ORIGINAL ARTICLE

Analysis of genetic parentage in the tawny owl (*Strix aluco*) reveals extra-pair paternity is low

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Abstract We have investigated genetic parentage in a Swiss population of tawny owls (*Strix aluco*). To this end, we performed genetic analysis for six polymorphic loci of 49 avian microsatellite loci tested for cross-species amplification. We found one extra-pair young out of 137 (0.7%) nestlings in 37 families (2.7%). There was no intra-specific brood parasitism. Our results are in accordance with previous findings for other raptors and owls that genetic monogamy is the rule. Female tawny owls cannot raise offspring without a substantial contribution by their mates. Hence one favoured hypothesis is that high paternal investment in reproduction selects for behaviour that prevents cuckoldry.

Keywords Microsatellite · Tawny owl · *Strix aluco* · Extra-pair paternity

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Introduction

The recent development of molecular genetic markers has revealed that birds are rarely sexually monogamous, with extra-pair offspring found in approximately 90% of the species studied (Griffith et al. 2002). The frequency of extra-pair young (EPY) greatly differs between species and populations; this has been related to a variety of ecological factors and life-history tactics (reviewed by Westneat and Sherman 1997; Griffith et al. 2002; Roulin et al. 2004). Several hypotheses have been proposed to explain why in some species the level of extra-pair paternity (EPP) is high. For example, females may copulate with extra-pair males to obtain good genes, maximize genetic compatibility, avoid inbreeding depression, or increase offspring heterozygosity (Tregenza and Wedell 2000; Petrie and Kempenaers 1998; Küpper et al. 2004; Foerster et al. 2003).

A high level of EPP is usually found in socially monogamous colonial species and in territorial species that reproduce at high densities and/or synchronously, because females can easily engage in extra-pair copulation (EPC) with neighbouring males (Stutchbury 1998; Arlt et al. 2004). Arnold and Owens (2002) also proposed that males in long-lived species reduce parental investment if paternity was uncertain to fully invest in future reproductive attempts in which the certainty of paternity is high. This argument is particularly relevant for species in which males assume most of the parental duties, for example raptors and owls (Møller and Cuervo 2000; Møller 2000; Pechacek et al. 2005). Indeed, males of these species provide most of the food items for their brood and their female partners stay on the nest to warm the eggs and nestlings,

and distribute the food among the offspring. Accordingly, in raptors and owls the incidence of EPP is particularly low even in species breeding at high densities (Roulin et al. 2004).

Here we report the first study of alternative breeding strategies of the tawny owl *S. aluco*, a philopatric and socially monogamous raptor. The tawny owl is the most common owl in temperate forests in the Palaearctic with typically 0.5–1.0 breeding pairs per km^2 (Galeotti 2001). To examine genetic parentage in 37 families of a Swiss population we developed a system of cross-species amplified microsatellite loci.

Methods

General methods and study site

The study was performed in spring 2004 in a population of tawny owls breeding in nest-boxes in western Switzerland in an area of 600 km^2 located at a mean altitude of 660 m (range 480–900). For each nest, we recorded clutch size, hatching date, number of hatchlings and fledglings. Using heparinised Microvette CB 300 (Sarstedt, Sevelen, Switzerland) we collected a blood sample (20–100 μL) from the brachial veins of 35 female and 34 male parents and 137 hatchlings from 37 families. To calculate the allele frequencies in the population we also considered eight extra adults (six females and two males) which had lost their clutch. Blood samples were stored in Queen's Lysis Buffer (Seutin et al. 1991) at –20°C until DNA extraction in the laboratory with a commercial kit (Wizard Genomic DNA Isolation Kit, Promega, Switzerland).

