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NO EXTRA-PAIR FERTILIZATION IN FLAMMULATED OWLS DESPITE AGGREGATED NESTING

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Abstract. We used DNA fingerprinting to estimate the frequency of extra-pair fertilization in the Flammulated Owl (*Otus flammeolus*), a socially monogamous species often found nesting in aggregations. We observed owls on 44 territories, 42 of which were located in aggregations of 3 to 10 territories with a mean nearest-neighbor distance of 539 m (\pm 160 m). We

sampled a subset of these nests and compared band sharing and numbers of novel fragments among 17 social pairs and 37 offspring. Like other socially monogamous raptors, the Flammulated Owls we studied were genetically monogamous despite their relatively high breeding density. We suggest that the necessity of male parental care may be an important factor contributing to genetic monogamy in this species.

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Ausencia de Fertilización Extra-Pareja en *Otus flammeolus* a pesar de Nidificación Agregada

Resumen. Usamos huellas dactilares genéticas (i.e., DNA fingerprinting) para estimar la frecuencia de fertilización extra-pareja en *Otus flammeolus*, una especie social monógama frecuentemente hallada nidificando en agregaciones. Observamos lechuzas en 44 territorios, 42 de los cuales se encontraron en agregaciones de entre 3 a 10 territorios, con una distancia media al territorio vecino más cercano de 539 m (\pm 160 m). Muestreamos un subconjunto de los nidos localizados y comparamos las bandas compartidas y el número de fragmentos nuevos entre 17 pares sociales y 37 hijos. Al igual que otras rapaces sociales monógamas, los individuos de *O. flammeolus* que estudiamos fueron genéticamente monógamos, a pesar de la relativamente alta densidad de individuos presentes en la época de cría. Sugerimos que la necesidad de cuidado parental masculino puede ser un factor importante que contribuye a la monogamia genética en esta especie.

Studies of parentage using molecular techniques have revealed that in a large number of socially monogamous bird species, females are often fertilized by extra-pair males (Westneat et al. 1990). These observations have re-energized debate on the evolution and maintenance of monogamy and the function of alternative mating strategies. Disentangling cause and effect in the regulation of social systems has proven difficult (Petrie and Kempenaers 1998); however, the importance of particular factors has been suggested in a number of studies. For example, the occurrence of extra-pair fertilization (EPF), or lack thereof, has been variously attributed to breeding synchrony (Stutchbury and Morton 1995), breeding density (Møller and Birkhead 1993), mate guarding (Gowaty et al. 1989), age (Gowaty and Bridges 1991), and the importance of male parental investment (Gowaty 1996).

Several investigators report that breeding Flammulated Owls are often aggregated, and they have thus been considered semicolonial (Marshall 1939, Howie and Ritcey 1987, Arsenault 1999). For example, one study of habitat use found groups of 2–10 territorial males spaced as little as 50 m apart and approximately 500 m on average (Wright 1996). The causes and consequences of such aggregated nesting in Flammulated Owls have not been examined.

Clusters of breeding birds may function as “hidden leks” where high-quality males benefit from nesting close to other breeding pairs by copulating with females socially bound to other males (Wagner 1993, Hoi and Hoi-Leitner 1997). Females might benefit in this system by mating with high-quality males even when paired with a lower quality male. Therefore, the potential for EPF is presumably high for Flammulated Owls, as in other cavity nesting birds that breed close to conspecifics (Mumme et al. 1985, Hoffenberg et al. 1988, Gowaty and Bridges 1991). In fact, extra-pair copulation (EPC) has been observed in the Flammulated Owl (Reynolds and Linkhart 1990), suggesting that this could be a common mating strategy for this secretive species.

On the other hand, there is evidence in some avian species that males will reduce provisioning if paternity is uncertain (Sheldon et al. 1997). Therefore, females may risk losing parental care by seeking EPCs. Orians (1969) suggested, and experimental studies have found, that male provisioning is important to female reproductive success for many bird species (Gowaty 1996). In Flammulated Owls, social fathers are active parents that provide much of the food consumed by nestlings, as in all owls (Johnsgard 1988). Therefore, the females may be monogamous to maintain the support of their social mate.

