adults and offspring (Queller et al. 1993). Single-locus minisatellite markers (Bruford et al. 1992) are more easily quantified and could be more useful, but they are difficult to clone and isolate (Hanotte et al. 1991).

Hypervariable single-locus microsatellite markers may be the preferred marker of choice for such analyses for a variety of reasons. Microsatellite loci consist of a variable number of tandem repeats of very short nucleotide motifs, usually fewer than five nucleotides in length (Tautz et al. 1986). Microsatellites are highly polymorphic, with heterozygosity levels reaching 90% (Dietrich et al. 1992, Beckmann and Weber 1992, Ellegren et al. 1992, Hughes and Queller 1993), abundant, and widely-distributed in the vertebrate genome (Tautz 1989, Litt and Luty 1989, Stallings et al. 1991, Dietrich et al. 1992). As a consequence of their small size, generally less than 300 base pairs (Taylor et al. 1994), microsatellite loci can be easily amplified by the PCR (Polymerase Chain Reaction). Alleles can then be scored consistently, and compared unambiguously across gels. These characteristics make microsatellite markers extremely useful to studies of kinship in natural populations (Queller et al. 1993).

In birds, previous parentage studies have used microsatellite markers to study paternity in species that are socially monogamous (Ellegren et al. 1995, Primmer et al. 1995). No studies have been attempted on species that are brood parasites even though there is wide-spread interest in determining the genetic mating system of such species (eg. Ankney and Scott 1982). Therefore, the power of microsatellites in such an analysis and the value of various analytical approaches are unknown. Here, I report on the isolation and use of microsatellite markers for parentage and kinship studies in a population of Brown-headed Cowbirds (*Molothrus ater*) at Delta Marsh, Manitoba. This population is the subject of long-term ecological, behavioural, and genetic studies of interactions between cowbirds and their hosts (Gibbs et al. in press, Hobson and Sealy 1987, 1989, Weatherhead 1989, Briskie and Sealy 1989, 1990, Neudorf and Sealy 1992, Sealy 1995, Sealy and Neudorf 1995). I demonstrate that these parentage markers permit identification of paternal, maternal, and sibling relationships within this population of cowbirds to a high degree of certainty. Thus, studies of the mating system, reproductive success and host use at the individual level are now possible for this species.

MATERIALS AND METHODS

Study Area, Trapping, and Banding Techniques:

The University of Manitoba Field Station (Delta Marsh) is located just west of the Assiniboine River Diversion on the southern shore of Lake Manitoba. Within Delta Marsh, my study site encompassed an area of approximately 1000 by 400 m parallel to the shore of Lake Manitoba consisting of dune-ridge forest and marsh habitats. More details of the study area are provided in Gibbs et al. (in press).

Tissue or blood samples were collected from cowbird chicks and adults from 17 May - 5 July, 1994. Details regarding nest searching, egg/chick collection, and blood sampling are in Chapter Three. Adult Brown-headed Cowbirds were captured in corn-baited tunnel traps positioned throughout the ridge and marsh in areas where the trapping of cowbirds had previously proven successful. Once captured, adults were banded with a unique combination of three coloured leg bands and one numbered aluminum band issued by the U.S. Fish and Wildlife Service. To ensure that most adult cowbirds in the study site had been caught, trapping was maintained throughout the breeding season. In addition, unbanded cowbird sightings were recorded daily while either systematically walking through the study area at various times during the day or while remaining stationary at locations known to be visited by cowbirds.

Microsatellite Detection, Isolation, and Primer Construction:

A microsatellite-enriched genomic library was constructed from Brown-headed Cowbird DNA using the approach described by Dawson et al. (in preparation). Briefly, genomic DNA isolated from whole blood preserved in lysis buffer (Seutin et al. 1991) of 10 individuals was pooled and then digested with AluI, HaeIII, and RsaI restriction endonucleases. Fragments 350-450 bases in length were then purified from an agarose gel and cloned into the SmaI site of pUC18 plasmid vector using a ratio of 3:1 (insert:vector) in the ligation mixture. The resulting plasmid library was transformed into *Escherichia coli* strain XL1-Blue cells and screened with filter hybridization using [α-^{32}P] dCTP-labeled $(TG)_n$, $(TC)_n$, and $(GC)_n$ polymer tracts (Pharmacia) to identify clones containing microsatellite sequences. Primary and secondary screening of roughly 16,200 colonies produced 28 positive clones.

Once colonies containing positive clones were identified, they were grown up overnight at 37°C in 5 ml Terrific Broth medium (Sambrook et al. 1989) containing 50 µg/ml ampicillin. Plasmid DNA was isolated by an alkaline lysis miniprep (Sambrook et al. 1989) and sequenced using Dideoxy sequencing (Sequenase V.2.0 [USB]) and S^{35} incorporation protocols. DNA that could not be sequenced successfully using standard Sequenase 2.0 protocols was cycle sequenced using AmpliTaq Cycle Sequencing (Perkin Elmer) and P^{33} end-labeled sequencing primers. Sequencing reactions were run on 6% denaturing polyacrylamide gels at 38 W for 3-6 hours. Gels were then dried and exposed

to X-ray film (Kodak: BIOMAX) for 1-3 days. This information was used to design primers (using Primer Ver. 0.5) to five of these clones (*Maμ* 10, 20, 23, 25, and 29).

DNA Extraction, PCR Amplification, and Allele Scoring:

Allelic variability and heterozygosity of all five Brown-headed Cowbird loci and two additional Yellow Warbler (*Dendroica petechia*) loci (*Dpμ* 15b, 16) were investigated using DNA isolated from blood samples by organic solvent purification (Sambrook et al. 1989). Sixty-five presumed unrelated Brown-headed Cowbirds from Delta Marsh, Manitoba who were defined as being residents (see below) on the study area were genotyped using these loci. PCR amplifications were performed in 10 μl reaction volumes using 50 ng genomic DNA, 0.4 pmole of the forward primer end-labeled with [γ-^{33}P] deoxyadenosine 5' triphosphate (dATP) (Dupont), 0.4 pmole of unlabeled forward primer, 0.8 pmole of unlabeled reverse primer, 300μM dNTPs, 0.5 U Amplitaq, 0.0l M Tris-HCL pH 8.3, 0.05 M KCL, 2.5 mM $MgCl_2$ and annealing temperatures ranging from 53-63 degrees C. PCR products (3.5 μl) were resolved by electrophoresis on 6% denaturing polyacrylamide gels at 55 W for approximately 2.5 hours. Gels were then dried and exposed to BIOMAX X-ray film overnight. Product sizes were determined within and among gels by reference to a sequencing reaction of a known control template, an individual of known genotype, and a clone of known size for each locus. All three were run simultaneously on the same gel.

Genetic Characterization:

Microsatellite allele frequency variation among male and female cowbirds was compared using CHIRXC (a computer program that performs homogeneity tests for RXC contingency tables) (Zaykin et al. 1993). The probability of the null-hypothesis of homogeneity among categories was calculated by way of a randomization procedure for hypothesis testing using Monte Carlo simulation (Roff and Bentzen 1989). This method was employed because small sample sizes resulted in some cells containing less than five values. The above procedure generates a null distribution of random X^2 values. The ratio of the number of instances in which a random X^2 value was greater than or equal to the observed X^2 is calculated as the probability of homogeneity. Deviations of observed genotypic proportion from those expected under Hardy-Weinberg equilibrium were also calculated using CHIHW which is a X^2 test similar to that outlined above, but which estimates the probability of agreement with genotypic proportions predicted under Hardy-Weinberg equilibrium.

Exclusion Analysis:

Forty-four males, 21 females, and 61 chicks were involved in the exclusion analyses using the program PROBMAX which was provided by R. Danzmann, Department of Zoology, University of Guelph. These adults represented the resident population at Delta Marsh during the 1994 breeding season. A resident was defined as an individual that was seen at least four times over a seven day period or more than once

over a period of more than one week (Woolfenden et al. in preparation). This definition will minimize the number of transient cowbirds that are mistakenly included as residents. It will also minimize the number of true residents that are excluded from the resident population because of their inconspicuous behaviour. Paternity by a specific male or maternity by a specific female was excluded if he/she could not have contributed either allele found in the chick at one or more of the seven loci. A match was also excluded if a male and female contributed the same allele but the chick was not homozygous at that locus. Paternity and maternity exclusion probabilities were calculated for each locus using male only and female only allele frequencies respectively, using the equation:

$PE_i = (1 - a - b)^2$ (Chakraborty et al. 1988)

where PE_i is the probability of exclusion at the ith locus, and a and b are allele frequencies in the adult population (male or female) of the alleles found in the offspring. The combined probability of exclusion for all loci [PE(C)] as in Chakraborty et al. (1988) was calculated using the following equation:

$PE(C) = 1 - \Pi(1 - PE_i)$.

Morin et al. (1994) interpreted PE(C) as the percentage of randomly chosen males or females that could be expect to be genetically excluded for a given offspring (could not have contributed to this particular offspring genotype) within a particular population.

Sibling Analysis:

I examined sibling relationships of all chicks not matched to parents through exclusion analyses using estimates of pair-wise relatedness as described in the program KINSHIP 1.0 provided by D. Queller and K. Goodnight, Department of Biology, Rice University. This program calculates genetic relatedness among pairs of individuals using the following equation:

$$r = \frac{\Sigma\Sigma\Sigma (Py - P^*)}{\Sigma\Sigma\Sigma (Px - P^*)}$$ (Queller and Goodnight 1989).

The equation is summed over individuals, loci, and allelic positions (2 for diploids) where P* is the adult population frequency of the allele present at the current locus and allele position, Px is the frequency of the current allele in the current individual (0.5 or 1.0 for a heterozygote or homozygote respectively), and Py is the frequency of the current allele in the current individual's 'partner'. These values were then compared to 95% confidence intervals for full sibling relationships obtained using a simulation within the KINSHIP program which produces a null distribution of relatedness calculations based on 1000

randomizations. Confidence intervals (95%) for half sibling and unrelated pair-wise relatedness values were also calculated to determine levels of overlap between null distributions for various levels of kinship.

RESULTS

Genetic Characterization of Microsatellite Loci:

Table 1 summarizes the genetic characteristics of five Brown-headed Cowbird and two Yellow Warbler microsatellite loci in the Delta Marsh population of Brown-headed Cowbirds (see Appendix 1 for allele frequencies). The observed variability at all seven microsatellite loci was high with the number of alleles per locus ranging from 6 to 27. Size differences between the smallest and largest allele per locus ranged from 12 base pairs (*Maμ* 10) to 64 base pairs (*Maμ* 20). Expected heterozygosities (Nei and Roychoudhry 1974) varied from 0.6768 to 0.9234 with a mean (±SD) heterozygosity of 0.830 ± 0.031. All sequenced clones were identified with the $(TG)_n$ probe and contained no less than 10 tandem repeats. Four of the seven repeat sequences were interrupted (Table 1).

I tested for differences in allele distributions among male and female cowbirds and failed to reject the null hypothesis of homogeneity of male and female allele frequency distributions in all seven sex by locus pair-wise comparisons. Associated P values ranged from 0.088 for *Maμ* 10 to 0.859 for *Dpμ* 15b (Table 2). A pooled P value, calculated by combining probabilities across all seven loci as described by Sokal & Rohlf (1981: 779) was also non-significant ($G = 13.04$; $df = 14$; $P > 0.25$).

I also tested for deviations of observed genotypic proportions from those expected under Hardy-Weinberg equilibrium for data pooled for males and females and failed to reject the null hypothesis of agreement with Hardy-Weinberg equilibrium for all

seven loci. Associated P values range from 0.147 for *Dpμ* 16 to 0.998 for *Dpμ* 15b (Table 3).

Null Alleles:

Although the allele distributions of all original seven loci conformed to predictions based on Hardy-Weinberg equilibrium, 111 out of 3887 (2.86%) of all exclusions did not involve heterozygous genotypes and thus, potentially could involve the presence of a null or nonamplifying allele(s) (Pemberton et al. 1995). An individual possessing one null allele would appear homozygous at a particular locus or would fail to amplify at all if the individual is homozygous for the null allele. Therefore, unless identical in genotype at this locus, an individual with one null allele would be inappropriately excluded as being the parent of an offspring with a null allele of the same size because they would appear to be homozygous for different alleles. For example, in our data set, one or both potential parents of six chicks were excluded entirely on the basis of a mismatch involving a homozygous genotype at the *Maμ* 29 locus. However, when data from locus *Maμ* 29 was excluded, the combined probability of correctly identifying the parents of these six chicks was high, ranging from 0.9048 to 0.9974. Thus, it seemed likely that these parents were inappropriately excluded from the analysis and that a null allele(s) was present at this locus.

To test this possibility and to examine the possible existence of null alleles at two other loci, non-overlapping alternate primer sets were constructed for *Maμ* 29, *Maμ* 10,

and *Dpμ* 15 (see Table 1). I then used the new primer sets to genotype all adults which had been classified as homozygotes with each original primer set. Comparison of individual genotypes produced using *Maμ* 29 and alternate *Maμ* 29b primer sets confirmed the presence of two null alleles at the *Maμ* 29 locus (e.g. Figure 1). The null alleles were 170 and 172 base pairs in length and were present in 6.2%, and 1.6% of the adult population respectively. Thus, approximately 8% of the alleles at this locus are nonamplifying. In contrast, *Maμ* 10b and *Dpμ* 15a alternate primer sets showed no indication of the presence of additional null alleles.

Power of Exclusion Analysis:

Exclusion analyses identified paternity and maternity for 47 and 43 chicks, respectively. Two possible fathers were identified for each of three additional chicks (but see below). All sampled males and females were excluded as the parents of 11 remaining chicks. Figure 2 shows the distribution of the marker loci at which a particular adult (male and female combined) was excluded as being the genetic parent of a given chick. Overall most of the exclusions (88.91%) involved two or more heterozygous loci. However, this is a conservative estimate of the total number of exclusions involving two or more loci in general because exclusions that were based on homozygous loci were not included in the distribution.

Exclusion probabilities for each locus and for all loci combined were also determined as in Morin et al. (1994) for all chicks that had identifiable parents. Table 4

shows male and female mean exclusion probabilities for each locus (PE_m) and for combined loci ($PE(C)_m$). Male mean exclusion probabilities range from 0.1190 for Maμ 10 to 0.7365 for Maμ 29 with a mean combined probability of exclusion over all seven loci of **0.9964**. Female mean exclusion probabilities range from 0.1120 for Maμ 10 to 0.7303 for Maμ 29 with a mean combined probability of exclusion of 0.9948. Therefore, on average, for a given offspring 99.64% of all randomly chosen non-parental males in this population would be excluded as fathers for any given offspring and 99.48% of all randomly chosen non-maternal females would be excluded as mothers of any one offspring.

