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M. E. Koopman

David McDonald

University of Wyoming, dbmcd@uwyo.edu

G. D. Hayward

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### MICROSATELLITE ANALYSIS REVEALS GENETIC MONOGAMY AMONG FEMALE BOREAL OWLS

MARNI E. KOOPMAN,<sup>1</sup> DAVID B. McDONALD, AND GREGORY D. HAYWARD

*Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071 U.S.A.*

**KEY WORDS:** *Boreal Owl*; *Aegolius funereus*; *extra-pair fertilization*; *genetic monogamy*; *microsatellites*; *owls*; *paternity analysis*; *Strigiformes*.

Extra-pair fertilizations (EPFs) occur in over 86% of passerine species that have been studied but only in about 45% of nonpasserine birds (Westneat and Sherman 1997). EPFs have been documented at low rates in some diurnal raptors (Korpimäki et al. 1996, Negro et al. 1996) and owls might be expected to show similarly low rates due to their similar ecologies (e.g., reversed sexual dimorphism, necessity for male parental care, relatively low densities). Previous studies of owls, however, have shown strict genetic monogamy (Lawless et al. 1997, Marks et al. 1999, Müller et al. 2001, Arsenault et al. 2002), in which the male that provisions the nest is the genetic sire of all offspring.

The propensity for genetic monogamy may be influenced by many factors, including density and synchrony of breeding pairs (Birkhead and Biggins 1987, Stutchbury and Morton 1995, Westneat and Sherman 1997), level of parental care provided by males (Dunn and Hannon 1989), and longevity (Arnold and Owens 2002). Extra-pair matings may be energetically costly when individuals are widely spaced (Lawless et al. 1997), but not as costly when nesting pairs are clumped or aggregated. Westneat and Sherman (1997), in a review of 88 studies of 72 bird species, found a trend (within but not among species) of increasing frequency of EPFs with increasing nest density. However, among three species of owls that were found nesting in relatively high densities in some areas (Long-eared Owls [*Asio otus*], Marks et al. 1999; Little Owls [*Athene noctua*], Müller et al. 2001; Flammulated Owls [*Otus flammeolus*], Arsenault et al. 2002), genetic monogamy was absolute.

North American Boreal Owls (*Aegolius funereus*) inhabit subalpine forests of the Rocky Mountains as well as boreal forests of Canada and Alaska. They exhibit a range of nesting densities, depending on habitat quality and annual productivity. Male Boreal Owls have very large home ranges (229–3390 ha; Hayward et al. 1993), are not highly

territorial, and may nest as close as 0.5 km from one another in highly productive areas (M. Koopman and G. Hayward unpubl. data). Although multiple nests within 0.5 km is not very dense compared to some colonial nesting birds, this density is actually high relative to Boreal Owl home-range sizes (Hayward et al. 1993), nightly movements (Hayward et al. 1993), and the acoustical range of the male call (G. Hayward unpubl. data). Pair bonds are formed during winter, and pair activity centers around the nest cavity. Females begin to inhabit nest cavities as early as 2 wk prior to laying eggs (Hayward and Hayward 1993), and they remain in the nest cavity while the male provides food throughout the nesting period. As a result, little opportunity exists for encounters with other males, although this behavior does not necessarily preclude the possibility of extra-pair matings (Westneat et al. 1990).

In this study, we investigated whether Boreal Owls participate in extra-pair fertilizations, in addition to assessing the possible influence of nesting density. We address only genetic monogamy of females; males provisioning multiple nests were not investigated. Although male Boreal Owls may provision multiple nests during years of high resource abundance (Carlsson et al. 1987, Korpimäki 1989, 1994), females are still considered monogamous if they do not accept fertilizations from males outside the pair bond (EPFs). Sequential polyandry, in which a female mates with two males sequentially in one breeding season, has been documented in Boreal Owls (Solheim 1983) but was not the focus of this study.

#### METHODS

**Sample Collection.** We collected blood samples from family members for parentage analysis from six different areas (Fig. 1) and from unrelated individuals for calculation of baseline gene frequencies from eight areas (Fig. 1) from 1995–2001. In the Rocky Mountains, Boreal Owl nests were located in nest boxes on six national forests: Grand Mesa and San Juan (southern Colorado), Routt (northern Colorado), Medicine Bow (southern Wyoming), Beaverhead/Bitterroot (southwestern Montana), and Payette (central Idaho). We collaborated with other researchers who monitored nest boxes in Anchorage and near Fairbanks, Alaska. Nest boxes in national forests of the Rocky Mountains were placed in subalpine forest habitat along multiple logging roads and small highways at intervals of 0.5 km. Box systems consisted of 250–450 box-

