No evidence of extra-pair paternity in the Atlantic Puffin Fratercula arctica

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Although many of the ecological and behavioural mechanisms behind the evolution of extra-pair paternity (EPP) in birds are still poorly known (Westneat & Stewart 2003), Arnold and Owens (2002) found that 55% of the interspecific variation in EPP rates was linked to taxonomic family or order level. At that time, only one paternity study had been published for a member of the auk family (Alcidae), which documented that the social father was not the biological father in six (7.8%, 95% CI recalculated at 2.3–19.3%) of 77 families of Common Guillemot Uria aalge in a Welsh colony (Birkhead et al. 2001). Since then, similar studies for two other auk species have been published: Ibarguchi et al. (2004) found the corresponding rate for the congeneric species Brünnich’s Guillemot Uria lomvia to be two (7.4%, 95% CI calculated at 0.9–24.3%) of 27 families sampled on Coats Island in Hudson Bay, whereas Lifjeld et al. (2005) found no incidents (95% CI 0–10.9%) of EPP in 26 families of the Little Auk Alle alle on Spitsbergen. In the latter study it was, however, pointed out that the incidences of EPP in these three auk populations were not significantly different. This could be expected, as they all exhibit many traits associated with low frequency of EPP (Westneat & Stewart 2003); not only are they closely related, they are also long-lived species with low fecundity, high levels of paternal care and little sexual difference in plumage coloration (Cramp 1985, Gaston & Jones 1998).

In the present study, we investigate the rate of EPP in a fourth member of the auk family, the Atlantic Puffin Fratercula arctica. This species exhibits the same general life-history characteristics as the Uria guillemots and the Little Auk: it is socially monogamous, lays only a single egg per season and the populations experience high adult survival (about 93% annually in five European colonies; Harris et al. 2005). In contrast to the three other species, the Atlantic Puffin has elaborate head and bill ornaments and strikingly coloured feet. However, to the human eye there is no clear sexual difference in their colouring, and males only have (in terms of surface area) 10–15% larger bills than their mates (Tycho Anker-Nilssen and Tomas Aarvak, unpubl. data). We therefore predicted that the rate of EPP in this species is likely to be as low as for the other auk species studied so far.

METHODS

Study site and field work

Blood and tissue samples were collected in late June and early July of 2003 and 2004 from 12 and 26 different family groups (pair with chick) of Atlantic Puffins, respectively, on the islet of Værholmen (67°26′N, 11°53′E) in Røst, northern Norway. The Røst archipelago has one of the largest breeding populations of Atlantic Puffins in the world (Anker-Nilssen & Aarvak 2006). The study burrows were marked in the field with numbered sticks and inspected on up to six occasions in the weeks around hatching. No birds were sampled twice, but on four nests we only succeeded in capturing one of the adults. Due to generally poor conditions for breeding in 2003, when only 35% of the chicks survived to fledging (Anker-Nilssen & Aarvak 2006), hatching success in the study nests differed significantly between the 2 years (χ² = 4.14, P = 0.042). In 12 nests, we therefore had no other option than to sample tissue from the dead embryo within the abandoned egg. In the other 26 nests, blood was sampled from live chicks.

Blood from chicks and adults was sampled by puncturing the bird’s metatarsus vein with a sterile needle and collecting 25–50 μL of the emerging blood with a sterile micropipette. The sample was immediately transferred to and dissolved in 1 mL of Queen’s lysis buffer (Seutin et al. 1991) in an air-tight Eppendorf or cryo container and stored at room temperature for the genetic analysis 1.5–2.5 years later. From dead embryos, a small sample of muscle tissue (heart) was cut out with a sterile scalpel and stored in 96% ethanol at room temperature in similar containers for an equally long period.