Unassigned Offspring:

The 18 remaining chicks that could not be assigned both male and female parents from the sampled adults using exclusion analysis were examined using pair-wise relatedness analyses. Four of these chicks could be assigned to individual males and this information was used to aid in the construction of possible sibling groups. For each pair-wise comparison of these nestlings I calculated an r-value using the program KINSHIP 1.0. To determine the significance of the observed relatedness values, I generated 95% confidence intervals for 1) unrelated chicks, 2) paternal and maternal half siblings, and 3) full siblings (Table 5). Due to extensive overlap in relatedness distributions (Figure 3), confidence intervals were conservatively modified to 1) -0.2745 - 0.3069 for unrelated chicks, 2) 0.3070 - 0.5584 for half or full siblings, and 3) 0.5585 - 0.8214 for full siblings.

Therefore, a pair of individuals was interpreted as being unrelated if its r-value was less than or equal to 0.3069 (upper bound of 95% confidence interval for unrelated individuals), as being either half or full siblings if its r-value was greater than 0.3069 but less than 0.5584 (upper bound of 95% confidence interval for half siblings), or as unambiguous full siblings if its r-value was greater than 0.5584. I chose these criteria because I was most concerned with minimizing the occurrence of a Type I error in my data: for example, a half sibling misclassified as a full sibling or an unrelated individual misclassified as a half sibling. Note, however that 95 % confidence limits calculated from simulated null r distributions indicate that full siblings could hypothetically have an r-value as low as 0.13 whereas unrelated individuals could have r-values as high as 0.31.

Six unassigned chicks were identified as unrelated to any other chicks, whereas, based on the above criteria, 12 remaining chicks were placed into three distinct sibling groups which consisted of individuals with pair-wise r-values of between 0.2208 and 0.8509 (Table 6). Some of the r-values in all three groups fell within the confidence limits of interval 2 (0.3070 - 0.5584) which made them either half or full siblings. Since monogamy and an extremely low frequency of extra-pair fertilizations were the norm in this population of cowbirds (see Chapter Three), I assumed that most pairs with r-values falling within the confidence limits of interval 2 were full siblings. However, unambiguous unrelatedness or full sibship took precedence over this assumption if discrepancies occurred when assigning chicks to kinship groups. For example, chick 94-23 could be placed in a full sibling group with either 1) chick 94-15 ($r = 0.7446$), or, assuming that this

population is mainly monogamous, with 2) chicks 94-28, 75, 76, and 79 (r = 0.3401, 0.4177, 0.5243, and 0.3423 respectively). Chick 94-23 was grouped with chick 94-15 because their r-value indicated unambiguous full sibship whereas grouping chick 94-23 with chicks 94-28, 75, 76, and 79 involved making the assumption that all matings were monogamous.

Using these guidelines, siblings in group one (chicks 94-70, and 74) were classified as full siblings since they had an r-value of 0.5212. Group two (chicks 94-15, 23, 28, 75, 76, and 79) was subdivided into two full sibling subgroups consisting of 1) chicks 94-15, 23, and 2) 94-28, 75, 76, 79). Chick 94-23 was considered likely to be a full sibling to chick 94-76 (Table 6), based on the previous interpretation of r-values falling within interval two, but unrelated to chicks 94-75, and 79. Since chicks 94-75, 76, and 79 are unambiguously full siblings, chick 94-23 was not included as part of the full sibling group. Although chick 94-15 was also interpreted as likely a full sibling to chick 94-28, 75, 76, and 79, based on the assumption of monogamy and low extra-pair fertilization frequencies, it was grouped with chick 94-23 in a second full sibling subgroup because their r-value indicated unambiguous full sibship (Table 6). Group three (chicks 94-34, 38, 48, and 49) was also divided into two full sibling subgroups: 1) chicks 94-34, 38, 49, and 2) chick 94-48. Chick 94-48 was classified as being likely a full sibling of chicks 94-34, and 49 (r = 0.3468 and 0.3552 respectively) but it was unrelated to chick 94-38 (r = 0.2208). Since chicks 94-34, and 38, and chicks 94-38, and 49 are unambiguous full siblings (Table 6), they had to be grouped together. Therefore, chick 94-48 could not be included in a

subgroup with 94-34, and 49 because it was not a full sibling to the remaining member of the subgroup (94-38) Thus, chick 94-48 was placed into a subgroup of its own.

However, the use of an additional microsatellite locus *Cuμ* 10 (Gibbs, unpublished data), excluded one of two possible fathers in all three previously mentioned cases where paternity of a given chick was ambiguous. Therefore, chicks 94-34, 38, and 48 were determined to have the same father as chick 94-49. These chicks are at least half siblings. However, since I assumed that half siblings were rare in this population (monogamy assumption), chicks in subgroup 3.1 and 3.2 (Table 6) were placed together into one full sibling group (94-34, 38, 48, and 49).

In total, 12 chicks were placed into 4 full sibling groups of 2(94-70, 94-74), 2(94-15, 94-23), 4(94-34, 94-38, 94-48, 94-49), and 4(94-28, 94-75, 94-76, 94-79) individuals. The remaining six chicks were classified as unrelated.

DISCUSSION

Microsatellite Marker Sensitivity:

The high levels of variability of this microsatellite marker set (mean $H_{exp} = 0.83$) leading to high mean combined exclusion probabilities of 99.6% for males and 99.4% for females means that this set of genetic markers has great potential for high resolution parentage analysis in situations where the identity of neither parent is known.

Yet, several circumstances exist where the sensitivity of the above microsatellite markers may be compromised. Firstly, when using two markers concurrently for the same purpose, linkage of one marker to another may result in the power of the two markers being lower than what is expected by multiplying the individual powers (Primmer et al. 1995). Therefore, the combined power of exclusion may be less than indicated by PE(C). The probability of linkage increases with the number of loci scored, but decreases with increasing genome size. Given that the avian genome is large (approximately 1×10^6 KB) (Bachmann et al. 1972), and only seven loci were used, it seems unlikely that linkage occurs within this microsatellite marker set although this needs to be confirmed with segregation analysis. Additionally, Primmer et al. (1995) suggest that even if present the effect of linkage is outweighed by the heightening sensitivity of an expanded marker set.

Secondly, null (nonamplifying) alleles, sometimes found at microsatellite loci (see Koorey et al. 1993, Pemberton et al. 1995, Primmer et al. 1995) have the potential to decrease the sensitivity of a microsatellite marker for parentage studies. Offspring

possessing null alleles will be classified as homozygous at the respective locus and may be incorrectly matched to potential parents. Adults possessing null alleles will be unjustly excluded as possible parents unless the sibling does not carry the null allele. Null alleles will also produce lower relatedness values between siblings resulting in reduced sensitivity when detecting kinship groups.

Since no confirmed cowbird families were available, null alleles could not be detected through cases of uniparental inheritance in verified pedigrees (Callen et al. 1993). As an alternative, I detected the presence of possible null alleles by identifying instances where individuals matching at six loci were excluded as possible parents due to a probabilistically unlikely mismatch involving a homozygous genotype at a seventh locus. The presence of a null allele(s) was verified and accounted for by constructing non-overlapping alternate primer sets which were then used to amplify the same microsatellite. Two null alleles were detected at one locus (*Maμ* 29). Unlike the results of Primmer et al. (1995), these alleles were not the most common alleles at that locus and this method allowed for the detection of more than one null allele per locus. This method of dealing with null alleles is of particular value when a limited number of microsatellite markers are available due to the need to use all markers.

An alternate method of identifying the presence of null alleles involves the identification of deviations of genotypic frequencies from Hardy-Weinberg expectations in the form of a heterozygote deficiency (Chakraborty et al. 1992, Morin et al. 1994, Pemberton et al. 1995, Blouin et al. 1996, Richard et al. 1996). It is important to note

that this approach did not detect the presence of null alleles at locus *Maμ* 29 in a preliminary analysis of the data before the identification of two null alleles present in 6.2% and 1.6% of the adult population at this locus. These results indicate that testing for deviations from Hardy-Weinberg Equilibrium may not be a powerful method of determining the presence or absence of null alleles at a given locus.

In general, I feel that null alleles will not prove to be a major impediment to parentage analysis using microsatellites because 1) most exclusions tend to involve heterozygous loci (> 98 %; Figure 3), and 2) at least based on these results, null alleles may occur at low frequencies in this population (but see Primmer et al. 1995).

It is also possible that mutations at microsatellite alleles could result in false parental exclusions. It has been estimated that the average mutation rate for 30 dinucleotide repeats is approximately 5.6×10^{-4} per locus per generation in *Homo sapiens* (Goldstein et al. 1995). Assuming that mutation rates are similar in avian species and given that most exclusions in our analyses were based on at least two loci, the probability of a false exclusion occurring due to mutation is approximately 3.0×10^{-7}. Thus, it is not likely that mutations had any significant effect on exclusion results.

R-value Distribution Overlaps and the Monogamy Assumption:

There is extensive overlap between r-value confidence intervals for unrelated chicks, half siblings, and full siblings for this population of cowbirds. In a similar study of wild mice (*Mus musculus*), Blouin et al. (1996) arbitrarily chose a point half way between

the means of two distributions as the cut-off for assigning individuals into one relatedness category over another as a method of dealing with distribution overlaps. I designated chicks with an r-value falling within the overlap between unrelated and half sibling confidence intervals as unrelated and those with an r-value falling within the overlap between full and half siblings to be half siblings. I chose these criteria because I was more concerned with minimizing the occurrence of a Type I error: in which half siblings would be misclassified as full siblings or unrelated individuals would be misclassified as half siblings. Individuals with r-values falling within the overlap between full and half siblings were subsequently classified as full siblings because I assumed that monogamy and extremely low frequencies of extra-pair fertilizations were the rule in this population of cowbirds.

There is the possibility that a small number of individuals were classified as unrelated in this analysis when they were actually related because I used modified 95% confidence limits as cutoff values for unrelated, half sibling, and full sibling categories. At least a 2.5% probability still exists that an incorrect classification of a relatedness value, marginal to either tail of a confidence interval, could occur. For example, using relatedness analyses, I determined chick 94-48, in kinship group 3.2 (Table 6), to be unrelated to chicks 94-34, 38, and 49 (kinship group 3.1) because its r-value with chick 94-38 (0.2208) fell within the overlap between unrelated and half siblings. Subsequent analyses using an additional microsatellite marker (*Cuμ* 10) showed that chick 94-48 had

the same father as chicks 94-34, 38, and 49. Thus, these chicks are at least half siblings and likely full siblings given the low frequency of half-siblings in this population.

My assumption of monogamy and a low extra-pair fertilization frequency made kinship analyses relatively simple in these relatedness analyses. However, this will not always be the case in other species. Birds with more complicated mating systems (e.g. Quinn et al. 1994) would result in complex kinship relationships involving potential parents and young and thus would include a greater number of ambiguous relatedness values between individuals. One way of dealing with this potential problem might be to increase the resolution of the kinship by increasing the number of markers used. For example, Blouin et al. (1996) found that data from 20 unlinked microsatellites from a wild mouse population could easily discriminate unrelated individuals from full siblings, and half siblings from full siblings or unrelated individuals better than 80% of the time. I explored this possibility by doubling the number of loci used for my analysis by duplicating the data for each locus (total loci = 14) and then recalculating the r-value confidence intervals for unrelated individuals, half siblings, and full siblings. This doubling reduced the overlap between full sibling and unrelated intervals completely while overlap between unrelated and half sibling intervals, and full sibling and half sibling intervals were reduced by approximately 0.2 units (45%). In addition, confidence intervals were shifted by -0.2 units resulting in more calculated r-values falling within the unambiguous full sibling category. Therefore, increasing the number of loci would increase the sensitivity of these relatedness analyses. A large enough marker set may allow unambiguous determination of

unrelated, half sibling, and full sibling individuals. For cowbirds, another alternative that may increase the resolution of the kinship analyses would involve assigning mothers to chicks based on egg markings and morphology assuming it is proven that eggs laid by an individual female have unique shell characteristics as has been documented by Dufty (1983).

Applications:

This microsatellite marker set will prove useful for studies of parentage and kinship in populations of Brown-headed Cowbirds and thus has the potential to address a wide range of outstanding questions related to individual behaviour in this species. For example, use of these markers will allow 1) direct quantification of the genetic mating system of this species which has been described as varying from strictly monogamous (Laskey 1950, Dufty 1982, Yokel 1986) to promiscuous (Nice 1937, Elliot 1980) in different geographic locations, 2) will permit accurate estimates of male and female reproductive success, and hence, patterns of mate choice and sexual selection within a population of this species, and 3) tests of the hypothesis that individual females are host generalists within cowbird populations.

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Table 1: Characteristics of seven passerine microsatellite loci used for parentage analysis in Brown-headed Cowbirds. The number of different alleles per locus was based on 65 individuals. T_{opt} refers to the annealing temperature at which PCR amplification was performed. H_{exp} refers to expected heterozygosity (Nei and Roychoudhry 1974). [1] Dawson et al. (submitted). [2] Non- overlapping primer sets used to amplify loci, *Dpμ* 15b, *Maμ* 10, and *Maμ* 29 to check for null alleles at these loci (see text). na = not applicable.

Locus	PCR product length (bp)	T_{opt} (°C)	Repeat motif	Primer sequences (5'-3')	Number of alleles	H_{exp}
Dpμ 15b[1]	131	53	$(AC)_{12}AT(AC)_2$	F - AGGATGAACAAATTATCAAGAGA R - GATAAATCACATAAGTGGGAACA	17	0.9124
Dpμ 16[1]	162	55	$(AC)_{12}$	F - ACAGCAAGGTCAGAATTAAA R - AACTGTTGTGTCTGAGCCT	10	0.6768
Maμ 10	160	63	$(TG)_{10}$	F - ATCCCTCATTTTGGCTCTTA R - GGAGGAGGTTTGCACAGT	6	0.6872
Maμ 20	147	55	$(GT)_{25}AT(GT)_2(T)_2(GT)_5$	F - TAAAAACAACAACAGCAAAC R - ACTCAACGCCTGTAGTAGTAA	18	0.8956
Maμ 23	157	61	$(TG)_3(T)_3(TG)_{15}$	F - TGCCAGTATTCTCTTGTGCTT R - CTGTGGGATGTAGGAATTGTG	9	0.7960
Maμ 25	132	55	$(AC)_{15}$	F - GTTGCTTCTTACCACCTATTC R - GTAACACAGATGAATGGATGA	27	0.9161

Table 1 (con't)

Locus	PCR product length (bp)	T_{opt} (°C)	Repeat motif	Primer sequences (5'-3')	Number of alleles	H_{exp}
Maμ 29	150	55	$(CA)_6GA(CA)_{14}$	F - CCCAAACCCTTCTATGATTC R - GATTCTGACAGCAAGGAGTCT	23	0.9234
Dpμ 15[2]	143	55	$(AC)_{12}AT(AC)_2$	F - GGCTGCAAACTCATTATCTC R - ATTGAGTCTGTCAGGTCCAG	na	na
Maμ 10b[2]	142	56	$(TG)_{10}$	F - AGAGACCCACTAGGCTCC R - CTGCTGGAGAACTTCGAG	na	na
Maμ 29b[2]	130	56	$(CA)_6GA(CA)_{14}$	F - GAACCAGATCTTTAAGGTCCT R - CAATTTGGCTGATTAAGTTCT	na	na

Table 2: Results of contingency table analyses testing for homogeneity of microsatellite allele distributions among male and female cowbirds. Chi-square values (degrees of freedom) for the comparison at each locus are given along with the estimated probability of homogeneity (± 95% CI) as determined by Monte Carlo testing (Roff and Bentzen 1989). The combined Chi-square value resulting from comparison across all seven loci is also shown (calculated as described by Sokal and Rohlf 1981, p. 779).

