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Nest parasitism in the barnacle goose: evidence from protein fingerprinting and microsatellites

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Geese are often seen as one of nature's best examples of monogamous relationships, and many social pairs stay together for life. However, when parents and young are screened genetically, some chicks do not match their social parents. Although this has often been explained as adoption of foreign young after hatching, conspecific nest parasitism is another possibility. We used nondestructive egg albumen sampling and protein fingerprinting to estimate the frequency and success of nest parasitism in a Baltic Sea population of barnacle geese, *Branta leucopsis*. Among the 86 nests for which we had the most complete information, 36% were parasitized, and 12% of the eggs were parasitic. Almost 80% of the parasitic eggs were laid after the host began incubation. Hatching of these eggs was limited to the few cases where the host female incubated longer than normally because her own eggs failed to hatch. Conspecific nest parasitism in this population therefore seems mainly to be an alternative reproductive tactic of lower fitness than normal nesting. Comparison with DNA profiling of chicks (with 10–14 microsatellites) and other evidence confirmed the suitability of protein fingerprinting for analysis of nest parasitism. It can often provide more data than microsatellites, if eggs are albumen-sampled soon after being laid, before most losses occur.

In spite of their apparently monogamous family life, goose families are not always composed of an adult female, her mate and their common genetic offspring. Often one or more chicks has genetic parents other than the social ones, male as well as female. Whether these extrapair goslings represent adoption of young or conspecific nest parasitism (CNP) is harder to determine. In CNP, parasites lay eggs in the nests of other females (hosts) of the same species, without sharing incubation or rearing of young. This is a widespread alternative reproductive tactic (Oliveira et al. 2008) among animals, being found in insects, fish and more than 200 species of birds (e.g. Brockmann 1993; Wisenden 1999; Yom-Tov 2001; Tallamy 2005). It is especially common among waterfowl, in extreme cases with parasitism in 95% of all nests (Semel & Sherman 1986).

The advantages for the parasitic female seem obvious: by laying costs of offspring care on others, she can use the saved energy for investment in more eggs or higher survival (Brown & Brown 1998; Åhlund & Andersson 2001; Hartke et al. 2006). For the host, extra eggs can lower hatching success and future fecundity (Visser & Lessells 2001; Hanssen et al. 2003, 2005; Milonoff et al. 2004). However, if costs are low in precocial species, they may be overcome if extra young also add benefits such as dilution of predation risk (Eadie et al. 1988), or social dominance for a larger family (Loonen et al. 1999). There may also be inclusive fitness gains if host and parasite, or adopter and adoptee, are closely related (Andersson 1984, 2001; López-Sepulcre & Kokko 2002). Host–parasite relatedness has been found in some insects and waterfowl (Andersson &

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Åhlund 2000; Loeb 2003; Roy Nielsen et al. 2006; Andersson & Waldeck 2007). To what extent adoptions are accidental or tactical choices is debated, as are fitness consequences of adoptions (Kalmbach 2006).

Scanning of goose families with DNA techniques cannot distinguish between CNP and adoption of young. To identify CNP unambiguously, we used protein fingerprinting of egg albumen (Andersson & Åhlund 2001) together with microsatellite genotyping of hatchlings and adults in a Baltic Sea population of barnacle goose, *Branta leucopsis*, determining the maternity of eggs and chicks. In this precocial species with strong female natal philopatry, families often contain extrapair young (Choudhury et al. 1993; Larsson et al. 1995). However, it is not clear to what extent they represent CNP or adoption of young. We aimed to clarify the occurrence of CNP. In addition, combining protein fingerprinting of eggs with microsatellite typing of chicks enabled us to compare for the first time these different molecular methods for analysis of brood parasitism.

METHODS

Study Area and Population

The barnacle goose colony on three small islands, Laus holmar off the east coast of Gotland, Sweden (57°17′N, 18°45′E), has been studied for more than 20 years. Since the first breeding attempt in 1971 the colony has increased rapidly (Larsson et al. 1988; Larsson & Forslund 1994; Black et al. 2007). Adult and juvenile geese were captured in mid-July each year on moulting localities by a rounding-up technique. Captured birds were individually marked to allow individual identification at a distance. In some years, blood samples (0.15 ml) from captured adults were taken from the wing vein and stored in ethanol. In cases where blood could not be sampled, a growing wing feather was taken from adult birds at capture. There are no indications that marking or sampling affects the birds negatively. The present study of brood parasitism was done in 2003 on Storholmen (39.7 ha), one of the three islands hosting the goose colony. Of 1860 pairs on Laus holmar in 2003, 1400 pairs nested on Storholmen. Research was approved by the Swedish Board of Agriculture.