Parentage analyses

Genomic DNA was extracted from the blood or tissue samples with E.Z.N.A. DNA Kit (Omega Bio-Tek, Norcross, GA, USA) and we assigned parentage of the young by using...
four polymorphic microsatellite markers previously developed from Whiskered Auklet Aethia pygmea (Dawson et al. 2005) and Scottish Crossbill Loxia scotica (Piertney et al. 1998). All individuals were also amplified with two additional microsatellite markers (Apy06 and Apy09; Dawson et al. 2005), but due to high levels (> 25%) of non-amplifying alleles they were excluded from the parentage analyses. Microsatellite loci were amplified by polymerase chain reaction (PCR). Each 10-μL reaction consisted of about 30 ng of genomic DNA, 0.5 μL of each primer (forward primers were fluorescently dyed), 0.1 μM dNTP mix (ABgene, Epson, UK) and 0.2 units of DNA polymerase (DyNAzyme, Finnzymes, Espoo, Finland) in the manufacturer’s buffer (final concentrations of 10 mM Tris HCl, 1.5 mM MgCl2, 50 mM KCl, 0.1% Triton X-100). PCR was run on a GeneAmp 9700 Thermocycler (Applied Biosystems, Foster City, CA, USA). The PCR profile used consisted of an initial denaturing step at 94 °C for 5 min, followed by 35 cycles consisting of 94 °C for 30 s, X °C for 30 s (where X was 55 °C for Apy02, Apy12, Apy14, and 50 °C for Lox1) for 30 s, and 72 °C for 30 s. PCR was performed in a GeneAmp 9700 Thermocycler (Applied Biosystems). The PCR products were sized using a capillary automated ABI 3100 sequencer (Applied Biosystems) and allele binning with GENEMAPPER v3.0 analytical software (Applied Biosystems). Marker polymorphism was calculated with CERVUS v3.0 (Kalinowski et al. 2007). The four microsatellite markers constituted a powerful marker set for parentage analyses in Atlantic Puffins, with a combined exclusion probability of 0.986 for the first parent and 0.997 for the second parent (Table 1). All samples amplified successfully on all four loci. A 95% confidence interval around the estimate of extra-pair paternity was calculated according to Rohlf and Sokal (1981).

### Molecular sex determination

Adults were sexed using a standard molecular method (Griffiths et al. 1998), in which the primers P2 and P8 amplify a single fragment in males and two fragments in females. PCR amplification was carried out in a total volume of 10 μL with reagents as above. Thermal cycling conditions included an initial denaturation step at 94 °C (5 min), then 35 cycles of 94 °C (60 s), 50 °C (30 s) and 72 °C (30 s), followed by a final step of 72 °C (7 min). The PCR products were separated by electrophoresis at 100 V for 45-60 min on 3% agarose gels stained with ethidium bromide, and the bands were visualized with UV light.

### RESULTS

Molecular sexing confirmed that all 34 pairs where both adults were sampled consisted of one male and one female as expected. Where only a single parent was sampled, it was the male in all four cases. Parentage was determined for all 38 young (12 embryos and 26 chicks) in an equal number of broods (Atlantic Puffins have only a single chick per brood). All nestlings shared an allele, with the genotype of their putative mother. Thus we considered all offspring to be true descendants of their putative mother. All except one nestling matched the genotype of their putative father at all four loci and were thus assumed to be sired through within-pair fertilizations. A single nestling had an allelic mismatch with the putative father at one locus. The large-sized paternal allele (442 base-pairs) of the young and the closest sized allele (438 base-pairs) of the putative father at this hypervariable tetranucleotide locus (Lox1) differed with a single repeat unit. As fragment length and repeat motif size are assumed to influence microsatellite mutation rates, and mutations at such hypervariable loci typically occur by an addition or deletion of one repeat unit through slippage replication (Ellegren 2000), we assumed the observed allelic mismatch to be the result of a mutation. Additionally, the genotype of this chick and that of the putative father matched at the two excluded loci (see Methods). We thus concluded that this chick was also sired by its putative father. Consequently, we estimated the rate of extra-pair paternity in the study population to be zero with an upper 95% confidence limit of 7.6%.