Locus	**X^2 Value (df)**	**P(± 95% CI)**
Dpμ 15b	10.80 (16)	0.859 (± 0.022)
Dpμ 16	9.06 (9)	0.448 (± 0.031)
Maμ 10	9.13 (5)	0.088 (± 0.018)
Maμ 20	16.86 (16)	0.467 (± 0.031)
Maμ 23	6.82 (8)	0.626 (± 0.031)
Maμ 25	24.05 (26)	0.634 (± 0.030)
Maμ 29	26.01 (22)	0.235 (± 0.026)
Combined	13.04 (14)	>0.25

Table 3: Results of contingency table analyses testing for deviations of observed genotypic proportion from those expected under Hardy-Weinberg equilibrium. Selander's D (an index of excess heterozygosity) and chi-square values (degrees of freedom) for the comparison at each locus are given along with the estimated probability of compliance with Hardy-Weinberg equilibrium (± 95% CI) as determined by Monte Carlo testing (Roff and Bentzen 1989).

Locus	**Selander's D**	**X^2 Value (df)**	**P(± 95% CI)**
Dpμ 15b	0.013	156.57 (136)	0.998 (± 0.003)
Dpμ 16	-0.007	50.09 (36)	0.147 (± 0.023)
Maμ 10	-0.015	11.93 (15)	0.482 (± 0.031)
Maμ 20	0.055	157.65 (153)	0.997 (± 0.003)
Maμ 23	0.001	31.90 (36)	0.449 (± 0.031)
Maμ 25	-0.027	406.16 (325)	0.989 (± 0.006)
Maμ 29	-0.086	252.49 (253)	0.994 (± 0.005)

Table 4: Mean exclusion probabilities (PE_m) for each locus and for combined loci ($PE(C)_m$) (± 95%CI) (calculating PE and PE(C) as described in Morin et al. 1994). Male calculations based on 47 cases where an adult male was identified as the male parent of a chick whereas female data based on 43 cases where an adult female was identified as the female parent of a chick.

	Male			Female		
Locus	**# of alleles**	**PE_m**	**$PE(C)_m$**	**# of alleles**	**PE_m**	**$PE(C)_m$**
Maμ 29	19	0.7365 (± 0.0034)	0.7365 (± 0.0034)	19	0.7303 (± 0.0103)	0.7303 (± 0.0103)
Dpμ 15b	17	0.7302 (± 0.0040)	0.9263 (± 0.0017)	14	0.6784 (± 0.0112)	0.9058 (± 0.0057)
Maμ 25	24	0.7031 (± 0.0062)	0.9797 (± 0.0006)	16	0.6783 (± 0.0144)	0.9715 (± 0.0021)
Maμ 20	16	0.6344 (± 0.0055)	0.9927 (± 0.0003)	15	0.6153 (± 0.0096)	0.9886 (± 0.0010)
Maμ 23	9	0.3648 (± 0.0063)	0.9952 (± 0.0002)	8	0.3426 (± 0.0213)	0.9924 (± 0.0008)
Dpμ 16	9	0.2258 (± 0.0086)	0.9959 (± 0.0002)	7	0.2411 (± 0.0202)	0.9941 (± 0.0007)
Maμ 10	5	0.1190 (± 0.0044)	0.9964 (± 0.0002)	5	0.1120 (± 0.0089)	0.9948 (± 0.0006)

Table 5: Lower and upper 95% confidence limits for relatedness values of various pedigree relationships. The probability that 2 individuals share an allele by immediate descent from their mother or father are indicated by r_m and r_p respectively.

			95% Confidence limits	
Relationship	r_m	r_p	**Lower**	**Upper**
Full Siblings	0.5	0.5	+0.1333	+0.8214
Half Siblings	0.5	0.0	-0.0737	+0.5584
Half Siblings	0.0	0.5	-0.0910	+0.5486
Unrelated	0.0	0.0	-0.2745	+0.3069

Table 6: Relatedness values for pairs of chicks in kinship groups two and three calculated as in Queller and Goodnight (1989).

Group 2	chick#	94-15	94-23	94-28	94-75	94-76	94-79
Subgroup 2.1	94-15	———					
	94-23	0.7446	———				
Subgroup 2.2	94-28	0.3401	0.2738	———			
	94-75	0.4177	0.2315	0.7352	———		
	94-76	0.5243	0.3520	0.3299	0.6422	———	
	94-79	0.3423	0.2669	0.6331	0.6703	0.5559	———

Group 3	chick#	94-34	94-38	94-49	94-48
Subgroup 3.1	94-34	———			
	94-38	0.8509	———		
	94-49	0.4084	0.5593	———	
Subgroup 3.2	94-48	0.3468	0.2208	0.3552	———

Figure 1: Microsatellite profiles for a family of Brown-headed Cowbirds obtained using primer set *Maμ* 29 (Profile 1) as well as alternate primer set *Maμ* 29b (Profile 2). Lanes containing individual chick profiles are numbered 1 - 6 whereas lanes containing maternal and paternal profiles are labeled with an M (mother) and F (father) respectively. Two size markers consisting of amplified fragments 130 and 150 bp in size are labeled with a '+'. Maternal and paternal alleles are identified by M1, M2 and F1, F2 respectively. One null allele present in both mother (M), and chicks 1, 2, 5, and 6 are also identified by M2 in family Profile 2.

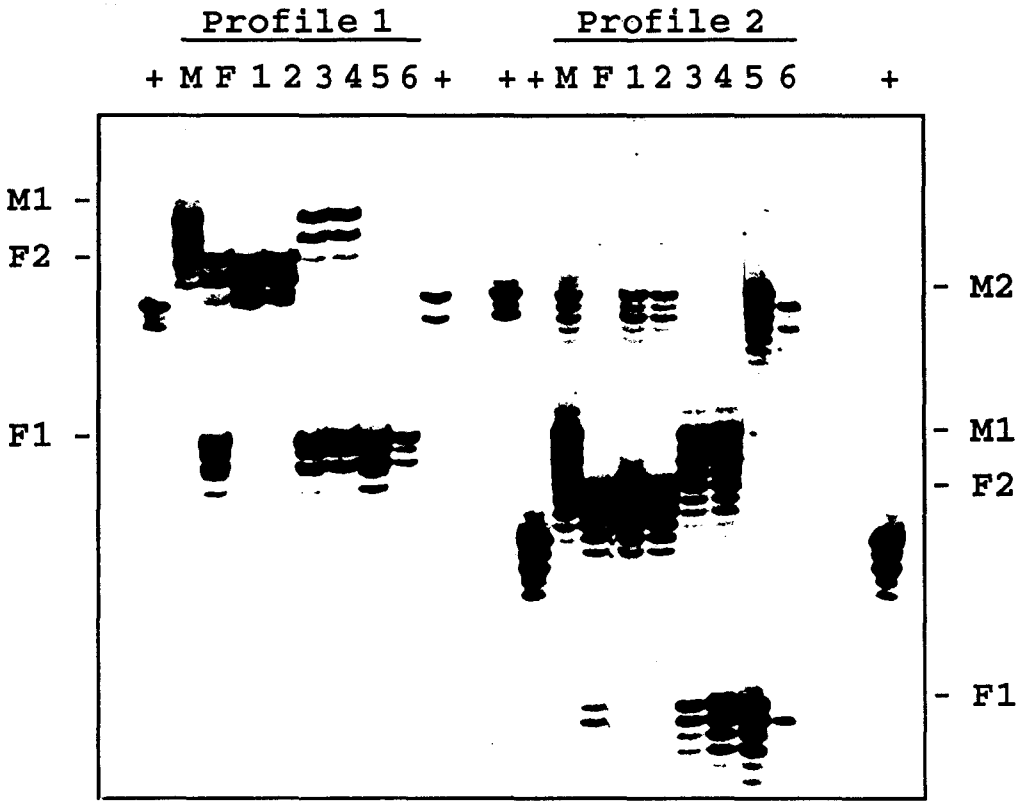

Profile 1
Profile 2
+ M F 1 2 3 4 5 6 +
+ + M F 1 2 3 4 5 6 +
M1 -
F2 -
F1 -
- M2
- M1
- F2
- F1

Figure 2: Total number of parental exclusions based on heterozygous loci accounting for the presence of null alleles at microsatellite locus *Maμ* 29.

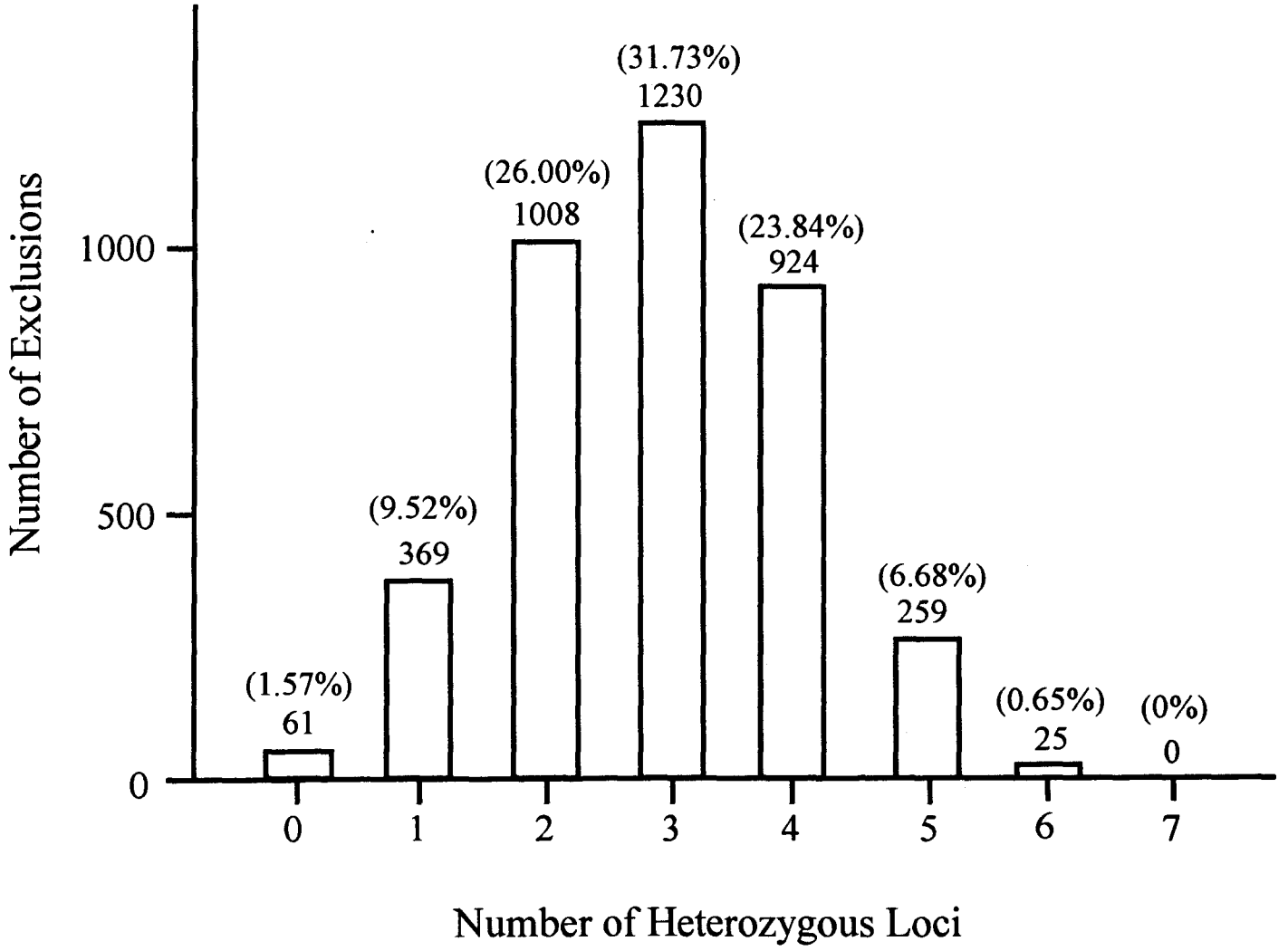
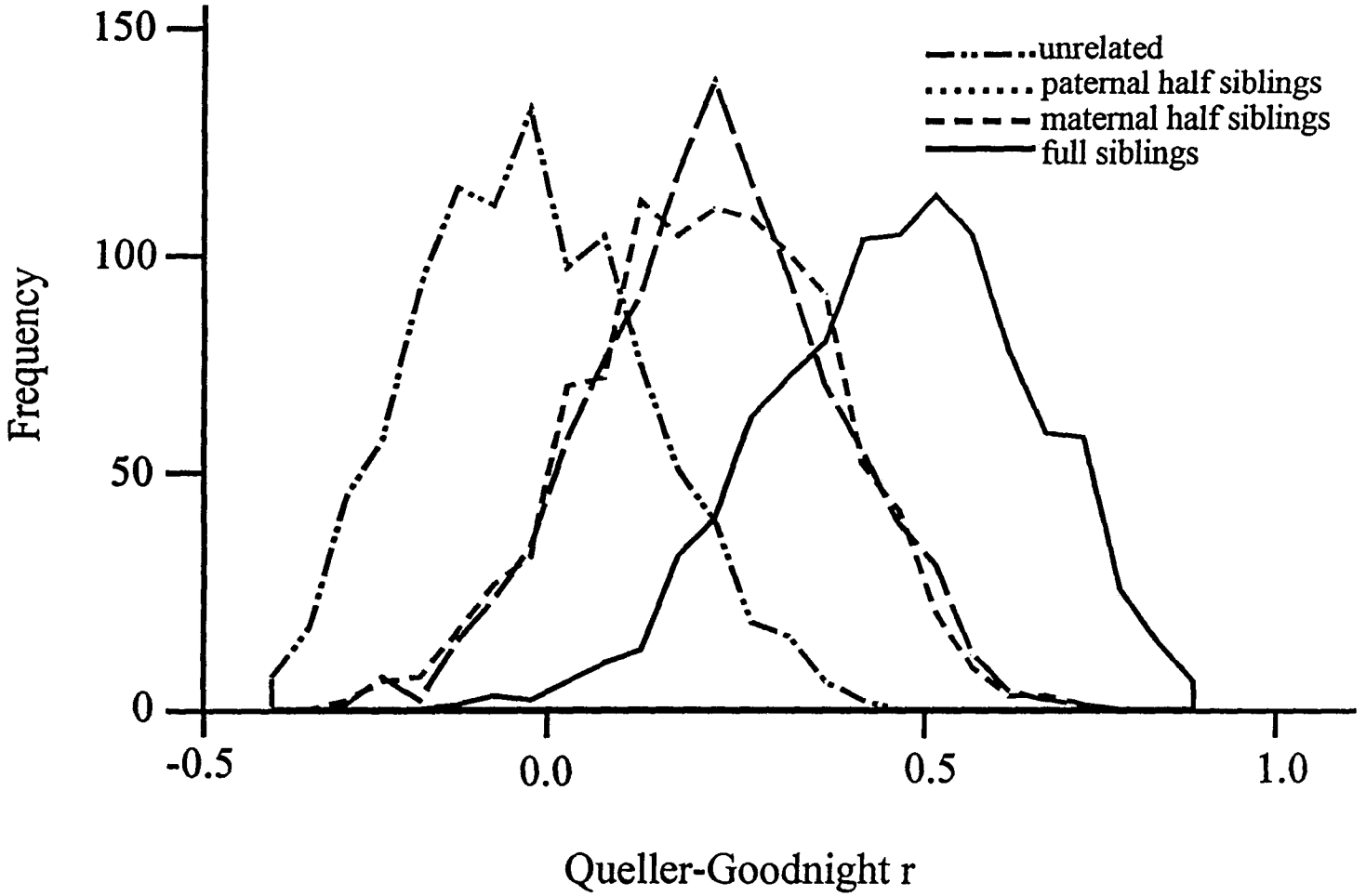