<sup>1</sup> Present address: Rocky Mountain Research Station, 240 W. Prospect, Fort Collins, CO 80526 U.S.A.; Email address: marnikoopman@yahoo.com

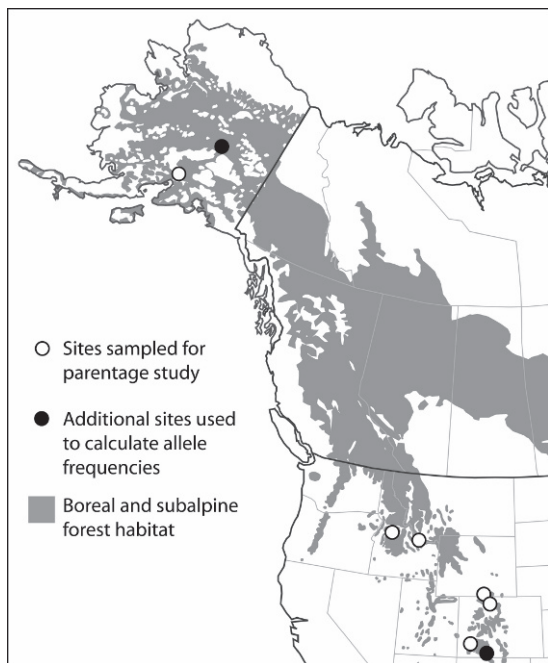


Figure 1. Collection sites for genetic samples used to examine genetic monogamy in North American Boreal Owls.

es per national forest and box use by Boreal Owls was, on average, only about 1%. Some groups of boxes appeared to attract nesting owls at higher densities than others, and consistently had two or more pairs of owls nesting within 2 km of each other. However, due to potential use of natural cavities for nesting, absolute nesting densities could not be estimated from nest-box occupancy data. Nevertheless, we believe that box use is a reasonable index for overall nesting density on the forests we monitored because we have observed higher rates of box use in older, more structurally complex stands despite higher cavity availability (G. Hayward and M. Koopman unpubl. data). For this study, we considered a Boreal Owl nest to be in a high density area if it was <1.5 km from the nearest known nest. Boreal Owls that nested 1.5–3 km from the nearest known nest were classified as medium density, and those that nested >3 km from the nearest nest were classified as low density.

We captured and banded adult females and males, and chicks. We captured adult females and chicks in nest boxes by blocking the entrance to the nest box during diurnal chick brooding periods. Males were captured at night as they brought food to the nest. We placed a trap on the outside of the nest box and the male entered through a one-way door. Thus, we were certain that the male we caught was feeding young at the nest. We collected a blood sample and banded each adult and each chick >14 d old with a U.S.G.S./Bird Banding Lab leg band.

**Genetic Analyses.** We developed and optimized 15 microsatellite loci (Koopman et al. 2004) and used seven that were polymorphic, had the highest resolution (least stutter

and nonspecific binding), and had no detectable null alleles. Because we sampled both parents and chicks from each nest, we were able to detect and discard loci with high-frequency null alleles. We recognized null alleles when we observed excess homozygosity, or when multiple females and their chicks were homozygous for different alleles. The seven primers used in this study included BOOW04, 06, 07, 13, 14, 18, and 19 (GenBank #AY257171-77). All loci were tetranucleotide repeats, except BOOW04, which was a dinucleotide repeat.

Blood was stored in Longmire's Solution (Longmire et al. 1988) at 10°C. DNA was isolated from samples using a Sigma GenElute mammalian DNA extraction kit. We used an MJ Research PTC-200 Peltier thermal cycler for the polymerase chain reaction (PCR). Each 10 µL PCR reaction contained 50–100 ng of genomic DNA, 0.15 µL Taq DNA Polymerase (Sigma), 1 µL 10× Buffer without MgCl (Sigma), 2 nmol dNTPs, 100 ng BSA, 1 pmol each forward and reverse primer, 0.3–0.7 M Betaine, 3.2 mM MgCl, and 0.25 pmol fluorescently labeled primer, all suspended in ddH<sub>2</sub>O. Annealing temperatures ranged from 44–62°C. PCR-amplified product was resolved by electrophoresis on a 25 cm, 7% polyacrylamide gel and genotyped using a Li-Cor 4200-S Automated DNA Sequencer. The DNA amplicons were detected by infrared laser. We assessed allele sizes using a 350 base pair genetic ladder and GeneImagIR™, V. 3.0 software was used to genotype individuals. For each locus, 50–80% of individuals were genotyped two or more times to confirm fragment sizes.

