

SHORT COMMUNICATION

Extra-pair paternity in waved albatrosses

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Abstract

We estimated the rate of extra-pair fertilizations (EPFs) in waved albatrosses (*Phoebastria irrorata*) on Isla Española, Galápagos, Ecuador, using multilocus minisatellite DNA fingerprinting. Waved albatrosses are socially monogamous, long-lived seabirds whose main population is on Española. Aggressive extra-pair copulation (EPC) attempts have been observed in the breeding colony during the days preceding egg-laying. Our genetic analyses of 16 families (single chicks and their attending parents) revealed evidence of EPFs in four families. In all cases males were the excluded parent. These data suggest that waved albatrosses have an unusually high rate of EPF relative to taxa with similar life histories. Future behavioural observations will determine the extent to which forced vs. unforced EPCs contribute to this high EPF rate.

Keywords: albatross, DNA fingerprinting, extra-pair fertilization, mating system, monogamy, procellariiformes

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Introduction

Many socially monogamous birds copulate outside the pair bond, often resulting in extra-pair fertilizations (EPFs) and mixed parentage within broods. The ultimate causation of females' participation in extra-pair copulations (EPCs) may involve a number of factors, including effects on the genetic quality of their offspring, insurance against mate infertility, and enhanced access to males' resources and services (Burley & Parker 1998). In contrast, only one selective influence has dominated discussions of the evolution of males' participation: the probability of gaining paternity of offspring outside the social family via EPFs (e.g. Gibbs *et al.* 1990). Abundant evidence, based on molecular assignment of parentage in wild birds, supports the view that males and females in socially monogamous systems may benefit from alternative mating tactics (Gowaty 1996; Gray 1998; Ketterson *et al.* 1998).

Colonially nesting species are predicted to have high EPF rates because proximity to potential copulation partners

is high (Møller & Birkhead 1993), yet Westneat & Sherman (1997) found no correlation between EPF rate and degree of coloniality. A stronger correlation with EPF rate is phylogenetic. Among avian taxa, variance in EPF rates tends to be higher in passerines than in nonpasserines (Fleischer 1996; Westneat & Sherman 1997). Variation in EPF rates for procellariiform taxa (tube-nosed seabirds) is limited, ranging from zero in fulmars (*Fulmarus glacialis*; Hunter *et al.* 1992), Cory's shearwater (*Calonectris diomedea*; Swatschek *et al.* 1994), and Leach's storm petrel (*Oceanodroma leucorhoa*; Mauck *et al.* 1995), to 9–13% in short-tailed shearwaters (*Puffinus tenuirostris*; Austin & Parkin 1996). Theoretical models examining effects on male parenting decisions suggest that EPF tolerance should be lowest in taxa with high adult survival, including typically long-lived procellariiform species (Mauck *et al.* 1999). Thus, theory and empirical data from related species and other colonially breeding birds suggest that EPFs in long-lived, colonially breeding seabirds, including our study species, the waved albatross, should be low.

Waved albatrosses (*Phoebastria irrorata*) are socially monogamous seabirds that nest in the Galápagos Islands, Ecuador. Annual survivorship for adults is high, averaging

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95% (Harris 1973). Females lay a single egg in minimal nest scrapes on the ground from mid-April to mid-May. Both females and males participate extensively in parental care throughout incubation and chick-rearing (Harris 1973). At the breeding site, individual males occupy small defended areas within the colony during the days preceding egg-laying (Harris 1973). Harris (1973) also reports that other individuals of either sex passing by these areas are frequently chased and attacked, and these attacks typically end with what appears to be a copulation attempt, although Harris suggests that the function of this behaviour is primarily territorial. Our anecdotal observations in the field match Harris' report: birds landing on Española are often chased down and these chases sometimes end in aggressive copulation attempts between birds that do not appear to be social mates (unpublished data). In addition to Harris' territoriality hypothesis, a potential function of these apparent copulation attempts is EPF. Here we used multilocus minisatellite DNA fingerprints to assess the rate at which EPFs occurred in waved albatrosses.