Sampling and Nest Success

During the breeding season (22 April to 14 June) we searched the area for new nests, checking them daily until 3 days after the last egg in the nest was added and incubation had started (except for 26–27 April and 16–23 May). We numbered new eggs with a nontoxic felt pen and sampled 0.3 ml of albumen through a 1 mm hole drilled 5–10 mm from the narrow end of the egg. The hole was sealed with cyanoacrylate glue (Loctite Superattack, Loctite Sweden AB, Göteborg, Sweden), with a droplet of activator (Loctite TAK PAK 7452) added to accelerate hardening. Samples were kept at −20 °C until electrophoresis. This procedure does not affect egg hatchability (Andersson & Åhlund 2001; Waldeck et al. 2004). While we sampled albumen, the pair usually remained in the neighbourhood and returned to the nest soon after we left. Albumen proteins are genetically variable among females, and their electrophoretic band patterns are useful both for identification of parasitic eggs and for estimation of host–parasite relatedness (Andersson & Åhlund 2000, 2001). An egg can be sampled up to a week after being laid, until the albumen becomes too viscous and easily contaminated with yolk. We noted differences in colour and shape between eggs in the nest, measured egg length and breadth with callipers, and noted any disappearances of eggs, usually caused by gull or fox predation. Foxes do not usually occur on Laus holmar, but during the 2003 goose breeding season a fox resided on the study island. We scored a nest as successful if one or more eggs hatched.

In cases where visited nests contained hatching eggs, we marked the emerging egg tooth with coloured nail varnish (IsaDora, www.isadora.com nontoxic) to be able to assign hatched chicks to individual eggs. We drew 25 μl of blood from the tarsal vein of hatched chicks. Hatching is synchronous and chicks usually stay in the nest for about 24 h. If they had already left we searched the nest for vascularized egg membranes, which can also be useful for microsatellite analyses.

Albumen Analyses

Albumen samples were analysed with isoelectric focusing (IEF) electrophoresis, using precast polyacrylamide gels with a fixed pH range (Immobiline DryPlate, GE Healthcare, www.gehealthcare.com see Andersson & Åhlund 2001 for details). Proteins applied to such gels migrate towards and come to rest at their isoelectric points, leading to high resolution of protein bands, which can also be compared between gels. These were stored dry at −20 °C and rehydrated in a solution designed to maximize band numbers and sharpness (Andersson & Åhlund 2001). The pH ranges used were 4–7, 4.2–4.9 and 4.5–5.4. Gels were loaded with 7 μl of crude albumen and run on an Amersham Biosciences Multiphor II System (GE Healthcare) with Electrophoresis power supply EPS 3501 for 6–14 h at 3000 V, 1 mA, 3 W and a cooling temperature of 10 °C.

DNA Analyses

DNA was isolated from blood, egg membranes, feathers and tissue using spin columns (Sigma, www.sigmaaldrich.com). We developed a panel of microsatellite primers to facilitate identification of true parents of hatchlings, using published primers from a range of geese, other waterfowl and passerines. PCR was done on a PTC-200 DNA Engine (MJ Research Inc., now Bio-rad Laboratoruis, www.biorad.com), comprising an initial denaturing step (94 °C–60 s) followed by 40 cycles of 94 °C (35 s), the annealing temperature (49–63 °C: see below; 40 s) and 72 °C (40 s). A final 2 min at 72 °C completed the run. Each 10 μl reaction contained 10–100 ng of sample DNA, 1 μl of 10× NH_4 buffer (Bioline, www.bioline.com), 2 mM of MgCl, 0.2 mM of each dNTP, 0.75 μM of each primer and 1 unit Biotaq polymerase (Bioline). Products were visualized by running on a 6% denaturing acrylamide gel and stained with silver (Bassam et al. 1991). We tested in total 32 microsatellites, of which 14 were polymorphic and possible to score, making them suitable for parentage determination. Details of these 14 microsatellite loci are listed in Table 1.

Three loci (Sfiμ5, Fields & Scribner 1997; Oxy6 and Oxy13 Muñoz-Fuentes et al. 2005) produced three or four extra bands in the same size range as the main product, making scoring of alleles unreliable. We overcame this by adding a further three or more bases (taken from the original sequence in Genbank www.ncbi.nlm.nih.gov) to both Forward and Reverse primers to increase specificity. This editing completely removed the additional products/bands, but it did not improve scoring of other markers with excessive stuttering. In two cases (Oxy6 and Oxy13) the original primer sequence had a 'PIGtail' (Brownstein et al. 1996) which was removed by adding the extra bases. Final primer sequences are listed in Table 2, 'a' denoting amendment from the original.

Tested on a panel of 70 presumed unrelated adult barnacle geese (54 females and 16 males), these 14 loci had a probability of 0.9983 of excluding an unrelated individual from paternity/maternity if the other parent was known (Chakravarti & Li 1983; Marshall et al. 1998). Five loci (CAUD012, Sfiμ11, Smo11, Smo8 and Ase46) were linked with the other nine but are still useful for parentage