Genetic parentage analysis

Because no microsatellite markers have been previously described in the tawny owl, we tested 23 and 26 microsatellite loci isolated from strigidae and passerines, respectively, for cross-species amplification and polymorphism (Appendix). Cross-species amplification tests were performed on blood collected in 2003 for the same studied population on eight nestlings from eight different broods. Polymerase chain reaction (PCR) was run in a 10 μL volume containing 10–50 ng genomic DNA, 1 μL *Taq* buffer, 0.5 U Ampli *Taq*Gold DNA polymerase (Applied Biosystems, Rotkreuz, Switzerland), 200 μmol L^{-1} of each dNTP (Amersham Pharmacia Biotech), 0.5 μmol L^{-1} of each primer, and a locus-dependent concentration of MgCl$_2$ (Appendix). PCR cycling conditions were 10 min at 95 °C, 40 cycles of 30 s at 95 °C (except for So8G11, with 35 cycles), 30 s at locus-specific annealing temperature, 45 s at 72 °C, followed by a final extension step for 60 min at 72 °C (GeneAmp 9700 thermal cycler; Applied Biosystems). PCR products were monitored for amplification by electrophoresis on ethidium bromide-stained 2% agarose gels, which were visualised by UV-transillumination. In total, 21 of the 23 strigidae loci and 18 of the 26 passerine loci were successfully amplified (Appendix), and PCR fragments of 11 loci which seemed to be polymorphic on agarose gels were then analysed by capillary electrophoresis on an ABI 3100 Genetic Analyser (Applied Biosystems) (Appendix). Out of those 11 loci, 9 were analysed for allele frequencies using 24 unrelated nestlings and for mendelian segregation using two families sampled in 2003. Final genetic parentage analysis using the blood samples collected in 2004 was restricted to six polymorphic loci that were in Hardy–Weinberg equilibrium and had a high exclusion probability for the candidate parents (Table 1). Exclusionary power of all six loci was 0.9936 for the first parent and 0.9995 for the second. Parentage assignment was performed with Cervus Version 2.0 (Marshall et al. 1998). The simulation was run with the settings: 10,000 cycles and 50 candidate parents; the proportion of sampled candidate parents was 0.72; the rate of typing error was set to 0.02. Because both social parents were known for most of the families analysed, we first used the program to control for mismatches between the nestlings' and their parents' genotypes. As a second step we ran the analysis without a known parent, including all sampled males and females, and then with the female assigned as known parent and all males as candidate parents.

Results and discussion

Nine of the 137 nestlings (6.6%) showed one mismatch with their putative genetic mother but no nestling was mismatched with its mother at more than one locus, suggesting that intra-specific brood parasitism does not occur in our population. In tawny owls only females incubate the eggs; because they breed in January and February, they can only go away from the nest for a very short spell during egg laying and incubation, otherwise eggs and small nestlings would freeze. During this period females are provided with food by their mate. The female's permanent presence in the nest hole leaves only very small opportunity for other females to parasitize nests during the egg laying and incubation period.

Table 1 Number of individuals analysed, size range in base pairs (bp), number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), exact P-values and SE of the Hardy–Weinberg equilibrium (HWE) test, exclusion probability for the first parent (ExP1), exclusion probability for the second parent when first parent assigned (ExP2), and estimated null allele frequency for nine cross-amplified microsatellite loci tested in unrelated tawny owls

Locus	No. individuals analysed	Size range (bp)	No. of alleles	H_O	H_E	HWE P-value	HWE SE	ExP1	ExP2	Estimated null allele frequency
Bb111	24	200–206	4	0.292	0.391	0.0735	0.0064	0.075	0.207	0.1812
Oe022	24	250–254	2	0.083	0.082	1	0	0.003	0.038	–0.0122
So15A6	**77**	**111–158**	**8**	**0.675**	**0.716**	**0.5144**	**0.0203**	**0.307**	**0.484**	**0.0274**
So8G11	77	126–158	8	0.545	0.729	0.0048	0.0019	0.32	0.495	0.1425
So13D8	**77**	**154–182**	**12**	**0.831**	**0.877**	**0.0833**	**0.0137**	**0.588**	**0.742**	**0.0228**
Oe2-57	**77**	**168–273**	**23**	**0.922**	**0.927**	**0.9665**	**0.0118**	**0.733**	**0.845**	**–0.0028**
Oe128	**77**	**301–321**	**6**	**0.805**	**0.827**	**0.3433**	**0.0084**	**0.464**	**0.64**	**0.0087**
Oe142	**77**	**228–268**	**10**	**0.818**	**0.766**	**0.6701**	**0.0215**	**0.37**	**0.548**	**–0.0379**
LOX1	**77**	**341–389**	**29**	**0.961**	**0.935**	**0.5785**	**0.0421**	**0.753**	**0.858**	**–0.0169**