Number of Exclusions
1000
500
0
(1.57%)
61
(9.52%)
369
(26.00%)
1008
(31.73%)
1230
(23.84%)
924
(6.68%)
259
(0.65%)
25
(0%)
0
0
1
2
3
4
5
6
7
Number of Heterozygous Loci

Figure 3: Probability distributions for relatedness values (r) of unrelated, maternal and paternal half siblings, and full siblings.


150
100
50
0
Frequency
-0.5
0.0
0.5
1.0
Queller-Goodnight r
unrelated
paternal half siblings
maternal half siblings
full siblings

Appendix 1: Allele frequency distributions of seven microsatellite loci assayed from the adult cowbird population at Delta Marsh.

Locus	Allele size (bp)	Sex: Male (N=44)	Sex: Female (N=21)	Pooled (N=65)
a) *Dpμ* 15b	161	0.0114	0.0	0.0077
	159	0.0114	0.0	0.0077
	157	0.0341	0.0238	0.0308
	153	0.0455	0.0952	0.0615
	151	0.0455	0.0476	0.0461
	149	0.0227	0.0238	0.0231
	147	0.0682	0.0952	0.0769
	145	0.1136	0.1429	0.1231
	143	0.1475	0.1190	0.1385
	141	0.0341	0.0	0.0231
	139	0.1023	0.0238	0.0769
	137	0.1364	0.1667	0.1462
	135	0.0227	0.0716	0.0385
	133	0.0341	0.0238	0.0308
	131	0.0455	0.0952	0.0615
	129	0.0568	0.0476	0.0538

Appendix 1 (con't)

Locus	Allele size (bp)	Sex: Male (N=44)	Sex: Female (N=21)	Pooled (N=65)
Dpμ 15b (con't)	125	0.0682	0.0238	0.0538
b) *Dpμ* 16	168	0.0114	0.0	0.0077
	160	0.0227	0.0	0.0154
	158	0.0227	0.0476	0.0308
	156	0.0114	0.0238	0.0154
	154	0.0341	0.0953	0.0538
	152	0.4431	0.2857	0.3923
	150	0.3750	0.4524	0.4000
	148	0.0682	0.0714	0.0692
	146	0.0	0.0238	0.0077
	144	0.0114	0.0	0.0077
c) *Maμ* 10	166	0.1023	0.0238	0.0769
	164	0.2273	0.2381	0.2308
	162	0.1932	0.2143	0.2000
	160	0.0	0.0714	0.0231

Appendix 1 (con't)

Locus	Allele size (bp)	Sex: Male (N=44)	Sex: Female (N=21)	Pooled (N=65)
Maμ 10 (con't)	158	0.4658	0.4524	0.4615
	154	0.0114	0.0	0.0077
d) *Maμ* 20	159	0.0114	0.0	0.0077
	153	0.0	0.0238	0.0077
	151	0.0114	0.0238	0.0154
	149	0.0341	0.0715	0.0462
	147	0.0455	0.0238	0.0384
	145	0.0794	0.1190	0.0923
	143	0.0794	0.1190	0.0923
	141	0.1023	0.0715	0.0923
	139	0.0114	0.0238	0.0154
	137	0.0455	0.0	0.0308
	133	0.0	0.0715	0.0231
	131	0.1818	0.1667	0.1769
	129	0.1364	0.0952	0.1231
	127	0.0341	0.0476	0.0384

Appendix 1 (con't)

Locus	Allele size (bp)	Sex		
		Male (N=44)	Female (N=21)	Pooled (N=65)
Maμ 20 (con't)	125	0.0682	0.0238	0.0538
	123	0.0114	0.0238	0.0154
	115	0.0227	0.0	0.0154
	095	0.1250	0.0952	0.1154
e) *Maμ* 23	165	0.0114	0.0	0.0077
	163	0.0114	0.0238	0.0154
	161	0.0114	0.0476	0.0231
	159	0.1591	0.1905	0.1692
	157	0.2273	0.1905	0.2154
	155	0.2954	0.3810	0.3231
	153	0.1591	0.0476	0.1231
	151	0.0341	0.0476	0.0384
	149	0.0908	0.0714	0.0846
f) *Maμ* 25	181	0.0114	0.0	0.0077
	179	0.0455	0.0	0.0308
	175	0.0114	0.0	0.0077

Appendix 1 (con't)

Locus	Allele size (bp)	Sex		
		Male (N=44)	Female (N=21)	Pooled (N=65)
Maμ 25 (con't)	173	0.0114	0.0	0.0077
	169	0.0226	0.0238	0.0231
	167	0.0	0.0476	0.0154
	165	0.0114	0.0	0.0077
	163	0.0114	0.0	0.0077
	161	0.0568	0.0952	0.0691
	159	0.0341	0.0238	0.0308
	157	0.0910	0.1191	0.1000
	155	0.0114	0.0	0.0077
	153	0.0114	0.0476	0.0231
	151	0.0341	0.0	0.0231
	149	0.0795	0.0476	0.0691
	147	0.0	0.0238	0.0077
	145	0.0114	0.0	0.0077
	143	0.0114	0.0	0.0077
	141	0.0	0.0238	0.0077
	139	0.0226	0.0476	0.0308

Appendix 1 (con't)

Locus	Allele size (bp)	Sex: Male (N=44)	Sex: Female (N=21)	Pooled (N=65)
Maμ 25 (con't)	137	0.0455	0.0476	0.0462
	135	0.0910	0.0476	0.0769
	133	0.0682	0.1991	0.0846
	132	0.0226	0.0	0.0154
	131	0.0226	0.0715	0.0384
	129	0.0568	0.0238	0.0462
	127	0.2045	0.1905	0.2000
g) *Maμ* 29	180	0.0	0.0476	0.0154
	178	0.0	0.0476	0.0154
	176	0.0114	0.0	0.0077
	174	0.0114	0.0238	0.0154
	172	0.1364	0.0953	0.1230
	170	0.0341	0.0238	0.0308
	166	0.0227	0.0	0.0154
	162	0.0114	0.0476	0.0231
	160	0.0	0.0238	0.0077

Appendix 1 (con't)

Locus	Allele size (bp)	Sex		
		Male (N=44)	Female (N=21)	Pooled (N=65)
Maμ 29 (con't)	158	0.0567	0.0714	0.0615
	156	0.0682	0.0	0.0462
	154	0.1477	0.0953	0.1308
	152	0.0454	0.0238	0.0384
	150	0.0114	0.0238	0.0154
	148	0.0682	0.0238	0.0538
	146	0.0454	0.0953	0.0615
	144	0.1023	0.1905	0.1308
	142	0.0567	0.0238	0.0462
	140	0.0	0.0238	0.0077
	138	0.0682	0.0476	0.0615
	136	0.0682	0.0476	0.0615
	132	0.0114	0.0	0.0077
	126	0.0227	0.0238	0.0231

CHAPTER 3

Female Host Specificity and the Mating System of an Obligate Brood Parasitic Bird, The Brown-headed Cowbird (*Molothrus ater*): Evidence From Parentage Analysis Using DNA Markers

ABSTRACT

This study combines genetic and behavioural observations taken in the field to examine the mating system, and host specificity of a Brown-headed Cowbird (*Molothrus ater*) population. Parentage analyses using genotypes from multiple microsatellite loci for males, females, and offspring studied show that eggs laid by an individual female are almost always fertilized by a single male, and that most eggs fertilized by a single male are laid by a single female. Examination of behavioural and genetic data indicate that the male observed most frequently with a female is usually the father of her offspring and that the female observed most frequently with a male is likely to be the mother of his offspring. Thus, these results show that monogamy is the predominant mating system in this population of cowbirds. Parentage and kinship analyses further suggest that most females usually lay their eggs in the nests of more than one host species, and that habitat type may

be an important factor in determining which host nests are parasitized by individual cowbirds. Behavioral data indicate a large overlap between individual male, and female non-feeding ranges but little overlap between female egg laying ranges. Overall, these results demonstrate that genetic and behavioural mating systems in this species coincide, that females may defend exclusive egg laying ranges, and that the previous characterization of cowbirds as host generalists is upheld.

INTRODUCTION

Interspecific brood parasitic birds lay their eggs in the nests of other avian species and abandon their offspring to the care of foster parents. Obligate interspecific brood parasitism is a reproductive strategy adopted by about 1% of all bird species (Rothstein 1990). The Brown-headed Cowbird (*Molothrus ater*) is the most common obligate interspecific brood parasite in North America. Its reproductive behaviour has been widely studied (for reviews see Friedmann 1929, Laskey 1950, Darley 1982, Payne 1973, Elliot 1980, Ankney and Scott 1982, Dufty 1982a, 1982b, Teather and Robertson 1986, Yokel 1986, 1989). In particular, two aspects of cowbird reproductive behaviour have received the most attention: 1) their mating system, and 2) female host specificity.

Cowbirds present an ideal system with which to study the evolution of mating systems and reproductive strategies. Cowbirds do not build nests or care for their offspring. Therefore, mating system theories suggest that there are no direct benefits in adopting a monogamous mating system in which both male and female parents can potentially provide assistance in raising offspring (Lack 1965, Payne 1977). A polygamous mating system where mate choice is based solely on genetic quality (Trivers 1972) may be more adaptive. However, some behavioural studies do suggest that cowbirds are monogamous (Laskey 1950, Dufty 1982a, 1982b, Yokel 1986). Therefore, cowbirds may be receiving indirect benefits (in addition to 'good genes') from a monogamous mating system that are not necessarily available in other mating systems.

Host choice of a brood parasite is also significant as it can influence a parasites' reproductive success (Friedmann 1963, Rothstein 1976, Payne 1977, Scott 1977). Choosing low-quality hosts may lead to a reduction in reproductive success through poor parenting (Rothstein 1976), while some high-quality hosts may decrease reproductive success because they eject parasite eggs (Rothstein 1975, Scott 1977). Cowbirds are generally accepted to be host generalists as a species but host use strategies adopted by individual females are not well known (but see McGeen and McGeen 1968, Elliot 1977, Fleischer 1985).

Below I review what is known about these features of cowbird behaviour and then discuss the use of genetic techniques to examine both behaviours within a single population.

Mating System:

The potential to increase fitness through parental care, despite the reduction of opportunities for additional matings, is a critical component of present hypotheses concerning avian mating systems (Hamilton and Orians 1965, Trivers 1972, Emlen and Oring 1977). It is estimated that greater than 90% of avian species are monogamous because each male and each female, on average, will leave more descendants than polygamous individuals or groups if they share in raising a brood (Lack 1968). Alternatively, polygamy should prevail in situations where parental requirements are minimal because less time is devoted to the incubation, feeding, and protection of

offspring and a greater amount of time is available to search for mates (Emlen and Oring 1977).

Brown-headed Cowbirds do not build nests of their own nor do they raise their own young and hence are relieved of the constraints of parental care. Therefore, their reproductive behaviour is not confined to a single nest and they are free to search for additional mates. Conventional theories suggest that these birds should be polygamous; however, the degree to which polygamy occurs depends on the ability of the cowbird to take advantage of an opportunity to pursue a polygamous relationship. This ability may be affected by several ecological factors such as the spatial distribution of resources (host nests) or the temporal distribution of mates (Emlen and Oring 1977).

Previous studies differ in their characterization of cowbird mating systems. Brown-headed Cowbirds have been reported to be monogamous (Laskey 1950, Dufty 1982a, 1982b, Yokel 1986), polygynous (Payne 1973), promiscuous (Elliot 1980), monogamous and polyandrous (Friedmann 1929), monogamous and polygynous (Teather and Robertson 1986), and monogamous and promiscuous (Nice 1937, Ankney and Scott 1982, Darley 1982). This variation in the mating system of the Brown-headed Cowbird is often explained as being due to the effects of ecological factors. Some of the more important factors invoked include sex ratio, host nest distribution, and cowbird population densities, which may vary geographically. For example, Teather and Robertson (1986) found that a male biased sex ratio may influence the mating system by weighting the advantage of mate guarding and ultimately select for monogamy. Alternately, they

suggest that the distribution of host nests may make territorial defence uneconomical, thus preventing a close association between male and female and leading to promiscuity. In addition, Yokel (1989) suggests that higher cowbird population densities may be associated with a greater degree of promiscuity.

A problem with most previous studies is that the results are based solely on behavioural observation of courtships (males and females seen associating on a regular basis), mate guarding, or following behaviours (i.e. Laskey 1950, Darley 1982, Dufty 1982b, Teather and Robertson 1986, Yokel 1989). Work on other avian species (Bray et al. 1975, Martin 1980, Roberts and Kennelly 1980, Ford 1983, Gibbs et al. 1990) suggests that these data are not sufficient for an accurate description of mating patterns (but see Yokel 1989).

The most comprehensive behavioural study to date based on observed copulations (Yokel 1986) suggests that cowbirds are generally monogamous in Eastern California. However, this study does not consider the possibility that all copulations may not be successful. For example, Westneat (1987) found that attempted extra-pair copulations were less likely to make cloacal contact than within-pair copulations in Indigo Buntings (*Passerina cyanea*), suggesting that observed copulations may not accurately estimate paternity of offspring.

