

MATING SYSTEM AND SOCIAL BEHAVIOR OF RUSTY BLACKBIRDS ON
YUKON FLATS NATIONAL WILDLIFE REFUGE

By

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A Thesis Presented to

The Faculty of Humboldt State University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Natural Resources: Wildlife

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May 2013

ABSTRACT

THE MATING SYSTEM AND SOCIAL BEHAVIOR OF RUSTY BLACKBIRDS ON YUKON FLATS NATIONAL WILDLIFE REFUGE

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Many aspects of the breeding biology of the rapidly declining Rusty Blackbird (*Euphagus carolinus*) are unknown. I used behavioral observations and genetic analyses to gain a better understanding of their mating system, on Yukon Flats National Wildlife Refuge, Alaska, USA. Four polymorphic microsatellites developed for other avian species (QmAAT21, QmAAT37, Aph54, and Mp2-43) were used to assess rates of extra-pair paternity, polyandry, and egg dumping. Behavioral observations were employed to identify the social mating system and parental nest investment in relation to genetic contributions. In contrast to previous studies, my results indicate that male Rusty Blackbirds are not socially monogamous; over 15% of nests belonged to polygynous males. There was no evidence of polyandry or egg dumping, but extra-pair paternity (EPP) occurred in $\geq 33\%$ of nests. There was no correlation between the proportion of young that a male sired in a nest and either feeding rate or nest defense. Further studies are needed to investigate the role of environmental and social factors on mating systems and the rates of polygamy and EPP in Rusty Blackbirds.

ACKNOWLEDGMENTS

I thank my committee members, T. Luke George, Susan M. Sharbaugh, and Mark Colwell, for their knowledge, guidance, and editing during the duration of this thesis.

Thanks to my field crew for their wholehearted enthusiasm and dedication to the project: Joel Scurr, Jacob Armiger, Lizzie Condon, T. Jesse Sargent, Louise Pless, Robert Brown, Megan Elrod, Mathias Herriges, J. Eric “Peaches” Caubo, Judy Williams, Frank Williams, Johanna “Asia” Andrecka, Nicole Pearce, Katie Eberhart, Travis Armiger, Willow Outwaithe, Shayna Hart, and Jeff Walters. Thank you to Anthony Baker at the Biology Core Facility at Humboldt State University and Sandy Talbot and George Kevin Sage at the Molecular Ecology Lab, Alaska Science Center, USGS, for their expertise and help with genetics. Thanks to the Yukon Flats National Wildlife Refuge staff and Alaska Bird Observatory, who provided logistical support and the opportunity to conduct this research. Additionally I’d like to thank the other graduates students in the Wildlife Department at Humboldt State University for their guidance and moral support. Last and not least I would like to thank my husband and family for their love and unconditional support throughout. Thanks to the Alaska Wildlife Diversity Program (Project T-9-1-9) and Alaska Department of Fish and Game, especially David Tessler, for funding for this project.

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INTRODUCTION

Mating systems are a description of the social behavior and genetic reproduction of individuals in a population (MacManes *in review*). Mating systems in many bird species were originally described based only on field observations of social behavior (Wink and Dyrce 1999). Because many bird species maintain pair bonds during the breeding season, most species were thought to be socially monogamous (Griffith et al. 2002), an exclusive association between a male and female for the purposes of reproduction (Neudorf 2004). However, since the 1980s, application of molecular techniques to examine mating systems have become increasingly common (Wink and Dyrce 1999). DNA evidence has shown that genetically monogamous mating systems are atypical in passerines (Griffith et al. 2002). Additionally, social polygyny, where males mate with multiple females and then assume parental care for the nests, are found in only ~5% of all bird species, (Hasselquist and Sherman 2001); however, copulations outside of the social pair are common in different mating systems (Griffith et al. 2002). Rates of extra pair paternity (EPP), the proportion of offspring resulting from copulations outside the social mating system, have been found to average 11.1% of the offspring and 18.7% of the broods in socially monogamous species; only 14% of studied passerines have been found to be truly monogamous (Griffith et al. 2002). Both social behavior and genetic relatedness of parents and offspring must now be considered when classifying a mating system (Birkhead and Møller 1995, Griffith et al. 2002).