Deviation from HWE was tested by use of Genepop version 3.4 (Raymond and Rousset 1995). Loci finally selected for genetic parentage analysis are shown in bold

One nestling out of 137 (0.7%) mismatched its putative biological father at two loci, and was therefore regarded as an extra-pair fertilized offspring. Hence, only one out of 37 (2.7%) broods contained an EPY. This male young was the last-hatched nestling of a three-egg clutch. Use of the parentage assignment computation in Cervus did not enable identification of the genetic father of this nestling among the males sampled. The discovery of only 0.7% EPY in the tawny owl is in agreement with the hypothesis that long-lived species in which males invest substantial effort in rearing their brood should have low levels of EPP (Westneat and Sherman 1997; Marks et al. 1999; Mauck et al. 1999; Wink and Dyrcz 1999; Møller 2000; Møller and Cuervo 2000; Griffith et al. 2002; Arnold and Owens 2002; Roulin et al. 2004; Spottiswoode and Møller 2004).

At least two mechanisms can account for low EPP rates in owls and other raptors (reviewed by Roulin et al. 2004). First, because much paternal effort is crucial for the success of the brood, females are predicted to refrain from seeking EPC if males reduce their breeding effort when their paternity is in doubt (Osorio-Beristain and Drummond 2001; Arnold and Owens 2002; Neff 2003). Alternatively, high paternal investment may leave males with little time available to seek EPC. As the females must stay in their nest hole during egg laying, because of the low ambient temperature, males interested in EPC would have to go searching actively for sexual contacts by intruding in foreign territories, risking injury by the mate of the target female and loss of the resources needed for their within-pair offspring.

Experimental work is now required in which there is manipulation of both certainty in paternity (e.g. by simulating intrusion of another male into a territory) and paternal investment (e.g. by modifying brood size, which would result in males with reduced brood size having more resources for seeking EPC than males with large brood sizes).

Zusammenfassung

Genetische Vaterschaftsanalyse bei Waldkäuzen (*Strix aluco*) zeigt ein seltenes Auftreten von Fremdvaterschaft

Wir untersuchten die genetische Elternschaft in einer Schweizer Waldkauzpopulation (*Strix aluco*) mittels sechs polymorpher Mikrosatellitenloci. Diese waren aus 49 Loci ausgewählt worden, die ursprünglich für andere Vogelarten entwickelt worden waren und nun auf Amplifikation und Polymorphismus in Waldkäuzen getestet worden waren. Wir fanden einen Jungvogel unter 137 Nestlingen (0,7%) in 37 Familien (2,7%), der nur durch eine Befruchtung außerhalb des Paarbundes erklärt werden konnte. Intraspezifischer Brutparasitismus war in unserer Population nicht feststellbar. Unsere Ergebnisse entsprechen früheren Untersuchungen in anderen Greifvögeln und Eulen, nämlich dass diese Arten in der Regel genetisch monogam sind. Waldkauzweibchen können die Brut nicht ohne erheblichen Beitrag ihres Partners aufziehen. Das führt zur Hypothese, dass das hohe Mass des männlichen Aufwandes in die Reproduktion ein Verhalten selektioniert, das Partnerbetrug verhindert.

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