We scanned the data for mismatched genotypes among family members using the program Cervus (Marshall et al. 1998). Our data included known mothers, nestlings, and assumed fathers based on behavioral data. Following Neff et al. (2000), we calculated the probability that a mating between a known mother and a random male would result in offspring that were genetically consistent with those that were sampled. This analysis allowed us to assess whether we had sampled a sufficient number of heterozygous loci to detect extra-pair paternity. Using multiple measures, including Cavalli-Sforza chord distances (Cavalli-Sforza and Edwards 1967), the assignment test (Paetkau et al. 1995, Cornuet et al. 1999), and the program Structure (Pritchard et al. 2000), we consistently found no genetic structure among subpopulations of Boreal Owls (Koopman et al. 2007), supporting our decision to pool populations when calculating overall allele frequencies. Although genetic differentiation was practically nonexistent (overall  $F_{ST} = 0.002$ ), we were conservative and used two different calculations of allele frequencies as our baselines with which to compare putative fathers: one each for Alaska (calculated using 51 samples from unrelated Fairbanks and Anchorage owls) and the Rocky Mountains (calculated using 159 samples from unrelated Colorado, Wyoming, Montana, and Idaho owls).

## RESULTS

We collected blood samples from 109 nestlings, 29 adult females, and 30 adult males at a total of 32 nests in six different areas. The number of nestlings per nest ranged from two to six. We genotyped mated individuals and their offspring using seven microsatellite DNA markers. At two nests we were unable to capture the attending male, but we sampled the adult females. Each of these two nests had five

Table 1. Number (and proportion) of North American Boreal Owl nests sampled at high, medium, and low nesting densities.

SUBPOPULATION	HIGH	MEDIUM	LOW	TOTAL NESTS
Anchorage, AK	3 (0.43)	0 (0.00)	4 (0.57)	7
Payette, ID	3 (0.33)	1 (0.11)	5 (0.56)	9
Beaverhead, MT	0 (0.00)	2 (0.25)	6 (0.75)	8
Medicine Bow, WY	0 (0.00)	0 (0.00)	2 (1.00)	2
Routt, CO	0 (0.00)	0 (0.00)	3 (1.00)	3
Grand Mesa, CO	2 (0.67)	1 (0.33)	0 (0.00)	3
Total	8	4	20	32

to six nestlings, thus providing a reasonable likelihood of detecting more than two alleles of male origin at any one locus. Because we were primarily testing for paternity, the three nests for which females were not sampled still provided sufficient data to be included in the analysis. Of 32 nests sampled, eight were considered to be in areas of high nest density (Table 1).

We found no evidence for EPFs in any of the samples. Of 109 nestlings, only three had genotypes that were incompatible with those of their parents; two were inconsistent with the mother's genotype and one was inconsistent with the father's genotype. In each of the three cases, however, the genotypes of the chicks were inconsistent with the parental genotype at only one of seven loci. Extra-pair fertilizations are expected to result in discrepancies at multiple loci (Chakraborty et al. 1988). The three inconsistencies we detected are therefore most likely the product of mutation, null alleles, or human error (Chakraborty et al. 1988) rather than EPFs or egg dumping.

The number of alleles per locus varied from 3–11 ( $\bar{x} = 6$ ) and the frequency of the most common allele ranged from 0.25–0.83 ( $\bar{x} = 0.62$ ). Genetic diversity among 210 unrelated Boreal Owls was high (overall heterozygosity = 0.57). Observed heterozygosities ranged from 0.289–0.759. Using baseline genetic frequency data calculated for two different regions (Alaska and the Rocky Mountains), we calculated that the probability of a mating between known mothers and random males resulting in the genotypes of the offspring we sampled ranged from 0.003–0.186 ( $\bar{x} = 0.060 \pm 0.016$  [SE]). This level of certainty (81–100%) was adequate for our purposes because we had behavioral data to further support the paternity assignment (Marshall et al. 1998).

#### DISCUSSION

We found no evidence for EPFs among Boreal Owls in either the boreal forest of Alaska or in the Rocky Mountains of Idaho, Montana, Wyoming, and Colorado. Although the total number of nests sampled was small (32), the number of nestlings sampled was large (109), and the geographic extent of our sampling effort was quite extensive.

Our result provides further evidence that female owls are highly monogamous, both socially and genetically

(with the exception of sequential polyandry, in which a female acquires a new mate after completing a nesting attempt with a previous mate). No evidence has been found for EPFs in naturally occurring owl populations studied, including Eastern Screech-Owls (*Megascops asio*; Lawless et al. 1997), Little Owls (Müller et al. 2001), and aggregate-nesting Flammulated Owls (Arsenault et al. 2002), Long-eared Owls (Müller et al. 1999), or Burrowing Owls (Korfanta 2001). Within one isolated and drastically declining colony of Burrowing Owls, Johnson (1997) found evidence for EPFs among two of 31 progeny. However, assessing paternity in Burrowing Owls is complicated by the fact that chicks often move from one burrow to another (Johnson 1997, N. Korfanta pers. comm.), confounding attempts to correctly identify social and genetic parents.