Behavioral observations are used to describe mating systems components such as parental investment (nest defense and feeding of nestlings Møller 2000), pair bond (Poirier et al. 2003), extra-pair copulations (EPC) (Westneat et al. 1990), and egg dumping (Sorenson 1991). Pair bonds are defined as a long-term social interaction for the purposes of reproduction lasting longer than copulation (Westneat et al. 1990). Following Hasselquist and Sherman (2001) an EPC is copulation with an individual outside the social pair bond while egg-dumping is laying eggs in another individual's nest of the same species (Westneat et al. 1987). The latter two behaviors are often difficult to observe in the field but can be detected through genetic testing (Westneat et al. 1987). The presence of EPP among nestlings indicates that EPCs have occurred. However, EPP rates are not necessarily equal to rates of EPC because copulation does not insure fertilization (Sheldon 2002). Genetic methods can also establish that egg-dumping has occurred.

It is important to recognize that the behavioral characteristics of a mating system are dynamic and interrelated (Raven and Johnson 2001:557-561). For example, it has been hypothesized that males alter their level of parental investment based upon their genetic contribution to the nest in order to maximize their reproductive success (Raven and Johnson 2001:557-561, Sheldon 2002). These individual reproductive choices can have significant effects on productivity at the population level (Eadie et al. 1998:317-318). As a result, mating system parameters are increasingly being incorporated into population modeling and viability analysis (Walters et al. 2002, Laiolo et al. 2008, Jenouvier et al. 2010, Payne et al. 2011) to identify variables that limit population growth

(Gerber 2006). Knowledge of a species' mating system can therefore be beneficial to conservation and management of a declining species.

The Rusty Blackbird (*Euphagus carolinus*) is a medium-sized passerine that breeds in wetlands across boreal forests of North America (Avery 1995). Analyses of Christmas Bird Counts and North American Breeding Bird Surveys have shown that Rusty Blackbird populations have suffered a chronic decline since the early 1900's, with an acute decline by at least 88% since the 1960's (Greenberg and Droege 1999). The Rusty Blackbird is now identified as a high priority species on several conservation plans including the International Partners in Flight Initiative (Rich et al. 2004), Alaska Department of Fish and Game's State Wildlife Action Plan (Alaska Department of Fish and Game 2006), U.S. Fish and Wildlife Service's Birds of Conservation Concern (U.S. Fish and Wildlife Service 2008), and Red List of Threatened Species (BirdLife International 2007). Currently, no single factor has been identified to explain the species' decline (Greenberg and Matsuoka 2010). However, many aspects of the Rusty Blackbirds' basic ecology remain unstudied, such as precise identification of the mating system using genetic methods.

Knowledge of the mating system is necessary for management of the species, since predictive population models are more accurate when the mating system is correctly identified (Sæther et al. 2004). The ecology of the Rusty Blackbird, especially the combination of large home ranges with significant overlap between pairs (Powell et al. 2010) and low breeding densities (Matsuoka et al. 2010a), makes it difficult to accurately ascertain mating behaviors based exclusively upon field observations. The Rusty

Blackbird mating system was previously classified as socially monogamous (Orians 1985:157, Ellison 1990). However, their patchy breeding distribution and semi-coloniality (Avery 1995) may indicate irregular temporal or spatial distribution of resources across their breeding range. Uneven distribution of resources on the breeding grounds coupled with unequal parental care by males and females (Matsuoka et al. 2010b) present opportunities for resource monopolization, a precursor for polygamy (Emlen and Oring 1977, Ellison 1990).

During the 2009-2010 breeding seasons, I examined the mating system of a Rusty Blackbird population on the Yukon Flats National Wildlife Refuge, Alaska, using genetic analyses and behavioral observations. The objectives were to: (1) classify the mating system, (2) estimate rates of social and genetic polygamy, (3) examine behaviors such as egg-dumping and sexual promiscuity (evidence of extra-pair copulations) that could result in extra-pair paternal nestlings, and (4) examine the relationship between social and genetic polygamy, to determine if the behavior of adults is influenced by their genetic contribution to the nestlings.

METHODS

STUDY AREA

I conducted my study during May-June 2009 and 2010 near the Shack Lake complex on Yukon Flats National Wildlife Refuge (YFNWR), Alaska (66° 17' 26" N, 148° 07' 38" W). Wetlands along the Yukon River and its tributaries support some of the highest densities of breeding Rusty Blackbirds in Alaska (Matsuoka et al. 2010a). The Shack Lake complex is dominated by marshes and isolated stands of mixed spruce (*Picea glauca* and *P. mariana*) and willow (*Salix* spp.) interspersed with shallow lakes and ponds. The area has poor drainage and water levels are maintained by spring flooding with an annual precipitation of 17-25 cm (Gallant et al. 1995).