Extensive behavioural observation indicates that the classification of the Brown-headed Cowbird mating system is uncertain and may vary geographically. Genetic

techniques involving the identification of paternal, maternal, and sibling relationships will allow the direct quantification of cowbird mating systems.

Host Specificity:

Cowbirds lay their eggs in the nests of several different host species. Host specificity has important consequences for the fitness of an avian brood parasite (Friedmann 1963, Rothstein 1976, Payne 1977, Scott 1977) and can influence the evolution and complexity of the host-parasite relationship (Hamilton and Orians 1965). If offspring of some females are better adapted for one host species over another, or if parasites imprint on the hosts that raise them (Friedmann and Kiff 1985), then some preference should be observed in their selection of hosts (Fleischer 1985). The decrease in host reproductive success as a result of the parasitism results in selection for host defences such as rejection of the parasitic egg through egg ejection (Neudorf and Sealy 1992), clutch abandonment (Burgham and Picman 1989), or increased nest defense early in the nesting cycle (Burgham and Picman 1989, Briskie and Sealy 1989, Neudorf and Sealy 1992). Host rejection has a direct impact on the parasites' fitness and may select for new parasite defences such as egg mimicry (Rothstein 1990), egg removal, or a shorter incubation period (Briskie and Sealy 1990), especially if host choice is limited. This interaction between parasite and host may lead to the specialization of the parasite on one or a few hosts as a consequence of a heightened parasite-host arms race (Rothstein 1990).

Little is known about the laying habits and host selection of individual female Brown-headed Cowbirds (Friedmann 1963, Payne 1977). Although most behavioural studies suggest that cowbirds parasitize multiple host species (Friedmann 1929, p 177-188, Jones 1941, McGeen & McGeen 1968, Elliot 1977), a few suggest that some individuals may be host specialists (Walkinshaw 1949, McGeen & McGeen 1968).

The use of genetic techniques allows for the determination of host specificity of individual cowbirds (Fleischer 1985), and also examination of the ecological factors which may be important in host choice. For example, Mason (1986) found that while Shiny Cowbirds (*Molothrus bonariensis*) exploit both large and small host species, larger species are preferred.

Molecular Markers:

Recent developments in the use of DNA-based genetic markers have allowed the direct quantification of reproductive behaviour, and allowed inferences to be made regarding descent in birds (e.g. Quinn et al. 1987, Gyllensten et al 1990, Westneat 1990; Lifjeld et al. 1993, Gibbs et al. 1994). One class of genetic markers used for this type of analysis are the hypervariable single locus microsatellite markers. Single locus microsatellite markers are preferred for a variety of reasons (see Chapter Two); however, the ease at which they can unambiguously resolve parentage among individuals in natural populations is unprecedented. Although previous studies have used microsatellite markers to examine parentage in socially monogamous species of known paternity (Ellegren et al.

1995, Primmer et al. 1995), none have been conducted on brood parasites in which no a priori knowledge of the parentage of a particular offspring is available. Social parentage of brood parasitic birds is uncertain because these species lay their eggs in the nests of either their own or other avian species and do not care for their offspring upon hatching (Payne 1977, Rothstein 1990). Chapter Two reported on the characterization of seven microsatellite markers which resulted in paternity and maternity exclusion probabilities of 0.9964 and 0.9948, respectively in a marked population of cowbirds. Thus, the tools are now available to resolve unambiguously parentage among individuals in natural populations of Brown-headed Cowbirds.

Most studies to date present conflicting results on the reproductive behaviour of the Brown-headed Cowbird because individual female breeding activities are distributed among many host nests making egg laying and copulatory activities difficult to study. Thus, key characteristics of the behavioural ecology of both the mating system and host specificity remain unknown. Here I use recently developed DNA-based techniques to examine both aspects of cowbird behaviour. I report on the use of microsatellite markers to examine parentage, kinship, and host specificity in a Brown-headed Cowbird population at Delta Marsh over one breeding season. Combining these data with observations of adult social behaviour allows for the examination of the relationship between genetic and social pairs. Furthermore, it allows the examination of host preference of individual females, providing insight into the mating and egg laying activities of individuals in this population.

MATERIALS AND METHODS

Study Area:

This research was conducted at the University of Manitoba Field Station (Delta Marsh) which is located just west of the Assiniboine River Diversion on the southern shore of Lake Manitoba (Figure 1). Within Delta Marsh my study site encompassed an area of approximately 1000 x 400 metres parallel to the shore of Lake Manitoba and consisted of distinct but adjacent dune-ridge forest and cattail marsh habitats. This study site was selected for several reasons:

1) Cowbird parasitism rates are extremely high (Robertson and Norman 1977, Weatherhead, 1989), thereby increasing the likelihood that an adequate sample size can be obtained. 2) Heavily parasitized Red-winged Blackbird (*Agelaius phoeniceus*), and Yellow Warbler (*Dendroica petechia*) hosts occupy distinct but adjacent habitats which extend along most of the southern shore of Lake Manitoba (Weatherhead 1989). Comparison of cowbird chicks from two ecologically distinct habitats (dune-ridge forest/marsh) allows for examination of host specificity and some of the ecological factors which may be important in host choice by cowbirds. 3) Because Red-winged Blackbirds and Yellow Warblers occupy distinct habitats, habitat preference (if present) and host preference may be confounded. The presence of a substantial number of parasitized Song Sparrow (*Melospiza melodia*) nests in Red-winged Blackbird habitat (cattail marsh)

allows for the examination of the importance of habitat type vs host species in cowbird nest preference.

Trapping, Banding, and Sampling techniques:

Tissue or blood samples were collected from cowbird chicks and adults from 17 May - 5 July, 1994. Nests at the study site were located, flagged, and monitored daily for Brown-headed Cowbird eggs. Emphasis was placed on finding Red-winged Blackbird, Yellow Warbler, and Song Sparrow nests as they are known to be heavily parasitized at this study site. Cowbird eggs were collected and incubated for up to ten days to ensure adequate tissue development prior to storage in sealed plastic bags at -20^{o}C. Blood samples (15-50 *u*l) were taken from the jugular vein (see Hoysak and Weatherhead 1991) of nestling cowbirds no less than 4 days post-hatching and were stored in 200-500 *u*l of 1XQueens Lysis Buffer (0.01M Tris/ 0.1M EDTA/ .01M NaCl/1%n-laurylsarcosine, pH 7.5) at 4^{o}C.

Adult Brown-headed Cowbirds were captured in corn-baited tunnel traps positioned throughout the marsh in areas where the trapping of cowbirds had previously proven successful. Once captured, adults were banded with a unique combination of three coloured leg bands and one numbered aluminum band issued by the U.S. Fish and Wildlife Service as described in Chapter Two. Blood samples (15-100 *u*l) were taken from the jugular or brachial veins and stored in 200-1000 *u*l of lysis buffer respectively at 4^{o}C.

Adult weights and flat wing (shoulder to longest primary tip) measurements were also recorded and birds were aged as described in Pyle et al. (1987).

Field Observations:

Observations of individual birds were recorded while either systematically walking through the study area at various times during the day, or remaining stationary at locations known to be visited by cowbirds. Behavioural observations included date, time, period of observation, band combinations of participants, vocalizations, behaviour, and locations of birds sighted to the nearest 1/8 quadrat (5000 sq. metres). The non-feeding ranges (area in which an individual confined all activities except feeding (see Dufty 1982b)) of individual males and females were plotted on a map. Range sizes were estimated by joining the outermost points of the observations of individual cowbirds (Darly 1983, Dufty 1982b) and areas were determined using a computerized digitizer (Roff and Hopcroft 1986).

Behaviour of individual cowbirds was classified into the following categories: 1) Courtship [copulation, mate guarding (Darley 1982), male high intensity song spread accompanied by bowing (Yokel et al. 1991) or close following (Darley 1982, Teather and Robertson 1986) behaviours] 2) Aggressive [one or a combination of chattering (a female aggressive vocalization; Dufty 1982a), bill pointing, chasing, or a male placing himself between female and an interloper while directing song spreads at the intruding male (mate guarding)] 3) Non-aggressive [individuals come within 10m of each other without

displaying aggressive behaviours (Teather and Robertson 1985); lone male gives low intensity 'song spread' with accompanying 'accent song' from a high perch; or foraging behaviour]. These data were used to classify social 'pair bonds' between males and females.

Microsatellite Loci Genotypes:

Five oligonucleotide primers designed from *Molothrus ater* genomic DNA and two from *Dendroica petechia* genomic DNA were used to amplify simple sequence repeats in *Molothrus ater* genomic DNA using the polymerase chain reaction (PCR) (see Chapter Two). Genomic DNA was isolated from whole blood or tissue samples (Sambrook et al. 1989) from a total of 65 presumed unrelated adults and 61 chicks. PCR products (3.5 μl) were resolved by electrophoresis on 6% denaturing polyacrylamide gels at 55 W for approximately 2.5 hours. Gels were then dried and exposed to X-ray film overnight. Product sizes were determined within and among gels by reference to a sequencing reaction of a known control template, an individual of known genotype, and a clone of known size for each locus. All three were run simultaneously on the same gel.

Mating System and Fecundity:

The Delta Marsh resident population (1994 breeding season) of 44 males and 21 females, and 61 chicks were examined using a marker set consisting of seven microsatellites. Paternity and maternity exclusion probabilities for this marker set were

determined to be 0.9964 and 0.9948 respectively (see Chapter Two) for this population of cowbirds. A resident was defined as an individual that was seen at least four times over a seven day period or more than once over a period of more than one week. This definition was adopted to minimize the number of migrant cowbirds that are mistakenly included as residents. It will also minimize the number of true residents that are excluded from the resident population because of their inconspicuous behaviour. Paternity by a specific male or maternity by a specific female was excluded if he/she could not have contributed either allele found in the chick at one or more of the seven loci. A match was also excluded if a male and female contributed the same allele but the chick was not homozygous at that locus.

I examined the kin relationships of all chicks not matched to parents through exclusion analyses to each other using estimates of pair-wise relatedness (r) as calculated in Queller and Goodnight (1989) (see Chapter Two). For each pair-wise comparison of nestlings I calculated an r value using the program Kinship 1.0. Finally, significance of the relatedness values was established by generating 95% confidence intervals for 1) unrelated chicks, 2) paternal and maternal half siblings, and 3) full siblings using a simulation within the Kinship 1.0 program (see Chapter Two).

To examine whether social pairs (males and females thought to be paired based on behavioural interaction) and genetic pairs are the same, I compared the frequency of consorts (any interaction between a male and a female involving courtship behaviour (Yokel 1986, 1989, Yokel and Rothstein 1991) between males and their genetically

determined mates with the frequency of consorts between the same males and other females. If more than two females were involved, the number of observed consorts between a male and his genetically determined mate was compared to the number of observed consorts between that same male and the female seen most frequently consorting with him, excluding his genetically determined mate. A similar test was performed to compare the frequency of consorts between females and their genetically determined mates to the frequency of consorts between those females and other males.

Host Specificity:

Analysis using genetic markers (Chapter Two) allowed for the determination of host nests parasitized by individual females. Host specificity at the individual level was investigated using a Fisher's exact test. Given that cowbirds are widely accepted as host generalists, the possibility that females are host specialists was tested using the following criteria: 1) Females who laid their eggs in the nests of more than one host species were considered to be generalists. 2) Females who laid their eggs exclusively in the nests of one host species were designated as host specialists for this analysis. Females that laid at least three eggs were included in the analysis. Host preference at the population level was also examined by comparing the proportion of host nests of a particular species that were parasitized with the proportion of total nests found belonging to that species. All eggs (fertile and infertile) were included in this analysis.

Habitat Specificity:

Habitat specificity at the individual level was investigated using a Fisher's exact test similar to that used to test for individual host specificity, but testing the probability that females parasitize nests exclusively in one habitat. Again, females that laid at least three eggs were included in the analysis.

RESULTS

Mating System:

Exclusion analyses identified both paternity and maternity for 43 of the chicks and paternity only for four additional chicks (see Chapter Two). Paternity was also determined for another three chicks using a supplementary polymorphic Swainson's Thrush (*Catharus ustulatus*) locus (*Cuμ* 10) (Gibbs, unpublished data). All sampled males and females were excluded as the parents of eleven remaining chicks.

Genealogical relationships among the eighteen remaining chicks that could not be assigned both male and female parents were examined using pair-wise relatedness analyses. Seven of these chicks could be assigned to individual males; this information was also used to aid in the construction of kinship groups. Given that this population is mainly monogamous (see below), I assumed that any half siblings with an r value falling within the overlap between 95% confidence intervals for full and half siblings (see Chapter Two) were likely full siblings. I also assumed that any two individuals with an r value within the overlap between unrelated and half sibling 95% confidence intervals for r were unrelated. Sibling analyses determined that four distinct groups of full siblings were present: 1) 94-70, 72, 2) 94-15, 23, 3) 94-28, 75, 76, 79, and 4) 94-34, 38, 48, and 49. The remaining six chicks were classified as unrelated. See Chapter Two for more specific details as to the criteria used.

The number of eggs identified as belonging to individual females in this cowbird population (banded and unbanded) ranged from 1 to 13 with a mean of 2.85 ± 2.80 (mean ± SD). Twelve known females were responsible for laying 43 of the 61 eggs collected and eleven males were responsible for fathering the 43 eggs (Table 1). In all cases, females were monogamous and produced offspring with only one male. Two males fathered chicks from eggs laid by two females. In these cases, where a male had more than one sexual partner, I considered the female that produced the greater number of offspring with that male to be his primary mate. Therefore, two out of 43 (4.7%) chicks collected from the study area were the product of a mating between a mated male and a female that was not his primary mate. It is not known whether these two chicks (94-035 and 94-025) were produced though polygynous relationships, where males were simultaneously mate guarding and maintaining pair bonds with two mates, or whether they were the result of extra-pair fertilizations between mated males and unmated females. Overall, these results are consistent with those found by Darley (1968) in London, Ontario and Yokel (1986, 1989) in eastern California which suggest that all females and most mated males are monogamous while a low number of males are polygynous.

A significantly greater percentage of females (90.91%) were observed to consort more frequently with their genetically determined mates than with other males (Wilcoxon matched-pairs signed-ranks test $T = 0$, $n = 10$, $P < 0.0025$). A significantly greater percentage of males (60.0%) were observed to consort more frequently with their genetically determined mates than with other females (Pratt matched pair signed-ranks test

$T = 0$, $n = 6$, $P \leq 0.05$). These data suggest that there is substantial overlap in the social and genetic mating systems of cowbirds as suggested by Yokel 1986, Yokel et al. 1991. Unlike the patterns observed in some other blackbirds (e.g. Gibbs et al. 1990), social pairs and genetic pairs were the same.