The purpose of this study was to better understand the mating system of Flammulated Owls by estimating the frequency of EPF using multilocus DNA fingerprinting. Furthermore, we wanted to explore the possible effects of the observed pattern, such as breeding density and male parental care.

METHODS

STUDY AREA AND FIELD METHODS

Our nine study areas were located between 2000 m and 2700 m elevation in lower montane coniferous forests in the Black Range and the San Mateo, Magdalena, and Zuni Mountains of western New Mexico (34°30'N, 108°00'W). These are xeric forests characterized by ponderosa pine (*Pinus ponderosa*) and Gambel oak (*Quercus gambelii*) with grassy understories and scattered shrubs. Remnant narrowleaf cottonwoods (*Populus angustifolia*) are scattered throughout the riparian areas. In our study areas, ponderosa pine intermingles with quaking aspen (*Populus tremuloides*) and Douglas-fir (*Pseudotsuga menziesii*) at higher elevations, and with pinyon pine (*Pinus edulis*) at lower elevations.

Study areas were defined by 1–3 survey transects located in suitable Flammulated Owl habitat (McCallum 1994). Survey transects were chosen based on topography so that study areas were covered systematically. Study area size was calculated by assuming owls were detected within 0.5 km on either side of transects. Transects in five study areas were located along roads or trails in drainage bottoms where the survey area encompassed the ridgeline on both sides of the drainage. One transect was located on a mesa and two were located on slopes with subtle topography. In six study areas, transects were aligned parallel to one another so that the width of the study area was greater than 1.0 km.

We conducted nighttime surveys from May through June 1996–1998 by stopping every 0.5 km along transects to listen for Flammulated Owl calls. If no owls were heard, we imitated or played a recording of a male territorial call to elicit a response. Each transect was surveyed at least twice in a breeding season and in at least two consecutive years. Surveys were not conducted when wind speed was greater than approximately 5 km hr⁻¹.

We spot-mapped locations of owls observed during surveys and territory monitoring (i.e., nest searching and repeated nighttime visits) to estimate the number of males and their territory boundaries. Nearest-neighbor distance was calculated as the distance between

TABLE 1. Density of Flammulated Owls in nine areas of western New Mexico.

| Mountain range | Study area | Size (ha) | Number of territories | distance (m) Mean \pm SD | Number of nests sampled |
|----------------|--------------|-----------|-----------------------|-------------------------------|-------------------------|
| Black | Doagy | 300 | 1 | >1.5 km | 1 |
| | Burnt Cabin | 300 | 1 | >1.5 km | 1 |
| San Mateo | Big Pigeon | 250 | 5 | 580 \pm 76 | 3 |
| | West Red | 350 | 6 | 758 \pm 306 | 1 |
| | L. Bear Trap | 350 | 7 | 600 \pm 100 | 1 |
| | U. Bear Trap | 350 | 7 | 500 \pm 200 | 2 |
| Magdalena | Water | 175 | 3 | 567 \pm 115 | 1 |
| Zuni | CWG | 200 | 4 | 438 \pm 144 | 1 |
| | Surprise | 175 | 10 | 330 \pm 177 | 6 |
| Mean | | 272 | 6.0 | 539 \pm 160 | 1.9 |
| Total | | 2450 | 44 | | 17 |

the estimated center of adjacent male territories. All means are reported \pm SD.

SAMPLING AND DNA FINGERPRINTING

Adult Flammulated Owls were captured with mist nets at nests and nestlings were removed from nest cavities. Many nest cavity entrances were large enough to reach nestlings by hand. A "trap door" was cut in the cavity wall for nests with smaller entrances. Owls were banded with U.S. Fish and Wildlife Service aluminum numbered bands. Up to 250 μ L of blood was taken from the wing using heparinized hematocrit tubes, immediately mixed with lysis buffer (0.1 M Tris, 0.1 M EDTA, 2% SDS) and stored at approximately room temperature.