The study area was located near a permanent research camp established by YFNWR in 2006. Two study sites were established and surveyed in 2009; one of the study sites was not used in 2010 because of low density of breeding Rusty Blackbirds. Each study site was approximately 300 ha.

FIELD METHODS

Rusty Blackbird nests were found and monitored using standardized nest searching protocols (Martin and Guepel 1993, Martin et al. 1997), with active nests monitored every three to four days. During the period when females were laying eggs, adults were captured in mist nets by displaying a stuffed con-specific specimen and playing Rusty Blackbird songs 30-100 m from the nest. Adults were also captured by placing two mist nets around an active nest. Juveniles and adults were captured

by placing a net array in a communal feeding location. All adults were sexed based upon plumage and presence of brood patches or cloacal protuberances and uniquely banded with a combination of three colored plastic bands and one aluminum United States Geological Survey (USGS) band. Blood was taken for genetic analysis from the brachial vein, placed in lysis buffer solution (Longmire et al. 1997) and stored under ambient conditions until the end of the breeding season. Birds were held briefly, until bleeding stopped, and then released. After release, birds were observed to confirm that they resumed normal behavior.

Nestlings and juveniles were banded with a USGS aluminum band and a plastic color band for cohort year when they were 5-11 days old. A blood sample was taken for genetic analysis from the brachial vein and handled as previously described. After the field season, blood samples were archived at -80°C at the Molecular Ecology Laboratory, US Geological Survey, Anchorage, Alaska.

Nests that were associated with at least one color-banded adult were selected for behavioral observations to quantify feeding rates and identify the social parents, those that fed nestlings and defended nests. Adult behaviors were quantified using focal observations at each nest during three 30-minute intervals during days 5-12 of the nestling stage. For these observations, the observer used a spotting scope placed at least 30 m from the nest. If the adults were disturbed by the approach of the observer, feeding observations were not collected until the adults resumed feeding the nestlings. Because we sometimes could not observe the adults actually feeding nestlings (due to thick vegetation and birds using multiple feeding paths to approach the nest), a feeding event

was defined as an individual approaching within 5 m of the nest with food and observed leaving without food.

To assess nest defense behavior, response of adults to the presentation of a nest predator at each nest was recorded. Nest defense observations were conducted after feeding observations were collected on each nest to minimize potential biases caused by the nest predator presentation. A stuffed Great Horned Owl (*Bubo virginianus*), mounted on a tripod, was placed >25 m from the target nest. Although Great Horned Owls were not a common predator in the study area, they are common throughout most parts of the breeding and wintering range. The mount was covered with a pillowcase until the birds resumed normal behavior (usually going off to feed) and then uncovered. For 3 min after the first individual approached within 15 m of the mount, observers recorded the total number of all males and females acting aggressively towards the mount (i.e., diving, calling, or “striking” the mount), the number that approached within 15 m of the mount, and the identity of banded individuals. All procedures were approved by Humboldt State University’s Institutional Animal Care and Use Committee (#08/09.W.73-A).

GENETIC ANALYSIS

Genomic DNA was isolated from red blood cells using Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, California). Extracted DNA concentrations were diluted to <25 ng/ml with elution buffer AE. PCR-amplified microsatellites were used to assess parentage of all sampled nestlings. Initially, eight individuals were screened at 28 microsatellite loci known to be variable in other passerine species (Appendix A). Five

loci (four with trinucleotide repeat motifs and one with a dinucleotide repeat motif) were selected for further analysis based upon levels of genetic diversity, apparent absence of PCR artifacts such as false alleles and allelic dropout, and measured reproducibility of PCR products. Chosen loci included QmAAT5, QmAAT21, QmAAT37 (Hughes et al. 1998), APH54 (Westneat and Mays Jr. 2005), and Mp2-43 (Otter et al. 1998).