Population Structure and Fecundity:

Exclusion analyses were used to determine whether known (i.e. banded) individuals produced offspring, and relatedness analyses were used to determine the number of unbanded parents that produced offspring in the population. For example, chicks 28, 75, 76, and 79 were determined to have the same parents through relatedness analyses. Therefore, one male and female were added to the breeding population even though they were not actually seen at the study area. The six additional unrelated chicks were assumed to have one unique unidentified male and female parent each. Thus overall, the breeding population consisted of 22 females (12 known, 10 unknown) and 20 males (14 known and 6 unknown). Only 12 out of 21 (57.14%) banded females and 14 out of 44 (31.82%) banded males in the resident population produced offspring. Based on trapping data from previous years, all males in the resident and breeding populations were at least three years of age and all females were at least two years of age. No yearling cowbirds were seen in the study area.

The ratio of trapped males to females in this study area was 2.2:1 (69 males:31 females). This sex ratio is significantly different from unity ($X^2 = 14.44$, $P = 0.0001$).

The ratio of known banded resident males to females was 2.1:1(44 males: 21 females) and is also significantly different from unity ($X^2 = 8.14$, $P = 0.004$).

Mate Choice:

I used two sample t-tests to compare weight and wing length of banded male breeding and non-breeding residents. There was no significant difference in weight (mated: 52.27 ± 2.36 (mean ± SD); unmated 51.92 ± 2.26 (mean ± SD); T= -0.47; df = 42; P = 0.64) or wing length (mated: 112.61 ± 2.48 (mean ± SD); unmated: 113.23 ± 3.01 (mean ± SD); T = 0.67; df = 43; P = 0.51). Similar two sample t-tests comparing weight (mated: 42.33 ± 2.40 (mean ± SD); unmated 41.36 ± 2.85 (mean ± SD); T= -0.82; df = 18; P = 0.43) and wing length (mated: 100.25 ± 2.60 (mean ± SD); unmated: 100.44 ± 2.13 (mean ± SD); T = 0.18; df = 19; P = 0.86) of female breeders and non-breeders were also not significant. A comparison of the ages of male breeders and non-breeders (Mann-Whitney U = 669.5, mated = 14, unmated = 30, P = 0.900), and female breeders and non-breeders (Mann-Whitney U = 85.5, mated = 12, unmated = 9, P = 0.2861) were not significant.

In addition to producing no known offspring, only two out of 30 (6.45%) males and one out of nine (11.11%) females classified as non-breeders were observed to consort with members of the opposite sex on more than one occasion. Thus, it is unlikely that

these individuals were breeders that were classified as non-breeders. Therefore, breeding birds did not differ from non-breeding birds with respect to age, weight, or wing length.

Non-feeding and Egg Laying Ranges:

Female non-feeding ranges varied from 0.13 ha to 8.68 ha (n=12) with a mean (± SD) of 2.90 (± 2.55) ha (Figure 2). In contrast, areas in which females laid their eggs (egg laying ranges; n=5) varied from 0.04 ha to 0.60 ha with a mean (± SD) egg laying range area of 0.31(± 0.24) ha (Figure 3). Four additional individual females laid all of their eggs in fewer than three nests. Therefore, egg-laying range areas could not be calculated for these females. Quantifiable female egg laying areas did not overlap, although two cowbirds parasitized the same host nest at egg laying area boundaries in three cases. A female's egg laying range intersected her non-feeding range in all but one case and was usually within the combined area of a female's and her mate's non-feeding ranges. However, a female's egg laying area was not always a subset of her non-feeding range.

Male non-feeding ranges varied from 0.72 ha to 9.81 ha (n=11) with a mean (± SD) area of 3.46 (± 2.78) ha (Figure 4). They usually encompassed the egg laying range and intersected the non-feeding range of their partner. In one case, a 'polygynous' male's non-feeding range intersected both partners' non-feeding ranges and contained all eggs laid by both females. There was no significant difference in size between male and female non-feeding ranges (Mann-Whitney U = 140, male = 11, female = 12, P = 0.644).

However, male ranges were larger than their mate's ranges in eight out of twelve cases. This agrees with the observations of Teather and Robertson (1988) and Darley (1983). The mean (± SD) percent overlap between any two female non-feeding ranges was 11.11 (± 21.75) %, whereas the mean (± SD) percent overlap between any two male non-feeding ranges was 13.72 (± 21.39) %. The mean (± SD) percentage of a single non-feeding range overlapped by other non-feeding ranges of same sex individuals was 68.38 (± 36.30) % for females and 76.04 (± 23.08) % for males. These data suggest that neither males nor females have exclusive non-feeding ranges, although females usually have exclusive egg laying ranges (Figure 3).

Host and Habitat Use:

Of the 62 nests that were parasitized in the study area, five were initially found to contain more than one cowbird egg laid by the same female and two were both found to contain two eggs laid by different females. Six nests were also parasitized at least once more after the initial cowbird egg was removed. Of these, five (83.33%) were parasitized by the original female. In total, 10 of 13 females (76.92%), that laid more than one egg, parasitized the same nest on more than one occasion.

Host use by individual females laying three or more eggs is shown in Table 2. Although sample sizes are small, the data suggest that individual cowbirds are host generalists, parasitizing several different species of hosts. A Fisher's exact test failed to show that the number of individual host specialists in this population was greater than zero

(P=0.103). I also examined host preference at the population level by examining the null hypothesis that the number of host nests of a particular species parasitized is proportional to the total number of nests of that species found. All eggs (fertile and infertile) were included in the analysis but eggs laid in the same nest were counted as a single parasitism event as this resulted in a more conservative test in this case. The hypothesis of proportionality was rejected ($X^2 = 34.86$, $P<0.0001$). A greater number of Song Sparrow nests were parasitized (20.69 % of total number of parasitized nests) than expected (6.5 %), but a smaller number of Yellow Warbler nests were parasitized (29.91 % of total number of parasitized nests) than expected (42.02 %). Thus, these results support the hypothesis that most individual females parasitize many host species. However, the female population is choosing host nests in a non-random fashion.

Habitat types in which individual females laid their eggs are shown in Table 3. Seven out of nine (77.78 %) females parasitized nests only in marsh habitat while two out of nine females (22.22 %) parasitized nests in both marsh and ridge habitat. A Fisher's exact test showed that the number of individual females parasitizing nests in one habitat only was greater than zero (P=0.001). Thus, females appear to be choosing habitat type in a non-random fashion.

DISCUSSION

Fecundity:

Captive Brown-headed cowbirds have the potential to lay approximately 40 eggs during their 2- to 3-month breeding season (Scott and Ankney 1980, 1983, Fleischer et al. 1987). Therefore, hypothetically, 880 eggs (22 breeding females x 40 eggs) should have been collected from host nests in this study area. Given that only 90 cowbird eggs were collected in total, females were either 1) laying fewer than 40 eggs per season, 2) dumping eggs when no host nests were available, or 3) parasitizing an unknown host in the study area. Since egg shells attributed to egg dumping have never been found on or near the study site (Sealy, pers. comm.), and all female non-feeding ranges are located within the boundaries of this study site, it seems unlikely that females are egg dumping. Since only 90 eggs were collected, if female cowbirds were laying at maximum fecundity then 790 out of 880 (89.77 %) eggs in this study area would have to have been laid in the nests of another host species that was overlooked. The Common Yellowthroat (*Geothlypis trichas*) is the only other acceptor host species in the area that could potentially accommodate these eggs. However, daily nest searching efforts located only one Common Yellowthroat nest in 1994 and it was not parasitized. It is possible that Common Yellowthroats are utilized as hosts by Brown-headed Cowbirds. However, it is not likely that there is a sufficient number of nests to accommodate 790 eggs in the study area.

Therefore, it is likely that cowbirds are laying substantially fewer than 40 eggs per season. Female reproductive success may be limited by 1) the lack of availability of suitable host nests, and 2) exclusion from prime host nest areas by other breeding females (see range overlap section). Only 62 out of 307 nests (20.20%) in this study area were parasitized by female cowbirds. Therefore, this area does not likely contain a sufficient number of suitable host nests (22 females X 40 eggs X (100/20.20) = 4356 nests) for each female to accurately time 40 successful parasitisms in one breeding season or female reproductive output is limited to less than 40 eggs through some intrinsic limitation. In situations where host nests are limiting, selection should favour maximizing the time spent nest searching. Female defense of host-rich areas would also be adaptive.

One mechanism for this reduction in female reproductive output could be an increase in the length of rest periods between 'clutches'. For example, one female at this study site produced 13 eggs which were laid in two main 'clutches' separated by a 13 day rest period. Although Ankney and Scott (1980) found no nutritional cost of egg laying, Holford and Roby (1993) suggest that fecundity of Brown-headed Cowbirds can be limited by dietary calcium. Therefore, calcium intake may be controlling the fecundity of individual females. This seems unusual for this population of cowbirds because nine out of 12 female non-feeding ranges border the southern shore of Lake Manitoba where aquatic organisms with calcified shells are abundant. However, female cowbirds may obtain their calcium supplement while searching for food outside of their non-feeding range where calcium is limited.

Non-feeding and Egg Laying Range Overlap:

Female non-feeding ranges (0.20 ha to 8.68 ha) were substantially smaller than those estimated in a similar fashion in Southwestern Ontario (0.90 ha to 13.40 ha) by Darley (1983) and those estimated in Kingston, Ontario (mean ± SD = 9.89 ± 2.69 ha) using radiotelemetry (Teather and Robertson 1985). These differences may be a result of variations in host densities. Female non-feeding ranges overlapped extensively (mean ± SD = 68.38 ± 36.30 %) and did agree with the results of both Darley (1983), and Teather and Robertson (1985). In contrast, female egg laying ranges were considerably smaller (0.03 ha to 0.60 ha) than their non-feeding ranges. Egg-laying ranges did not overlap with the exception of a few shared nests at egg laying range boundaries. Therefore, females may exclude other females from egg laying areas. Female non-feeding range boundaries may overlap because a female spends most of her time searching for and monitoring suitable host nests. Males do not share in any defensive duties (Darley 1983) and females do not have the time to effectively defend a large non-feeding range by themselves. However, the availability of suitable host nests is an important factor in a female's reproductive success (Yokel 1989). Therefore, exclusive use of nests in a small area would be beneficial if they were economically defendable (Brown 1964). Although not observed at this study site, several studies have reported the defense of breeding ranges through female aggression (Dufty 1982a, 1982b, Darley 1983). It would be particularly adaptive for a female to defend an egg laying range because of the substantial amount of effort that is invested in locating and monitoring nests. The discovery that a

single female is usually responsible for most multiple parasitisms of a single nest also supports the theory that females defend exclusive egg laying ranges and that host nests may be limiting. These results contradict Fleischer (1985) who found that laying areas overlapped 75% on average in eastern Kansas. However, it appears that most of this overlap can be accounted for by the overlap between measurable egg laying areas of just two females in Fleischer's study area and this value may not be an accurate representation of egg laying range overlap in general. This overlap may also be a result of the clumping of host nests on Fleischer's site as it contained intermittently grazed pasture land.

Male non-feeding ranges (0.72 ha to 9.81 ha) were substantially smaller than those observed in Southwestern Ontario (0.4 to 25.0 ha) by Darley (1982) but still overlapped extensively (mean ± SD = 76.04 ± 23.08 %). They usually encompassed the egg laying range and overlapped the non-feeding range of their partner. Since this study as well as others (Darley 1983, Teather and Robertson 1985, 1986) show that individual female non-feeding ranges overlap extensively with each other, and that female non-feeding ranges overlap extensively with their mates' non-feeding ranges, male non-feeding ranges will undoubtedly overlap. This situation should promote mate guarding because males cannot exclude other males from their own or their mates' non-feeding ranges.

Why is Monogamy Adaptive?:

Mating system theories state that polygamy should be more common in situations where parental requirements are minimal (Emlen & Oring 1977). Although female

Brown-headed Cowbirds must devote a substantial amount of time and energy to egg laying and nest searching, neither males nor females are constrained by parental care because their offspring are reared by the host. Therefore, cowbirds should adopt a polygamous mating system given that the degree to which multiple females are economically defendable is high, and that females can take full advantage of opportunities to pursue polygamous relationships (see Emlen and Oring 1977). For example, a male will have a higher reproductive fitness if he can successfully monopolize and copulate with two females instead of one. One would also expect male and female mate choice to be based on genetic quality (indirect benefits) as neither males nor females stand to gain any direct benefits through parental care.

Contrary to this prediction, the Delta Marsh population of cowbirds is mainly monogamous possibly because the following assumptions of polygamy mating system theories are violated: 1) multiple females are not economically defendable due to a male-biased sex ratio, overlapping male non-feeding ranges, and a lack of temporal distribution of receptive females, and 2) the constraint of nest searching may prevent females from taking advantage of any opportunities to pursue polygamous relationships.

In past literature, the mating system and the intensity of male mate guarding was explained in part by the population sex ratio. This ratio was based on the number of males and females trapped in a given area (Darley 1982, Dufty 1982a, 1982b, Teather and Robertson 1986). However, this ratio may not be an accurate estimate of the actual sex bias or intensity of sexual selection in the population. Some of the trapped birds may be

migrants and may not be members of the resident population. Therefore, I compared the number of banded resident males to the number of banded resident females observed at the study site. The sex ratio (2.1:1) was significantly different from unity and indicates that intermale competition is likely high in this population. This is further supported by the discovery that only 40% of all resident males breed. Present hypotheses state that monogamy via mate guarding becomes increasingly adaptive to males as the sex ratio of a population becomes increasingly male biased (Whittenberger and Tilson 1980). My results are consistent with this theory.

However, Emlen and Oring (1977) suggest that the intensity of sexual selection and the mating system may be better illustrated by the operational sex ratio (OSR; a measure of the degree of monopolizability of mates). Emlen (1976) describes the OSR as the average ratio of fertilizable females to sexually active males at any given time. A male biased OSR would promote polygyny whereas a female biased OSR would favour polyandry. Naturally, a 1:1 OSR would promote monogamy because fertilizable females or sexually active males are not in excess in the population.

Although the OSR is difficult to determine, it may be estimated in species such as the Brown-headed Cowbird, where female receptivity is synchronous and spans the entire breeding season, by the ratio of known female residents to known sexually active male residents in the population. Since all males in this population are at least three years old, I assumed that all resident males were sexually active. Therefore, the estimated OSR of this population is equal to the sex ratio of the resident population (2.1:1) and was significantly

different from unity. Thus, the intensity of sexual selection should be great and polygyny should be favoured due to the excess of non-breeding males in the population. Genetic and behavioural data both disagree with this hypothesis. The Delta population of cowbirds is monogamous. Therefore, factors other than the degree to which mates can be monopolized, estimated by the operational sex ratio, must be influencing a male's decision to mate guard and the mating system of this population of cowbirds.