Extracted DNA was cut with Hae III restriction enzyme and loaded onto 1% agarose gels (20 \times 25 cm gel, 1 \times TAE buffer, 35 V, 48 hr). A sizing standard (Lifepoint, Lifecodes Corp., Stamford, Connecticut) was run in every lane with DNA samples. Separated DNA fragments were transferred to neutral charge nylon membrane with the TurboBlotter system (Schleicher and Schuell, Keene, New Hampshire). The membrane was incubated in blocking solution, hybridized to two alkaline-phosphate labeled oligonucleotides (Jeffreys' 33.15 and 33.6, Lifecodes Corp.), washed, and incubated in assay buffer using Lifecodes products and protocols. CDP-star substrate (TROPIX, Bedford, Massachusetts) was applied and the membrane exposed to medical X-ray film. A detailed description of lab protocol is described in Arsenault (1999).

DNA fragments detected by each probe and size standard fragments were hand drawn on acetate overlays using a light board for analysis of banding patterns. Portions of the fingerprints that were distorted or unclear (due to smudging, spotting, etc.) were not considered for the analysis. Band sharing between parents and offspring, the male and female of each pair, siblings, nestlings, and neighboring males, and between unrelated adults in each population (all relationships were putative) were calculated as twice the number of shared bands divided by the sum of the number of bands in both individuals (Lynch 1991). We calculated band sharing between each juvenile and the closest neighboring male (that we sampled) because these males were potential genetic parents. Band-sharing

values were compared between groups using the Mann-Whitney *U*-test with SYSTAT 7.0 (SYSTAT 1992). Exclusion analysis was based on band sharing and novel fragments.

We scored DNA fragments between approximately 3500 and 25 000 bp in size for each owl. A mean of 25 \pm 6.2 and 19 \pm 6.0 fragments were scored per individual for Jeffreys' 33.15 and 33.6 probes, respectively.

RESULTS

We found Flammulated Owls on 44 territories in the areas that we surveyed. Owls nested close to one another with a mean nearest-neighbor distance of 539 \pm 160 m (Table 1). We sampled 17 social pairs and all of their 37 offspring (mean 2.2 \pm 0.8 offspring per pair) from nine locations in four mountain ranges (Table 1). Two of the nests sampled were solitary with a nearest-neighbor distance greater than 1.5 km (Table 1). Fifteen of the nests sampled were in aggregations of 3 to 10 territories (Table 1) and had a mean nearest-neighbor distance of 396 \pm 198 m. All of the nests in each of the study areas were active simultaneously.

The mean band sharing for adult pairs was 0.32 \pm 0.07 (Table 2), and this was significantly lower than band sharing between parents and their offspring (0.61 \pm 0.11; Mann-Whitney *U* = 5028, *P* < 0.001; Table 2). The distributions of band sharing in these two groups are nearly exclusive (Table 2); therefore our data are useful in analyzing parentage (Westneat 1990). The mean band sharing between unpaired adults within each population was 0.28 \pm 0.10 (Table 2), very similar to that for mated pairs. The 99% upper confidence limit for all comparisons between adults was 0.33, and the lowest parent to offspring band sharing value was 0.39. Mean band sharing between parents and offspring was not different from that between siblings (0.61 \pm 0.14; Mann-Whitney *U* = 4099, *P* = 0.78; Table 2). Juveniles had an average of 5.4 \pm 2.3 paternal-specific bands and 9.7 \pm 4.5 maternal-specific bands corresponding to a probability of 2.1 \times 10⁻³ for assigning the male parent incorrectly and 1.6 \times 10⁻⁵ for assigning the female parent incorrectly (Bruford et al. 1992). Therefore, the probability of false inclusion of extra-pair young was low. We detected only five novel bands across all parent to offspring comparisons

TABLE 2. Relatedness of Flammulated Owls in western New Mexico. The proportion of bands shared and number of novel fragments were determined for five comparison groups, using multilocus DNA fingerprinting. The sample consisted of 17 social pairs and 37 nestlings.

| Comparison | n^a | Band sharing | | | Novel fragments | |
|------------------------------|-------|--------------|------|-----------------|-----------------|-----------------|
| | | Min. | Max. | Mean \pm SD | Total | Mean \pm SD |
| Parent to offspring | 148 | 0.39 | 0.87 | 0.61 \pm 0.11 | 5 | 0.03 \pm 0.18 |
| Nestling to neighboring male | 73 | 0.09 | 0.50 | 0.30 \pm 0.08 | 330 | 4.50 \pm 2.2 |
| Sibling to sibling | 54 | 0.36 | 0.91 | 0.61 \pm 0.14 | — | — |
| Male to female (pairs) | 34 | 0.13 | 0.42 | 0.32 \pm 0.07 | — | — |
| Adult to adult (unpaired) | 37 | 0.12 | 0.50 | 0.28 \pm 0.10 | — | — |

^a Number of pairwise comparisons.