Polymerase chain reactions (PCR) amplifications were carried out using singleplex, direct-labeled reactions (locus Mp2-43) or universal-tailed (the remaining three loci) reactions. The final volume (10 uL) of each sample contained 2–100 ng of genomic DNA, 0.2 mM dNTPs, 5.0 *p*moles unlabeled primers and 1.5 *p*moles universal IRD-labeled primer for loci QmAAT5, QmAAT21, QmAAT37 and APH54 or 3.6–4.0 *p*moles unlabeled primers and 0.4 *p*moles IRD-labeled primer for locus Mp2-43, 1.0 mg BSA, 1X PCR buffer (Perkin Elmer Cetus I), and 0.3 units Amplitaq DNA polymerase (PE Biosystems, Forest City, CA). For QmAAT5 and APH 54, 1 μ L 5M Betaine was added in the PCR reaction. PCR reactions for QmAAT 21, QmAAT 37 and Mp2-43 began at 94°C for 2 min, followed by 40 cycles of 94°C for 15 sec; 50°C for 15 sec; 72°C for 30 sec. PCR reactions for QmAAT5 and Aph54 were 94°C for 2 min followed by 40 cycles each of 94°C for 30 sec; 50°C for 30 sec; 72°C for 60 sec. A 30 min extension at 72°C concluded each reaction.

The fluorescently labeled PCR products were electrophoresed on a 48-well 6% polyacrylamide gel on a LI-COR 4200LR or IR² DNA automated sequencer (LI-COR, Lincoln, NE). To size fragments, two to three of the initially screened individuals were

scored against a fluorescently-labeled M13 sequence ladder of known size, and used as size standards on each gel, typically occupying six lanes on each gel. Based on these standards, genotypes for each individual were determined using GeneImagIR™ 4.05 software (Scanalytics, Inc.).

To ensure quality control, 15% of the samples were extracted, amplified, and genotyped in duplicate. Further, 50% of the samples were re-amplified and electrophoresed. In addition, samples were processed again (a minimum of three times) when family relationships determined using genetic data were inconsistent with field data, or where multiple paternity or egg dumping was suspected. Sterile handling techniques were used with all DNA and all procedures were performed with positive and negative controls to provide evidence of replication without contamination.

STATISTICAL ANALYSIS

Genetic Variation

Genetic variation was used to assess the validity of the markers used for further analysis, such as parentage assignment, which assumed no linkage disequilibrium and that all alleles were in Hardy-Weinberg equilibrium. Only adult samples were used to minimize the potential bias of related individuals (Marshall et al. 1998) and therefore better represent the entire population. Mean number of alleles per locus (A), observed heterozygosity (H_o), and estimated heterozygosity (H_e) were quantified with Microsatellite Toolkit (Park 2001). Exact tests of Hardy-Weinberg equilibrium, analysis of linkage disequilibrium of the microsatellite loci, and F_{is} , [correlation of genes within

individuals within populations indicating levels of inbreeding (Weir and Cockerham 1984)] were calculated using GENEPOP Ver.4.0.1 (Raymond and Rousset 1995, Rousset 2008). Probability of exclusion of parentage for the population was calculated with CERVUS Ver. 3.0 (Marshall et al. 1998, Kalinowski et al. 2007). Probability of exclusion is the probability that two unrelated individuals drawn at random from the population would be expected to have alleles in common at every locus (Paetkau and Strobeck 1998). Tests involving multiple comparisons were corrected for the increased likelihood of making a Type 1 error using the sequential Bonferroni adjustment (Sokal and Rohlf 1995).

Genetic Mating System, Extra-pair Paternity Rate, and Egg Dumping

Not all adults within the study population were sampled for genetic analyses. Parentage and sib-ship assignments were used to calculate the percent of extra-pair paternity and to evaluate the presence of genetic polygamy (egg dumping). Potential parent–offspring and sib-ship genetic relationships were determined using COLONY version 2.0 (Wang 2004, Wang and Santure 2009) and CERVUS (Marshall et al. 1998, Kalinowski et al. 2007). COLONY infers parentage and sib-ships jointly using a group-likelihood approach; by contrast, CERVUS calculates likelihoods between pairs of individuals (dyads) (Jones and Wang 2010). COLONY’s algorithm partitions the individuals into family groups and calculates the maximum likelihood based upon Mendelian rules and the manual adjustment of parameters to assign individuals to family groups. One medium-length run was executed in COLONY assuming the following prior

parameters from the known histories of individuals in the population: known allele frequencies calculated from the adults only, both sexes polygamous (meaning half sibs can exist in the population), assigned known maternal sibships, no paternal sibships known, no offspring with excluded mothers, fathers, and no excluded maternal or paternal sibships.