Darley (1982), and Dufty (1982a, 1982b) suggest that male cowbirds are not territorial. This is consistent with observations in the Delta population which show extensive overlap in male non-feeding ranges at this study site. Overlapping non-feeding ranges prevent males from having sole access to females within their non-feeding range at any given time. With no guarantee that searching for additional females will result in additional copulations, it would be more adaptive for males to mate guard to ensure their own copulatory success and prevent other males from copulating with their mates. Mate guarding behaviour has been observed in other populations of cowbirds (Dufty 1982b). Therefore, overlapping male non-feeding ranges may influence the decision to mate guard and the mating system.

The temporal distribution of mate receptiveness may also have an impact on the mating system. If females in a population become sexually receptive in unison, there is little potential for individual males to monopolize mates and little potential for polygyny regardless of the spatial distribution of resources (Emlen and Oring 1977). Brown-headed Cowbird females are receptive for the entire breeding season (approximately eight weeks;

Ankney and Scott 1980). Therefore, it may be more productive for males to defend and copulate with their receptive mates for the entire breeding season than to risk getting cuckolded themselves while searching for additional mates. This is particularly adaptive if the sex ratio is male biased (Wittenberger and Tilson 1980) because the chances of finding a second mate are low due to intensive intermale competition (Thornhill 1976).

Monogamy may be 'preferred' from the males' point of view because multiple females are not economically defendable. The majority of males may reproduce most successfully by defending exclusive access to single females. This decision is likely influenced by an extended period of simultaneous female receptiveness as well as by overlapping male non-feeding ranges suggested by Dufty (1982a) and by the male-biased sex ratio suggested by Wittenberger and Tilson (1980), Dufty (1982a), and Teather and Robertson (1986). All these factors drastically reduce the ability of a male to monopolize more than one mate and favour monogamy.

Males do not guard their mates constantly to enforce fidelity (Yokel 1986). Therefore, females could copulate and produce offspring with other males besides their mate if monogamy is not adaptive. However, consistent with Yokel (1989), this female behaviour was not observed in my study. Contrary to the suggestions of Darley (1982), this monogamous relationship was not entirely enforced by mate guarding. Consort data show that a female's main consort was her mate. Therefore, it seems as though females are partly responsible for maintaining the monogamous relationship and must be receiving some benefit from the relationship besides a genetic contribution. Yokel and Rothstein

(1991) found no evidence to substantiate the claim that males protect their mates from harassment nor do males participate in nest searching activities (Hann 1941, Norman and Robertson 1975). I suggest that females adopt a monogamous relationship, not because they are receiving assistance from their mates, but because this mating system indirectly maximizes the time available to them for nest searching activities.

The availability of suitable host nests in which to place eggs is an important factor in the reproductive success of female cowbirds (Yokel 1989). However, equally important is a female's ability to utilize them. As with monogamous species who must devote a large amount of time caring for their young, female cowbirds must devote a substantial amount of time searching for high quality host nests in which to lay their eggs. This is particularly true if cowbirds have the potential to lay up to 40 eggs in a season (Ankney and Scott 1980). Searching for additional mates and possibly maintaining pair bonds with them would result in less time for nest searching. If a female has already obtained a high quality mate and host nests are abundant, then her ability to find nests and the amount of time that she devotes to nest searching are the main factors influencing her reproductive fitness. To maximize fitness, a female should spend as much time as possible searching for nests while spending just enough time consorting with her mate in a monogamous relationship to strengthen pair bonds and ensure the fertilization of her eggs. I suggest that the pursuit of polygamous relationships would only result in a reduction of the available nest searching time and lower female reproductive fitness.

The mating system of this population of cowbirds can be characterized by one hypothesis for monogamy described by Wittenberger and Tilson (1980). This hypothesis suggests that mate guarding is the best method for males to maximize reproductive success in nonterritorial species especially when the sex ratio is male-biased. This theory has been used to explain monogamy in other Brown-headed Cowbird populations (see Darley 1982, Dufty 1982a, 1982b) and can describe monogamy at this site. However, it does not suggest why a monogamous relationship is adaptive to females. I suggest that monogamy, and mate choice based on genetic quality are adaptive for females because they maximize the chances of producing fertile offspring and also the time available for nest searching activities (see above). A limiting number of suitable host nests in this study area and exclusive egg-laying ranges indicate that there is female competition for access to host nests. Extensive overlap in female non-feeding ranges may also indicate that females do not have time to effectively defend large ranges because their time is spent searching for nests.

Mate Choice:

Since Brown-headed Cowbirds do not care for their young and males do not provide females with any active investment contributions (any male action increasing a female's reproductive success) (Yokel and Rothstein 1991), female choice of a 'high quality' mate should reflect genetic quality and not the amount of resources or assistance that a male can provide his mate (Trivers 1972). Yokel and Rothstein (1991) suggest that

dominance is an important correlate of male mating success and that song type, age, and body size (dominance indicators) may be cues for genetic quality. I found no significant difference in age or body size (wing length and weight) between male breeders and non-breeders that might serve as an indicator of genetic quality. Therefore, if female choice occurs in this population then it may be on the basis of additional characteristics not considered in this study such as song type or other traits correlated with male condition.

Host and Habitat Specificity:

Similar to Fleischer (1985), I found little evidence to suggest that individual females strictly chose the nests of one host species over another despite the non-random parasitism patterns of the population and one female laying all 13 of her eggs in Red-winged Blackbird nests. However, this lack of detected individual host specificity may be a consequence of my choice of analyses due to small sample sizes. For example, my test for individual host specificity had its limitations as it gave an individual laying 13 eggs the same weight as an individual laying three eggs and considered a female to be a host generalist even if she laid just one egg in the nest of a second host species. A more accurate test of individual host specificity would involve larger individual egg sample sizes and separate X^2 tests for each female cowbird to test whether individual females lay a significant number of eggs in the nests of more than one host species.

This population of female cowbirds parasitized several host species in a non-random fashion. Proportionately more Song Sparrow nests and fewer Yellow Warbler

nests were parasitized than expected. Thus, the population seems to be showing some degree of specificity when choosing host species to raise their young. A possible explanation for this behaviour is that these females have a host preference when preferred nests are available but will lay in any nest as required. Mason (1986) produced similar conclusions for another widely accepted host generalist, the Shiny Cowbird (*Molothrus bonariensis*), which preferred large hosts over small ones while still exploiting both. The tendency for Yellow Warblers to bury cowbird eggs (Sealy 1992, 1995) makes it an inferior host because the cowbird must closely time parasitism events with the laying cycle of the host (Weatherhead 1989). Cowbird eggs laid in the pre-laying period (before the day on which warblers lay their first eggs) are likely to be buried or abandoned (Sealy 1992). In addition, Song Sparrow nests may be more valuable to a cowbird because of their apparent inconspicuousness to predators in my study area (usually sunken into the ground and concealed by tall grass) and their obvious resistance to wind damage. Therefore females may preferentially lay in Song Sparrow nests because the chances of chick survival are higher, but will parasitize Yellow Warbler nests if no Song Sparrow nests are available.

The number of individual females parasitizing nests in one habitat only was significant. Most (seven out of nine) females preferred marsh habitat over ridge habitat even though four of these seven females' non-feeding ranges were composed of both habitats and one was exclusively composed of ridge habitat. Similar behaviour is reported for another obligate brood parasite, the Common Cuckoo (*Cuculus canorus*). The

cuckoo, a semi-generalist, parasitizes several host species whose nest sites are similar to their main hosts (Moksnes and Røskaft 1995). However, because Song Sparrow and Red-winged Blackbird habitat is distinct from Yellow Warbler habitat at my study site, cowbird habitat specificity could be a result of the differential ability of females to locate nests of different species. For example, some females may find it easier to locate Red-winged Blackbird (marsh host) nests and will therefore parasitize them more frequently than Yellow Warbler (ridge host) nests making it appear as though marsh habitat is preferred. However, most females (six of nine) laid eggs in the nests of more than one host species.

In summary, distinguishing between host and habitat preference at this site is problematical because both factors are closely interrelated. With my data, it is impossible to exclude either variable (host nest type or habitat) as factors influencing host choice by female cowbirds. Overall, however habitat seems to be a slightly better predictor of which host nests are parasitized by an individual female.

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Table 1: Brown-headed Cowbird families as determined by exclusion analyses as described in Chapter Two. [1] Refers to an unbanded female determined to have produced offspring through relatedness analyses as described in Chapter Two. [2-9] Refer to eggs laid in the same nest. [10] Refers to situations where a male is either polygynous or is monogamous and has successfully obtained an extra-pair fertilization (EPF).

	Parents					
Mating System	**Female**	**Male**	**Chick #**			
Monogamous	0991-15912	0991-06887	94-007[2]	94-008[2]	94-009[2]	94-010
			94-013	94-017	94-043[3]	94-050
			94-052	94-053	94-060[4]	94-061[4]
			94-072[5]			
	0991-15917	0991-06827	94-014	94-021[6]	94-022[6]	94-002
			94-026	94-081[6]		
	0991-15915	0991-15510	94-012[7]	94-016[7]	94-020	94-066
			94-067			
	1391-90832	0991-06864	94-005	94-084[8]	94-090[8]	
	1391-90842	0991-06810	94-011	94-037	94-040[3]	
	0991-15918	0991-06842	94-002	94-031		
	1391-90831	0991-06811	94-001	94-003		
Polygynous / Monogamous + EPF[10]	1391-90839	0991-06829	94-042	94-062	94-073[5]	
	0991-15908	0991-06829	94-035			
	1391-9081	0991-06854	94-018[9]	94-019[9]	94-029	
	Unknown[1]	0991-06854	94-025			
N/A	1390-90801	0991-06883	94-077			
	1391-90804	0991-06803	94-058			

Table 2: The number of eggs (greater than or equal to three) laid by individual females in Red-winged Blackbird (RW), Song Sparrow (SS), Yellow Warbler (YW), and other host nests.

	Number of Eggs			
Mother	**RW**	**SS**	**YW**	**Other**
0991-15912	13	0	0	0
0991-15915	3	2	0	0
0991-15917	0	3	3	0
1391-90811	0	3	0	0
1391-90832	3	0	0	0
1391-90839	2	1	0	0
1391-90842	1	1	1	0
Unknown #1	0	2	1	0
Unknown #2	2	0	1	1
Host Nest Frequency	0.4731	0.0599	0.3862	0.0808

Table 3: The number of eggs (greater than or equal to three) laid by individual females in host nests found in marsh and ridge habitats.

	Number of Eggs	
Mother	**Marsh**	**Ridge**
0991-15912	13	0
0991-15915	5	0
0991-15917	4	2
1391-90811	3	0
1391-90832	3	0
1391-90839	3	0
1391-90842	2	1
Unknown #1	3	0
Unknown #2	3	0

Figure 1: Map showing University Field Station in relation to the Lake Manitoba shoreline at Delta Marsh, Manitoba. Study site is inset.

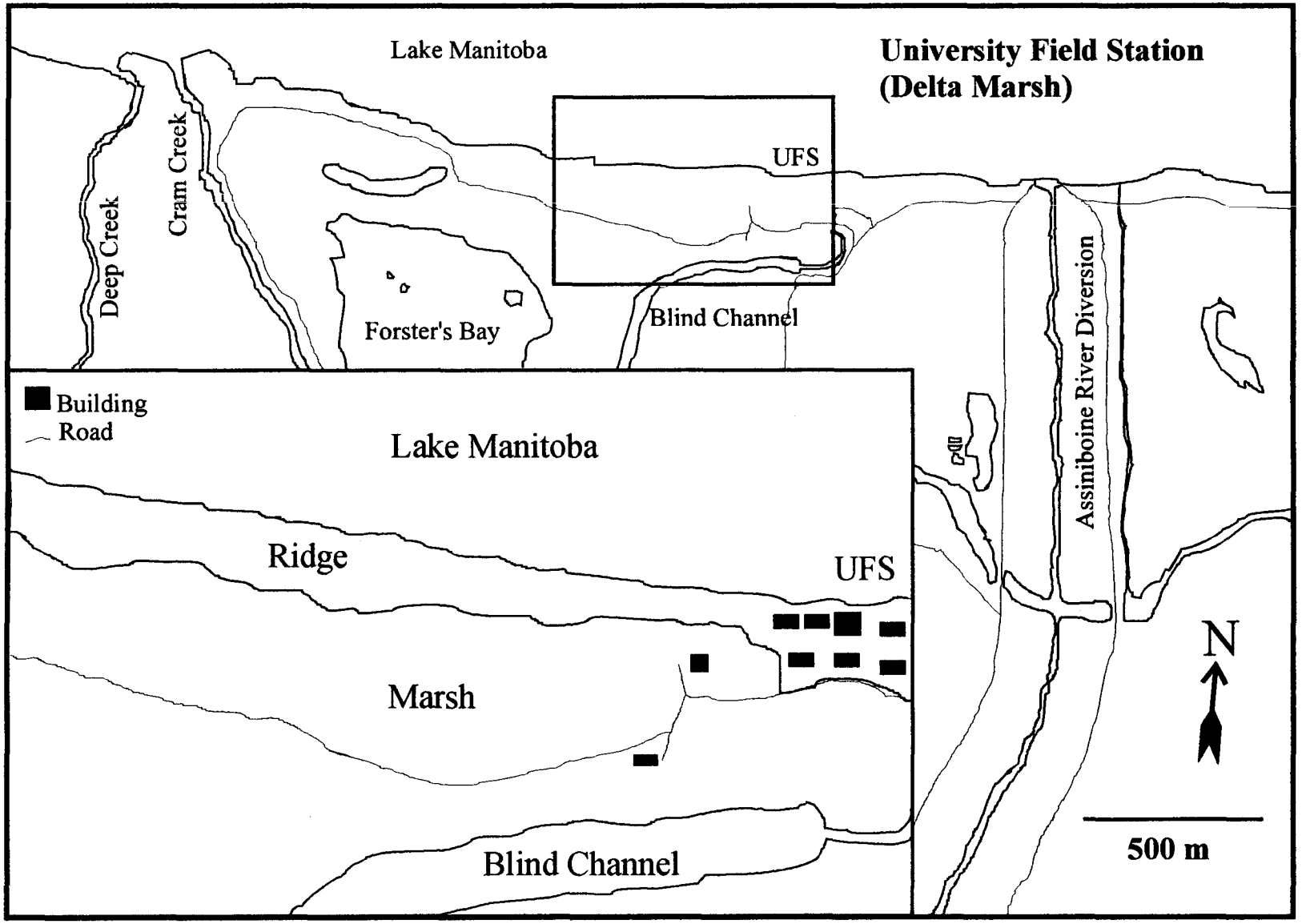

Lake Manitoba
University Field Station
(Delta Marsh)
UFS
Deep Creek
Cram Creek
Forster's Bay
Blind Channel
Assiniboine River Diversion
Building
Road
Lake Manitoba
Ridge
UFS
Marsh
Blind Channel
N
500 m

Figure 2: Map showing overlap of non-feeding ranges of twelve resident female Brown-headed Cowbirds at Delta Marsh, Manitoba.