Methods

We studied waved albatross parentage at the eastern end of Isla Española, Galápagos Islands, their principal breeding site (Anderson & Cruz 1997). During the hatching period in early July 1996 we collected whole blood samples of approximately 1 cc from 17 hatchling offspring and their social parents. We considered an adult as a social parent if it brooded the hatchling. We saw no evidence among our sampled families nor elsewhere on the island that hatchlings were brooded by more than one male and one female. We drew the blood samples from the brachial vein with a sterile syringe and stored them in Queen's lysis buffer (Seutin *et al.* 1991), in the field at ambient temperature and later at 4 °C.

In the laboratory, the blood samples were incubated for 8 h at 65 °C, after adding 300 µg of Proteinase K. The DNA was extracted several times in phenol/phenol: chloroform: isoamyl alcohol, then dialysed against TNE₂ (0.01 M Tris-HCl pH 8.0, 0.01 M NaCl, 0.002 M EDTA). DNA (4–5 µg) was digested with excess *Hae*III restriction endonuclease at 37 °C. Resulting fragments were separated on a 0.8% agarose gel (22 cm) at 20 V for 65 h until all fragments smaller than 1600 base pairs were run off, and then transferred to nylon by Southern blot in 10× SSC buffer. The probe (33.15; Jeffreys *et al.* 1985) was radiolabelled by random primer extension with [³²P] dCTP. Hybridizations were run overnight at 62 °C in 1.5× SSC, 0.1% SDS, 5× Denhart's solution, and 6% w/v polyethylene glycol. Hybridized filters were washed 4×30 min at 62 °C in 1.5× SSC, 0.1% SDS, and exposed to X-ray film at –20 °C.

Digested samples were run as family groups, with putative parents flanking their offspring's lane, thus minimizing

scoring errors resulting from distance between lanes under comparison (Piper & Parker Rabenold 1992). No dyadic comparisons were performed across more than three lanes. We assessed the parentage of each chick by estimating its genetic similarity to the putative parents as the proportion of fingerprinting bands shared (band-sharing analysis), and by assessing whether all bands in the offspring lane could be accounted for in the fingerprints of the putative parents (unattributable band analysis). One chick sample did not provide readable bands, so parent-chick comparisons were made in only 16 families.

Band-sharing values were calculated as $2N_{AB}/(2N_{AB} + N_A + N_B)$, where N_{AB} is the number of bands shared by individuals A and B, N_A is the number of bands unique to A, and N_B is the number of bands unique to B (Wetton *et al.* 1987; Lynch 1990). Band-sharing values for all dyads consisting of putative parents and offspring were evaluated by comparison to distributions of band-sharing values for first-order relatives and unrelated birds. The set of 16 dyads of first-order relatives came from mother-offspring comparisons. The set of 31 dyads of unrelated individuals came from comparisons of mated pairs ($n = 16$) and nonmates ($n = 15$), with no individual being used in more than two dyads. The probability of paternity misassignment was calculated as X^P , where X is the proportion of bands shared among unrelated males, and P is the average number of exclusively paternal bands (see Parker Rabenold *et al.* 1991). We also calculated the probability of misassigning an uncle as father as s^P , where s is the proportion of bands shared between siblings (Parker Rabenold *et al.* 1991).