When nests contained only two siblings ($n=3$ nests), nests were also run in CERVUS. COLONY is less accurate at assigning relationships with reasonable confidence when the number of nestlings is ≤ 2 (Wang and Santure 2009). To assess the likelihood of parentage (i.e., assign paternity or maternity) CERVUS calculated likelihood-odds ratio (LOD) scores (the sum of log likelihood ratios at each locus) for potential parent–offspring pairs. The potential parent–offspring pair with the highest LOD score was the most likely parent.

For both CERVUS and COLONY, delta scores (the highest LOD score minus the second highest LOD score) were calculated to assign parentage and family cohorts at the 0.8 and 0.95 statistical confidence levels. These analyses also allowed for scoring errors, the presence of null alleles, and mutation rates. In addition, paternity and maternity were assessed for all nestlings by comparing all nestlings to all sampled males and females for potential parentage. For calculations involving potential father–offspring or mother–offspring pairs with a known parent, the mother’s or father’s genotypes were included, respectively, in the analyses. Thus, potential parent–offspring pairs were identified with field observations and genetic data considering non-exclusion and likelihood.

Relationship between Social and Genetic Mating Systems

Sib-ship cohorts assigned from COLONY determined the number of males genetically contributing to a nest. The relationship between feeding rates of males and the proportion of offspring they sired in a nest based on genetic analyses was examined using a Spearman rank correlation. Spearman rank correlation was also used to examine the association between the number of males defending a nest and the number of males that genetically contributed to a brood.

RESULTS

In four instances (n=8 of 53 nests), color-marked males were socially bigamous. Each of the males tended two broods within a breeding season simultaneously and initiated a second nest with a new female, within 500 m of their first nest. Males' first nests were close to fledging when the second nest had three to four day old nestlings. There were no observed instances of social polyandry. All females (n=53) were observed to only build, incubate, and attend one nest of chicks. There were no observations of extra-pair copulations.

A total of 162 individuals (54 adult and 108 nestlings) were genotyped at four of the five loci (Table 1). The presence of null alleles was detected in locus QmAAT5 by Micro-Checker (Oosterhout et. al. 2004) and therefore this microsatellite was omitted from analyses. The remaining four loci displayed no evidence of linkage disequilibrium and all were in Hardy-Weinberg equilibrium ($\chi^2 = 6.24$, $P=0.62$). The probability of exclusion of parentage for the population was 0.02. F_{is} values for the four loci ranged from 0.0101 to 0.877 (Table 1). Only chicks belonging to known nests (2009, n=29 nests; 2010, n=25 nests; n=103 chicks over both years) and genotyped at ≥ 3 loci were included in parentage analysis. Four fledged chicks belonging to unknown nests and a single fledged chick (belonging to a known nest that was too high to band chicks while in the nest) were excluded (n=5).

Paternity and sib-ship assignment confirmed three of the four socially polygynous males as genetic fathers of each of their nests. They fathered $\geq 60\%$ of the chicks in their nests they socially attended but did not father the majority, $\geq 60\%$, in any further nests.

One nest was not sampled for genetic paternity because the nestlings were predated within the first four days of hatching and I did not take a blood sample of the chicks prior to predation. Socially monogamous males were assigned to a single nest (did not father $\geq 60\%$ of the nestlings in a nest aside from the nest they fed and nest defended). Maternity was assigned to 82.6% of chicks (85 of 103) (at 95% confidence level). The remainder of the chicks belonged to a nest where the social mother was not sampled for genetic analysis. Multiple maternity (polyandry) and egg dumping were not detected in any of the sampled nests.

Paternity was assigned to 46.6% of chicks (48 out of 103 chicks) with $\geq 80\%$ C.I. Potential multiple paternity, where some nestlings were not assigned to the social father of the nest or were assigned to more than one male, was detected in 41.7% (10 out of 24) of nests. These potential extra-pair paternal offspring accounted for 21.4% (22 out of 103) of all young

Sib-ship assignments detected multiple paternity (the presence of half siblings) in 33.3% of the nests (8 out of 24 nests) and 13.6% of the chicks (14 out of 103) (at the 95% confidence level). In two nests, a minimum of three males sired the young in each brood. The four nests with fewer than three chicks in a nest were analyzed with both CERVUS and COLONY to ascertain instances of EPP. There were no conflicting results between the two programs; nestling were assigned to the same males.