Avian taxa demonstrate a variety of mating systems ranging from complete social and genetic monogamy to widespread EPFs. Among passerines, social monogamy with biparental care is the most common mating system and EPFs are widespread (>86% of surveyed passerine species showing some EPF with values as high as >70% of nestlings produced; Griffith et al. 2002). Diurnal raptors, with general ecology similar to that of owls, might be expected to resemble owls in their mating strategies, but two studies of diurnal raptors (Eurasian Kestrels [*Falco tinnunculus*] and Lesser Kestrels [*Falco naumanni*]) revealed that 3–5% of chicks were fathered outside the pair bond (Korpimäki et al. 1996, Negro et al. 1996).

While genetic monogamy among female Boreal Owls is not surprising, considering that EPFs have never been reliably demonstrated among strigiforms, it is surprising to find an avian taxon with no evidence for EPFs when many others show at least low levels (Korpimäki et al. 1996, Negro et al. 1996, Westneat and Sherman 1997). For all species, the benefits of EPFs must be considered with respect to the associated costs. We suspect that genetic monogamy among female owls derives from the fact that owls experience very few benefits from EPFs and the costs may be high. Whereas female passerines may benefit from association with neighboring males for increased protection, decreased competition, and provisioning for her and her young if her mate dies (Emlen 1978, Stacey 1982), lon-

ger-lived species, such as owls, would not benefit in this manner due to the low likelihood of male mortality during the nesting period. Genetic superiority among males in neighboring territories may provide impetus for females of some species to solicit EPFs (Smith 1988, Kempnaers et al. 1992). Among Boreal Owls, however, females tend to choose older, more experienced males over younger males (Laaksonen et al. 2002). Male Boreal Owls appear to become better mates as they age based on experience, rather than genetic superiority (Laaksonen et al. 2002).

Because of the high energetic investment by male Boreal Owls, assurance of genetic paternity likely is important. Among owls, males provide most of the care for both the female and the young during the nesting season (Mikkola 1983). Male Boreal Owls feed their mates for an extended period prior to nesting and throughout incubation and brooding, and males care for the chicks well beyond fledging (Hayward and Hayward 1993). The period of high male investment may be longer than 3 mo. Females may help feed younger chicks, but often leave the territory entirely when the chicks are ca. 20 d old (Hayward and Hayward 1993). Males may provide less care if females participate in EPFs, thereby causing the nest to fail. With such severe repercussions, such a strategy would not be adaptive.

It is tempting to attribute genetic monogamy in owls solely to long life and the necessity for high levels of paternal care. These two factors appear to provide sufficient impetus for females to forego EPFs and choose mates with more breeding experience. Nevertheless, some long-lived species with high male investment in parental care, such as diurnal raptors, have low levels of EPFs (Korpimäki et al. 1996, Negro et al. 1996) rather than strict genetic monogamy. We suspect that other factors, such as phylogenetic relationships and ancestral breeding strategies (Griffith et al. 2002) act as additional influences promoting an unusually strong prevalence of genetic monogamy in female owls.

#### ANÁLISIS DE MICROSATÉLITES REVELAN MONOGAMIA GENÉTICA EN HEMBRAS DE *AEGOLIUS FUNEREUS*

RESUMEN.—Utilizando datos de siete loci microsatelitales, evaluamos la paternidad genética de pichones de la especie *Aegolius funereus* en el oeste de los Estados Unidos, incluyendo a Alaska. No encontramos evidencia de fertilizaciones extra-pareja entre 109 pichones de 32 nidos. Estos nidos fueron muestreados en seis subpoblaciones diferentes, algunas de las cuales presentaban altas densidades de individuos nidificantes. Con base en nuestros resultados y en los de otros cuatro estudios, concluimos que las fertilizaciones extra-pareja son extremadamente raras en *A. funereus*, y que las hembras son social y genéticamente monógamas, con excepción de eventos de poliandria secuencial. Los machos podrían aprovisionar varios nidos cuando los recursos son abundantes, pero las hembras no participan en fertilizaciones extra-pareja. Aunque la densidad y la sincronía de la nidificación parecen influen-

ciar la frecuencia de las fertilizaciones extra-pareja en algunas aves paserinas, no parecen hacerlo en los búhos. Varios factores, entre los que se incluyen la longevidad y la alta inversión parental por parte de los machos, actúan para reforzar la monogamia genética de las hembras como la principal estrategia de apareamiento en los Strigiformes. [Traducción del equipo editorial]

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