Sex of social parents was determined by polymerase chain reaction (PCR) amplification of a sex-linked DNA sequence within the chromo-helicase-DNA-binding protein (CHD) locus (Ellegren 1996; Griffiths *et al.* 1996, 1998). The CHD locus has been localized on both W and Z chromosomes in a variety of bird species (Griffiths *et al.* 1998). In albatrosses, primers CHD2920 (5'-AGAAGATA-TTCTTGAAAGAGCCA) and CHD3120 (5'-GGCTCCT-STTCTTCCCCTT) were designed with reference to the chicken CHD-Z DNA sequence (GenBank Accession no. JF11; Funahashi *et al.* 1993; GenBank Accession No. AF004397; Griffiths & Korn 1997) and crane CHD sequences (W. Duan and P. Fuerst, unpublished data) to amplify an intron and flanking exon from both W and Z copies. The intronic size difference, however, was not significant in waved albatrosses, so the PCR products were heated to denaturation and allowed to re-anneal at a lower temperature to create a heteroduplex (Delwart *et al.* 1994) that can be detected after gel electrophoresis. PCR conditions were 95 °C for 3 min, followed by 38 cycles of 95 °C for 1 min, 57 °C for 2 min, and 72 °C for 2.5 min, then incubation at 95 °C for 5 min and finally at 72 °C for 30 min. The amplification was conducted in 25 µL reactions containing

400 μ M dNTPs, 1 \times PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl; BRL Inc.), 2 mM MgCl₂, 4 pmol of each primer and 25–200 ng of genomic DNA. PCR products were electrophoresed on a 3% agarose gel with 0.6 \times TBE buffer. Gels were run for approximately 1 h, and stained in 0.6 \times TBE with 3 μ g/mL ethidium bromide for 15 min. The gel was exposed to UV light to visualize DNA banding patterns. The sexes were distinguished by the numbers of bands individuals possessed: individuals with one band were identified as males while birds with two bands were classified as females.

Results

Band-sharing among first-order relatives (mother–chick) ranged from 0.45 to 0.80 (median = 0.59) and among breeding adults ranged from 0.05 to 0.57 (median = 0.21). Waved albatrosses show high natal philopatry (Harris 1973), so the among-adults comparisons might, by chance, include a small number of pairs of close relatives, accounting for the slight overlap of first-order relative and among-adult frequency distributions (Fig. 1).

Band-sharing in social father–chick dyads fell into two discrete groups. One group of 12 birds resembled first-order relatives closely (range 0.46–0.78, median = 0.65). The other group of four fell at the lower end of the among-adults distribution (range 0.00–0.24, median = 0.19). The social offspring of these four males were the only chicks with more than one unattributable band (Fig. 2); each of the four chicks showed at least five unattributable bands. We scored an average of 15.8 bands per lane. We estimate the probability of misassigning an unrelated male as father as 7.4×10^{-5} . The probability of mistakenly assigning an uncle as father is 0.039. Thus, on the basis of low band-sharing and high numbers of unattributable bands in their offspring, we conclude that these four males were not the genetic fathers of their putative offspring. We suggest that mutation can account for the one case of a chick with one unattributed band where the estimated rate of mutation was 1 in 253 or 0.004 mutations/locus/meiotic event.

Female putative parents paired with chicks that had five or eight unattributable bands still had high band-sharing values, between 0.45 and 0.80 (Fig. 2). We conclude that these social mothers were in all cases the genetic mothers of the chicks they were attending, even when the attending male was not the genetic father. At the population level, our estimate of the frequency of EPF ($4/16 = 0.25$) had a binomial 95% confidence interval of 0.07–0.52 (Zar 1996).

Discussion

These results indicate that waved albatrosses in our study population participate in effective EPCs: 25% of fathers examined were not genetically related to their putative offspring. We conclude that these results are consistent