Feeding observations were conducted on 24 nests. Of these, 18 had nestlings genotyped and analyzed for parentage. In the other six nests the chicks weren't sampled (because the nest was too high to safely remove the chicks), the male was not

sampled/color banded, or there was only the one chick sampled. There was no evidence that feeding rates of males was related to proportion of their offspring in the nest ($r_s = -0.183$, $df=16$, $P=0.466$) (Figure 1).

Nest defense observations were conducted on 20 nests, but only 18 nests were genotyped. One nest was excluded because only one chick was sampled, and another nest was too high to safely sample the nestlings. There was no evidence that the number of males defending a nest was related to the number of males that had genetically contributed to the brood ($r_s = -0.144$, $df=17$, $P=0.557$). Most often there was only one male and one female feeding at a nest (Table 2).

DISCUSSION

Seventy-two percent of passerine species are socially monogamous while <5% are socially polygynous (Hasselquist and Sherman 2001). Although the majority of Rusty Blackbird pairs in my study population were monogamous, 15.1% of located nests belonged to polygynous males; one male invested in two nests in 2009, and three males invested in two nests in 2010. This rate of social polygyny was likely biased low because of difficulties in banding a high proportion of individuals early in the season, which reduced the chances of observing and identifying multiple males at a nest, or ascertaining if males had multiple nests.

Field observations suggested that female Rusty Blackbirds were monogamous with no observed instances of sexual promiscuity, but it might have been detected if a larger percentage of males were color banded early in the breeding season. I found no evidence of social polyandry (females laying multiple clutches of eggs for different males), which is rare amongst all bird species (Emlen and Oring 1977).

Genetic monogamy is also uncommon in birds, with higher rates of sexual promiscuity found in socially monogamous species (Hasselquist and Sherman 2001, Griffith et al. 2002). Molecular analyses showed that female Rusty Blackbirds were either socially and genetically monogamous or socially monogamous and sexually promiscuous. Extra-pair paternal nestlings were found in nests of socially monogamous and socially polygynous pairs with the majority of nests with EPP nestlings (5 of 8) belonging to a polygynous male.

Although genetic analysis confirmed observations of social polygynous males, the analysis did not identify additional polygynous males. An estimated 40% of breeding males on the study sites were captured each year resulting in paternity assignment with a 80% confidence level and lack of further genetically identified polygynous males. Moreover, 21% (n=5) of the males captured for genetic analysis were males that did not have a known active nest on the study site. These males were not seen again after banding. Whether these male were breeding outside the study area or were males without a nest (i.e., floaters) is unclear but, they probably contributed towards a lower confidence interval for paternity assignment.

Across bird species, feeding rates of young by males are negatively correlated with EPP (Møller and Cuervo 2000), possibly because of the reduction in certainty of paternity as EPCs increase (Sheldon 2002). Within species, however, the relationship between male contribution to a nest and genetic relatedness is less clear. For example in Western Bluebirds (*Sialia mexicana*), males do not alter their parental care in response their genetic contribution to a nest (Dickinson 2001) but in other species such as Dunnocks (*Prunella modularis*) they do (Davies et al. 1992). In Rusty Blackbirds in this study there was no evidence that either feeding rates by males were related to the proportion of young a male sired in a nest or that the number of males defending a nest was related to the number of males that sired young in a nest. Thirty-three percent of nestlings were a result of EPP in this study, but usually only a single male was observed feeding and defending the nest. It is not clear if the lack of contribution in these cases is due to the inability of males to identify their young or if males do not contribute for other reasons

(Sheldon et al. 2002). In two cases, however, I observed two males feeding young at the same nest. Thus, there is at least the potential that multiple males may contribute to feeding young in a nest in some cases of multiple paternity.

Mating systems are diverse in the family Icteridae. In Red-winged Blackbirds (*Agelaius phoeniceus*), up to 90% of territorial males may exhibit polygyny, depending upon the population (Yasukawa and Searcy 1995). Extra-pair paternity is also common in Red-winged Blackbirds; between 23-48% of young are a result of EPP, and they exhibit low rates of egg-dumping at <1% (Yasukawa and Searcy 1995). Although EPP rates have not been examined in Yellow-headed Blackbirds, 50% of the copulations in that species (Twedt and Crawford 1995) were extra-pair copulations. The Rusty Blackbird's closest relative, the Brewer's Blackbird (*Euphagus cyanocephalus*), shows similar rates of polygyny as Rusty Blackbirds, ranging from 8-50% of the males depending upon the year (Williams 1952). However, Williams (1952) did not use molecular methods, so rates of polygyny may be higher for Brewer's Blackbirds. Low levels of egg dumping (3%) have been detected in Brewer's Blackbirds (Martin 2002); it is possible that low levels of egg dumping occurred in the population I studied but I was unable to detect it because of low sample size.