### DISCUSSION

If we assume our sample was fully representative, it predicts the true rate of EPP in the Atlantic Puffin to be less...
Carlo likelihood ratio test, the rate of EPP can be found within the Alcidae (Monte Carlo likelihood ratio test, $G = 7.92$, $df = 3$, $P = 0.068$, 95% CI 0.063–0.073; data from Birkhead et al. 2001, Ibarguchi et al. 2004, Lifjeld et al. 2005, this study). With only 168 family units of auks studied so far and only eight (4.8%) cases of EPP discovered, this result is certainly sensitive to the low sample sizes (Griffith et al. 2002). It is nevertheless worth mentioning that the overall rate of EPP for the two guillemot species (8/104), which usually breed in the open and virtually side by side with many other pairs in their colony, appears to be higher than that for the two hole-nesting species, Atlantic Puffin and Little Auk (0/64; Fisher’s exact test, $P = 0.025$). Although the difference is only marginally significant and must be treated with caution, one possible explanation could be that these hole-nesting auks have fewer opportunities for successful extra-pair copulations (EPCs) on land simply because they have less clustered nesting distributions within colonies and because males are unlikely to accomplish forced EPCs on the sea. In sharp contrast to Common Guillemots, Creelman & Storey (1991) found that male Atlantic Puffins devote much less time to mate guarding and have never been observed succeeding in, nor attempting to force, EPCs, and that the female apparently rarely if ever solicits EPCs. However, it is not known to what extent a female might seek EPCs outside her own neighbourhood, as has been demonstrated to be common in European Shags Phalacrocorax aristotelis (Graves et al. 1992). In another colonial seabird, the Northern Fulmar Fulmarus glacialis, which behaves more like Puffins by spending much time away from the colony during the pre-laying period, EPCs occur at a low rate, but intra-pair paternity is secured by repeated copulations after the last EPC (Hunter et al. 1992).

The striking ornamentation of Atlantic Puffins, together with their brightly coloured feet and gape, could suggest that signalling sexual quality is more important in this species than in the less conspicuous guillemots and Little Auk. Nevertheless, our results indicate that any species-specific differences in ability to assess individual quality is either outweighed by some other factor, or does not significantly affect the likelihood of EPP among the Alcidae. In contrast to the other Atlantic auks, Atlantic Puffins usually copulate at sea close to the colony (Cramp 1985). Even if this might facilitate extra-pair interactions and males do indeed attempt EPCs there, females are easily able to escape assaults by diving (Creelman & Storey 1991). This is also seen in several species of Aethia auklets, which most often copulate at sea (Hunter & Jones 1999). Even in Razorbills Alca torda, which usually copulate on shore, the males are rarely able to fully accomplish EPCs (Wagner 1992). As the highly gregarious breeding behaviour of auks provides plenty of opportunities for extra-pair interactions, the low rates of EPP among the species within this family indicate that female auks have little benefit from EPCs other than as an insurance against male infertility and, as indicated for Common Guillemots by Walsh et al. (2006), mate appraisal to allow faster re-pairing in case of mate loss early in the season.

Costs and benefits of EPP are not only different for the male and female bird, but certainly also affected by the ecology of their species (Westneat & Stewart 2003). Individual trade-offs associated with EPP and, consequently, the temporary rates of EPP within and between populations of the same species are therefore likely to differ with varying environmental conditions for breeding. With no cases of EPP revealed, however, our study of the Atlantic Puffin could provide no support for this assumption, despite the fact that the study population experienced very different hatching and breeding success in the 2 years as a result of highly variable food availability (Anker-Nilssen & Aarvak 2006). Unfortunately, studies of how variation in access to food (e.g. Graves et al. 1992, 1993) or other important resources affect, in concert, the time budgets and sexual behaviour of alcids (e.g. by altering their mate-guarding capacity, social interactions and opportunities for EPCs) are still virtually non-existent.

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REFERENCES


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