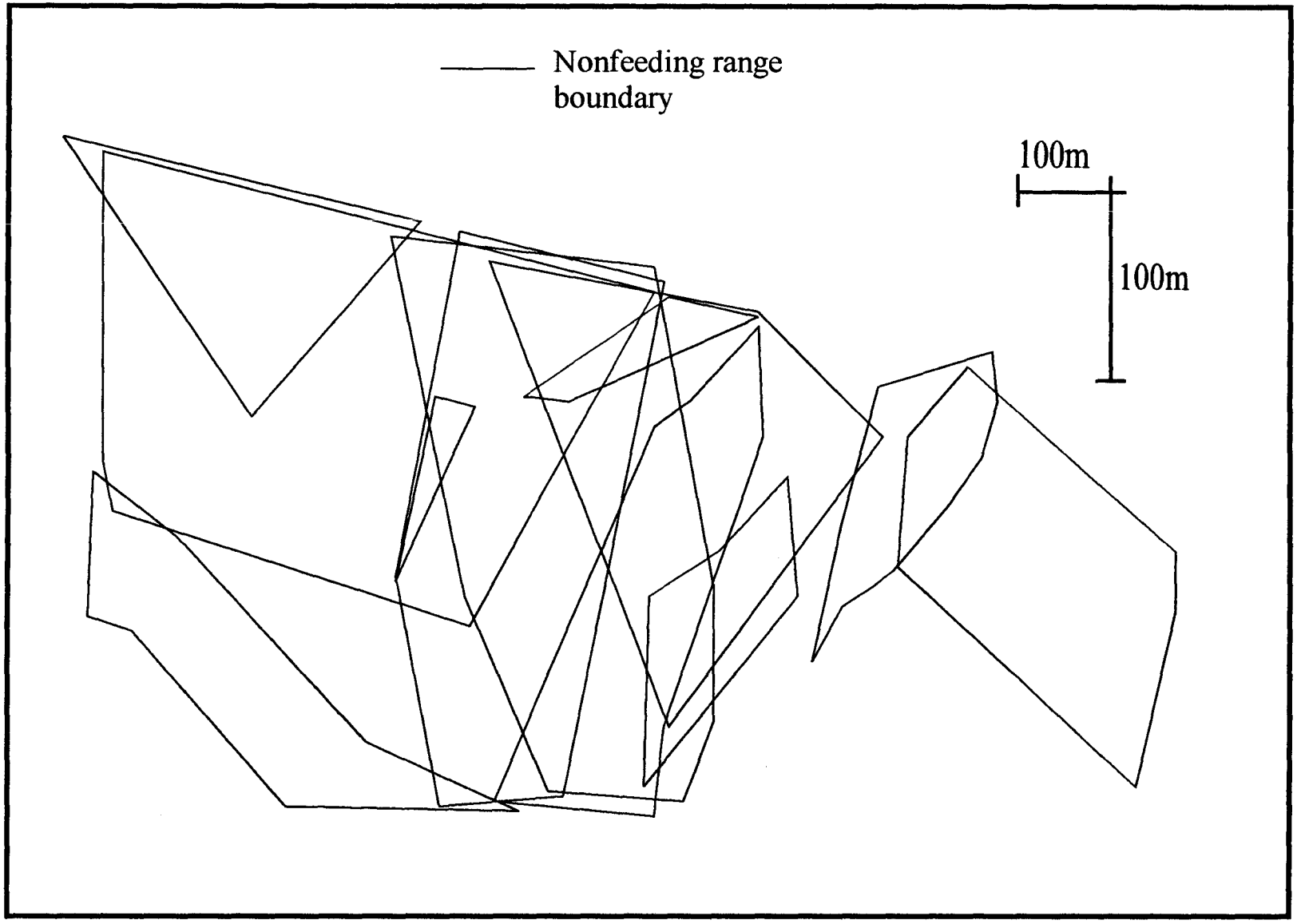

Nonfeeding range boundary
100m
100m

Figure 3: Map showing overlap of egg laying ranges of female resident Brown-headed Cowbirds at Delta Marsh, Manitoba who laid at least three eggs.

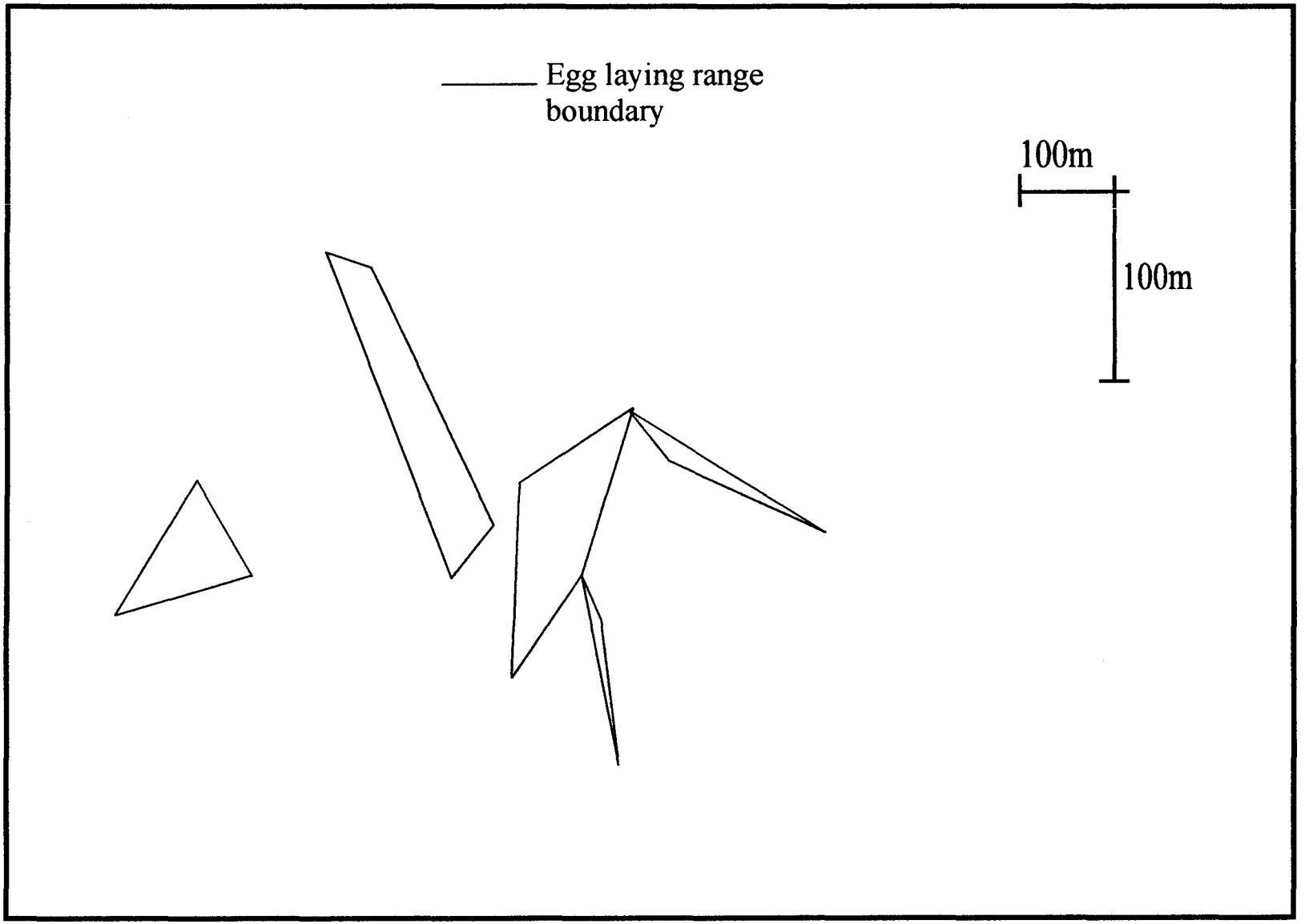
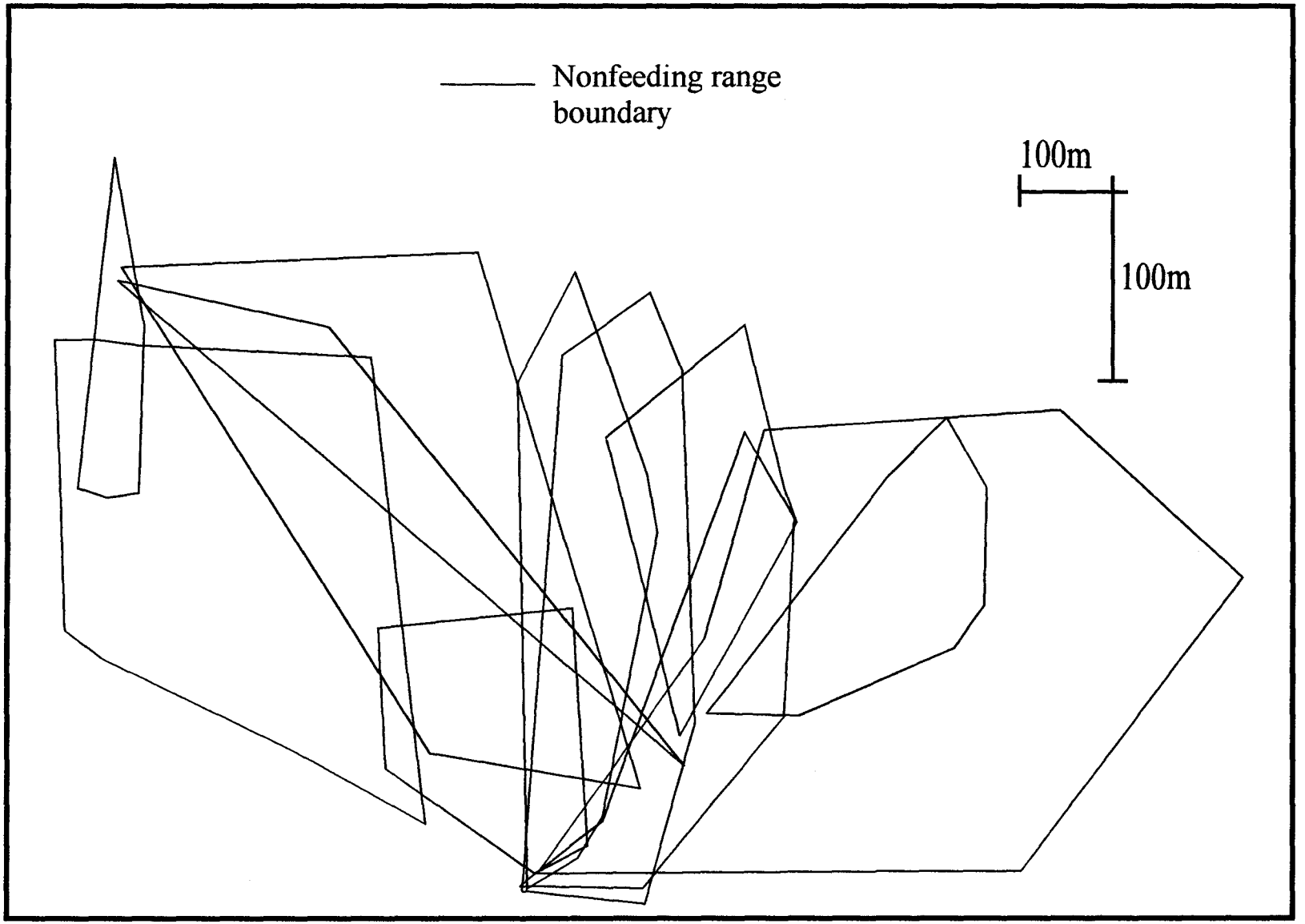

Egg laying range boundary
100m
100m

Figure 4: Map showing overlap of non-feeding ranges of eleven resident male Brown-headed Cowbirds at Delta Marsh, Manitoba.


Nonfeeding range boundary
100m
100m

CHAPTER FOUR: GENERAL CONCLUSIONS

In Chapter Two, I examined the usefulness of DNA microsatellite markers for parentage and kinship studies in the Brown-headed Cowbird. I have shown that a combined set of seven polymorphic microsatellite DNA markers can unambiguously resolve parentage among individuals in a population of Brown-headed Cowbirds at Delta Marsh, Maintoba. Although similar studies have been completed on other nonparasitic species, this is the first study to demonstrate that microsatellites are useful for high resolution parentage analyses in brood parasitic bird species where there is no a priori information available on male or female parentage.

In Chapter Three, I addressed the following questions concerning the reproductive behaviour of a Brown-headed Cowbird population at Delta Marsh, Manitoba : 1) What is the predominant mating system of this population?, 2) Are female cowbirds showing any signs of host specifity?, and 3) Do female cowbirds show a preference towards hosts in a certain habitat?

The genetic mating system of this population was mostly monogamous and matched the social mating system determined through behavioural observation. Although mating system theories suggest that these birds should be polygamous, I suggest that monogamy and mate choice based on genetic quality are adaptive for females because they maximize the chances of producing fertile offspring through maximizing the time available

to search for nests. I also suggest that monogamy is adaptive for males because multiple females are not economically defendable due to overlapping male non-feeding ranges, a male-biased sex ratio, and an extended period of simultaneous female receptiveness. Therefore guarding a single female maximizes male reproductive success.

Assuming that this population is monogamous from year to year, future studies should find that radio-tracked females spend a large amount of time localized in a small area of their non-feeding range. This area should coincide with a female's egg laying area as determined through genetic analyses. In addition, radio-tracked males should spend a substantial amount of time with their mates.

My results also suggest that individual female cowbirds are best described as host generalists although three out of nine females laid their eggs in the nests of a single host including one female who laid 13 eggs in the nests of Red-winged Blackbirds. This study was limited by the small number of eggs laid by individual females; therefore, additional study is suggested. Individual females may be showing a preference for host nests in one habitat over those in another.

Although genetic analyses has greatly enhanced our understanding of cowbird reproductive behaviour, some issues still remain unresolved. For example, is the mating system, and the degree of host and habitat specificity consistent from year to year at this site or does it change with host and parasite density? Do individual pair bonds last longer than one breeding season? How much time do females actually spend nest searching, and how much time do males spend mate guarding? Given that most previous host specificity studies assume that similar eggs are laid by the same female, it would also be interesting to

know whether eggs genetically determined to have been laid by the same female are quantifiably similar in appearance and morphology. Additional study of this population is required to answer these questions.

These microsatellite markers will also allow the quantification of the reproductive behaviour of other populations of cowbirds and will test the hypotheses that cowbird mating systems vary with host density, population density, and geographical location. Hence, the effects of these variables on other aspects of Brown-headed Cowbird reproductive behaviour such as reproductive success, mate choice, sexual selection, and host specificity can be examined.