Similar to what has been found in many other species, behavioral observations alone were not sufficient to understand the mating system of Rusty Blackbirds. Although this study established a foundation for understanding the Rusty Blackbird mating system, it is based on observations at one site over a short period of time. More studies are needed in other breeding locations to investigate whether the mating system of this species varies

across the species range and over time.

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TABLES

Table 1. Microsatellite diversity of loci in Rusty Blackbirds on Yukon Flats National Wildlife Refuge, Alaska.

Locus	No. of Alleles	Size Range (base pairs)	Ho ¹	He ²	F _{is} ³	Exclusion Probability ⁴	Exclusion Probability ⁵
QmAAT21	22	157-199	47	50.2	0.036	0.299	0.176
QmAAT37	20	147-207	48	49.8	0.047	0.284	0.165
Aph54	32	200-259	53	51.74	-0.010	0.202	0.113
Mp2-43	5	127-137	31	28.21	-0.031	0.877	0.784

¹Observed heterozygosity.

² Expected heterozygosity estimated from allele frequencies of the sampled individuals.

³ Correlation of genes within individuals within populations; high positive numbers indicate an excess of homozygotes a possible indicator of large amounts of inbreeding, and negative numbers indicate excess of heterozygotes.

⁴ Average probability of not excluding a candidate parent from parentage of an arbitrary offspring given only the genotype of that offspring.

⁵ Average probability of not excluding a candidate parent from parentage of an arbitrary offspring given the genotype of that offspring and of the known parent of the opposite sex.

Table 2. Nest defense observations of Rusty Blackbirds on Yukon Flats
National Wildlife Refuge, Alaska.

Females:Males ¹	Nests ²	Percentage (%) ³
1:1	10	45.5
0:1	2	9.10
1:2	2	9.10
2:1	2	9.10
1:4	1	4.55
1:0	5	22.73
Total	22	100

¹Ratio of number of females to males during a nest defense observation.

²Number of total nests that had specified ratio of females to males defending during nest defense observations.

³Percentage of total nests (22) that had specified ratio of females to males nest defending.