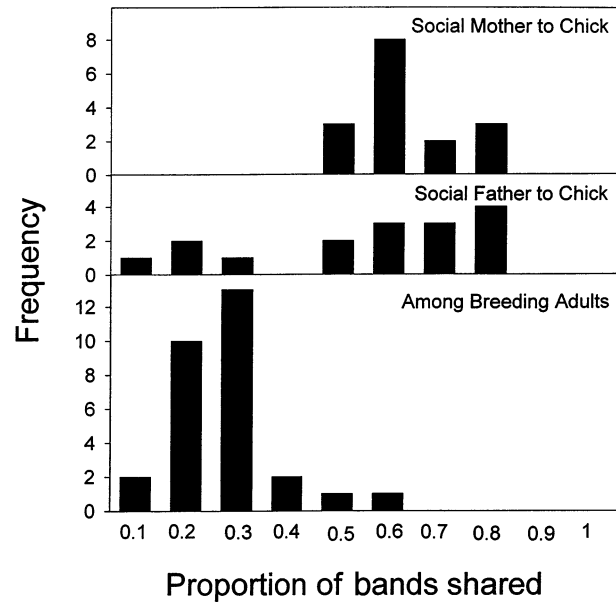


Fig. 1 Band-sharing among dyads of three types in waved albatrosses.

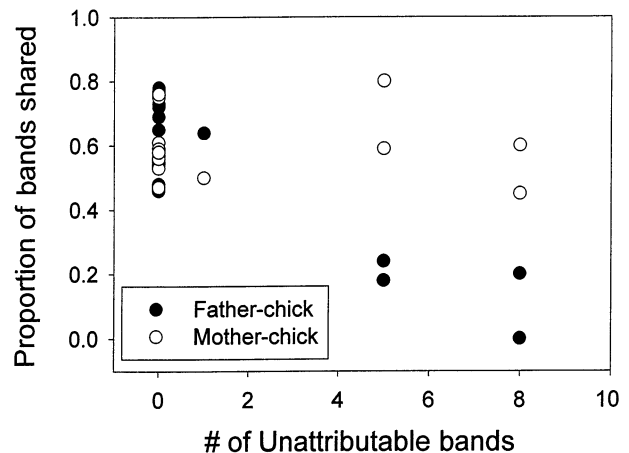


Fig. 2 Relationship between band-sharing and number of unattributable bands within waved albatross social families.

with EPFs rather than intraspecific brood parasitism as all excluded parents were males. EPFs are unusual in long-lived seabirds, yet long-lived waved albatrosses in this study have a relatively high EPF rate. Among taxa examined by Westneat & Sherman (1997), EPFs were more common and of higher average frequencies in passerines than in nonpasserines. Yet the 25% frequency of EPF for waved albatrosses in our study was greater than all other nonpasserine taxa and consistent with average values (0.18 ± 0.17) among passerines examined by Westneat & Sherman (1997). While the strength of our conclusions may be tempered by the small sample size of this initial study, our data do

suggest that waved albatrosses have an unexpectedly high rate of EPF.

A possible explanation for this unusually high EPF rate may be linked to this species' limited breeding distribution. Except for a few pairs on Isla de la Plata off the coast of Ecuador, waved albatrosses breed exclusively on Española, a situation that may promote high degrees of philopatry since birds always return to the same island to breed. This, in turn, may lead to locally high levels of relatedness among individuals if degrees of natal and breeding philopatry are high. An untested idea is that individual males may tolerate higher EPF rates because the cost of EPF to the social father is reduced significantly when the genetic sire is his close relative.

Our data are consistent with the hypothesis that males participate in aggressive EPCs that increase the number of offspring that they father via EPFs. However, Harris's (1973) hypothesis that observed copulation attempts are a component of territorial aggression, in which behavioural dominance is asserted over potential intruders of both sexes, can not be excluded by these data as attackers appear to treat individuals of both sexes in the same manner (Harris 1973). If both sexes are treated equally, Harris' behavioural dominance hypothesis may be the more parsimonious hypothesis, suggesting that EPFs are a byproduct of territorial aggression. Putative attacks, on the other hand, may serve two functions for attackers: potential intruders are inhibited by rushing males and territory owners attempt to enhance their reproductive success by copulating with passing intruder females. Currently, we lack detailed behavioural observations necessary to attribute these EPFs to aggressive EPCs.

Clearly, these questions would benefit from extensive behavioural observations of copulation behaviour in waved albatrosses. Such observations may also reveal whether, in addition to the conspicuous aggressive EPCs, there exist any other classes of EPCs initiated by females or males that may account for the surprising level of EPFs found in these birds.

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