(Table 2) and no more than one novel band in any particular parent to offspring comparison. In cases where a novel band was present, band sharing between parents and offspring was greater than 0.49. Comparisons between juveniles and neighboring males had an average band sharing of 0.30 ± 0.08 (Table 2). When neighboring males were tested as putative parents (with the juvenile's social mother), an average of 4.5 ± 2.2 novel bands (range = 0–9; Table 2) were present. In cases where band sharing between juveniles and neighboring males was greater than 0.35, there were at least two novel bands present. Based on these results, none of the social parents could be excluded as the genetic parents of their putative offspring, but all of the neighboring males could be excluded as the fathers of the offspring to which they were compared. Therefore, if we assume all of the 37 offspring sampled were from independent fertilizations, then EPF did not occur in more than 3% of all cases.

DISCUSSION

Nesting in proximity provides opportunity for EPC in Flammulated Owls. In fact, males have been observed moving into the territories of neighboring pairs during periods of female fertility, and in one case, a female was observed copulating with an extra-pair male (Reynolds and Linkhart 1990). However, our multilocus DNA fingerprinting results indicate that male Flammulated Owls do not successfully fertilize females other than their social mate in more than 3% of all cases. Therefore, owls appear to be socially and genetically monogamous, in spite of nesting proximity.

Cause and effect cannot be determined in this study, but the requirement for intensive male parental care in this species may be a key factor contributing to the maintenance of genetic monogamy. If females control the paternity of their offspring, then the costs and benefits to the female should influence whether or not EPFs occur. Existing theory suggests that females may benefit from EPCs by (1) gaining direct benefits from extra-pair males, such as foraging opportunities on their territories; (2) becoming familiar with local males so that re-mating may be quicker if their current mate dies or re-mates; and (3) obtaining "good genes" by copulating with high-quality extra-pair males when paired with a low quality male (Petrie and Kempenaers 1998). However, there is evidence for some birds that males will reduce provisioning of food if paternity is

uncertain (Sheldon et al. 1997). Therefore, females may risk losing paternal investment by seeking EPCs. If male provisioning is important for female reproductive success in the Flammulated Owl, then females might be monogamous to maintain the support of their social mate (Orians 1969, Gowaty 1996).

Male parental effort, in fact, is high in Flammulated Owls, as in all owl species (Johnsgard 1988). They produce only one brood per year and re-nesting is unknown. Their nesting period is long for a small, insectivorous bird: almost three months from egg laying to fledgling independence (McCallum 1994). Furthermore, average reproductive success is low: about two young fledge per pair per year (McCallum 1994). Males provision females extensively throughout courtship, copulation, and incubation, when females rarely, if ever, forage on their own (McCallum 1994). Males are also the sole providers through the first part of brooding, and contribute equally with females in provisioning nestlings thereafter (DPA, unpubl. data). After nestlings fledge, each is attended by one parent, and male provisioning continues until young are independent (Linkhart and Reynolds 1987). Therefore, male provisioning may well be critical to female reproductive success in Flammulated Owls, possibly contributing to genetic monogamy in this species.

However, some experimental studies of parental care have found no reduction in care by males who were not the fathers of all of the offspring they attended (Whittingham et al. 1993, Ratti et al. 2001). Thus, the relationship between male parental care and paternity is complex and factors such as age, offspring recruitment, and alternative activities must be considered (Whittingham et al. 1992, Westneat and Sherman 1993).

Like this study, DNA fingerprinting studies of other socially monogamous raptors, all of which have a high degree of male parental care, have detected low frequency of extra-pair fertilization (0–6%; Korpimäki et al. 1996, Negro et al. 1996, Lawless et al. 1997, Marks et al. 1999). Although these studies suggest a general pattern, comparisons with raptors exhibiting little male parental care would be useful to begin teasing apart the influence of phylogeny from the influence of male parental care in determining the frequency of EPFs.

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