FIGURES

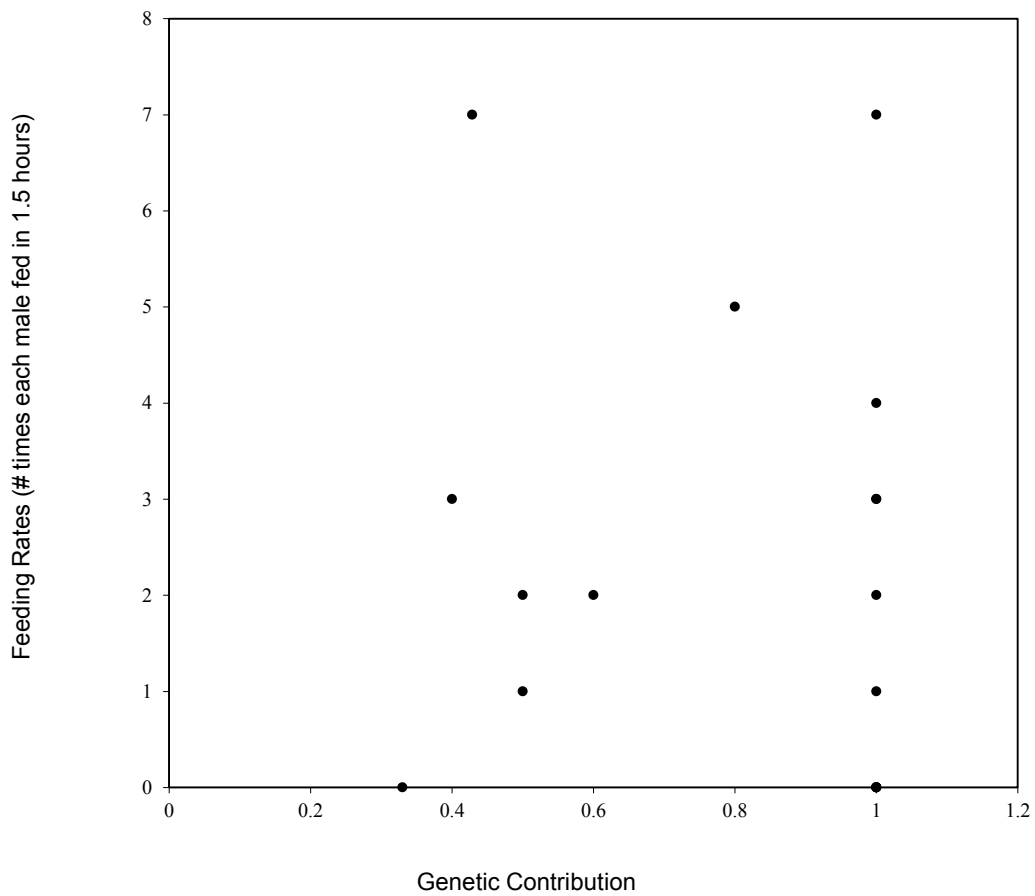


Figure 1. Correlation between a males' feeding rate and his genetic contribution to the nest (measured as the proportion of the offspring in the nest that they sired).

APPENDIX

Appendix A. Twenty-eight microsatellite markers screened for polymorphism in Rusty Blackbirds of interior Alaska

Primer name	Species developed for	Results ¹	Source
PAT MP2_14	<i>Poecile atricapillus</i>	V	Otter et al. 1998
PAT MP2_43	<i>Poecile atricapillus</i>	V	Otter et al. 1998
Escμ6	<i>Emberiza schoeniclus</i>	NV	Hanotte et al. 1994
MJG1	<i>Aphelocoma ultramarine</i>	V	Li et al. 1997
Mme8	<i>Melospiza melodia</i>	NV	Jeffery et al. 1999
Pca3	<i>Parus caeruleus</i>	V	Dawson et al. 2000
Pca4	<i>Parus caeruleus</i>	NP	Dawson et al. 2000
Pca7	<i>Parus caeruleus</i>	NV	Dawson et al. 2000
Pca8	<i>Parus caeruleus</i>	NP	Dawson et al. 2000
Pca9	<i>Parus caeruleus</i>	V	Dawson et al. 2000
Pdoμ5	<i>Passer domesticus</i>	V	Griffith et al. 1999
PmaCAn1	<i>Parus major</i>	NV	Saladin et al. 2003
PmaGAn11	<i>Parus major</i>	NSB	Saladin et al. 2003

¹ V – product, polymorphic
 NV- product, monomorphic
 NP – no product
 NSB – non-specific binding

Appendix A. (continued) Twenty-eight microsatellite markers screened for polymorphism in Rusty Blackbirds of interior Alaska

Primer name	Species developed for	Results ¹	Source
PmaGAn27	<i>Parus major</i>	NSB	Saladin et al. 2003
PmaGan28	<i>Parus major</i>	V	Saladin et al. 2003
PmaGAn31	<i>Parus major</i>	NSB	Saladin et al. 2003
PmaTAGn71	<i>Parus major</i>	NSB	Saladin et al. 2003
PmaTAGn86	<i>Parus major</i>	NV	Saladin et al. 2003
Pocc8	<i>Phylloscopus occipitalis</i>	V	Bensch et al. 1997
Ppi12	<i>Pica pica</i>	NP	Martinez et al. 1999
AAT5	<i>Quiscalus mexicanus</i>	V	Hughes et al. 1998
AAT31	<i>Quiscalus mexicanus</i>	NSB	Huges et al. 1998
AAT21	<i>Quiscalus mexicanus</i>	V	Hughes et al. 1998
AAT37	<i>Quiscalus mexicanus</i>	V	Hughes et al. 1998
APH54	<i>Agelaius phoeniceus</i>	V	Westneat and Mays Jr. 2005
Hru2	<i>Hirundo rustica</i>	V	Primmer et al. 1995
Lei160	<i>Gallus domesticus</i>	NV	Gibbs et al. 1997
Man13	<i>Manacus manacus</i>	NV	Piertney et al. 2002

¹ V – product, polymorphic
 NV- product, monomorphic
 NP – no product
 NSB –non-specific binding