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No evidence for extra-pair paternity in the western gull

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Abstract

The genetic mating system of western gulls *Larus occidentalis* breeding on Southeast Farallon Island, California, was determined using multilocus DNA fingerprints of 33 chicks from 22 broods. No extra-pair paternity (EPP) was found, despite extra-pair copulations (EPCs) occurring. This suggests that paternity guards are effective, and that females gain few genetic benefits from EPCs. The EPP in western gulls concurs with that of other seabirds, reinforcing the idea that seabirds generally have a monogamous genetic mating system.

Keywords: DNA fingerprinting, extra-pair copulations, *Larus occidentalis*, paternity, Southeast Farallon Island, western gull

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Introduction

An increasing number of DNA fingerprinting studies reveal that extra-pair fertilizations occur in most avian species, and that genetic monogamy is rare even though 80–90% of bird species are socially monogamous (Lack 1968; Møller 1986). Extra-pair paternity (EPP) varies considerably among species, from 76% of offspring in superb fairy wrens *Malurus cyaneus* (Mulder *et al*. 1994), and 44% in tree swallows *Tachycineta bicolor* (Dunn *et al*. 1994) to 0% in willow warblers *Phylloscopus trochilus* (Gyllensten *et al*. 1990). The reasons for the various levels of EPP between species are evidently complex and there is much discussion regarding this variation (e.g. Birkhead & Møller 1996; Gowaty 1996).

Western gulls are long-lived seabirds, with over 80% adult survival (Spear *et al*. 1987), long-term pair bonds and a low divorce rate. Similar to other gulls, they are socially monogamous (e.g. Bent 1921; Schreiber 1970), although rare instances of polygynous trios and female–female pairs have been recorded (Hunt & Hunt 1977). Males invest very highly in paternal care: they perform half of the incubation (Pierotti 1981; Gilbert 1996), over half of the chick-feeding (Pierotti 1981; Gilbert 1996), and feed their females prior to laying. This may predict a low level of EPP (Møller & Birkhead 1993; Parrott 1995); however, western gulls breed colonially and synchronously and mate guarding does not occur, leaving ample opportunity for extra-pair copulations (EPCs). Forced EPC attempts by males on females commonly occur in western gulls, and females occasionally accept them (Pierotti 1981; Gilbert 1996). Therefore, to determine the outcome of these EPCs and to determine the true genetic mating system of this species, DNA fingerprinting was conducted. The results are discussed in the light of paternity guards and female benefits. The level of EPP is compared with the observed frequency of successful EPCs (from Gilbert 1996), and comparison is drawn with EPP in other seabirds.

Methods

Study area and data collection

Detailed observations of western gull mating behaviour were made in 1994 and 1995 on Southeast Farallon Island (SEFI) by Gilbert (1996). SEFI is located 42 km west of San Francisco, California, USA (37°42′N, 23°00′W), and is part of the San Francisco Bay National Wildlife Refuge managed by the US Fish and Wildlife Service.

Under license, 0.4 mL of blood was collected by venipuncture from incubating adults and chicks aged 1–10 days. To prevent potentially sampling chicks unrelated to the female (due to brood parasitism or 'adoption', e.g. Holley 1981), eggs and chicks were individually marked at laying and hatching, respectively.

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DNA fingerprinting

Multilocus DNA fingerprinting was conducted in the Zoology Department at the University of Leicester, UK. The standard protocol described by Burke & Bruford (1987) and Bruford *et al*. (1992) was used.

DNA samples were run on 1% agarose gels with putative parents and offspring in adjacent lanes, and marker DNA (lambda/*Hin*dIII) in two peripheral lanes. DNA on filters was hybridized with radioactively labelled ^{32}P probe 33.6 and then probe 33.15 (Jeffreys *et al*. 1985a; b).

Bands on autoradiographs were considered shared between two individuals if they differed in migration distance by less than 0.5 mm (Bruford *et al*. 1992), even with some difference in intensity, to allow for variation in quality between autoradiographs.

For broods where both putative parents were sampled, an exclusion analysis was carried out (Burke & Bruford 1987): for each offspring, the number of unattributable bands (those not shared with either parent) was counted. If the frequency of the observed number of unattributable bands exceeded that from a Poisson distribution it was considered to indicate EPP. The number of unattributable bands was also compared with those found when the putative father was replaced by an assumed unrelated 'fake' father (the nearest other male to the chick on the autoradiograph; Warkentin *et al*. 1994). The exclusion analysis was conducted for each probe separately, to avoid pseudoreplication in case some fragments hybridized with both probes.

For all broods, genetic similarity was measured using the band-sharing coefficient (S) between two individuals, calculated from the formula: $S = 2N_{\mathrm{ab}}/(N_{\mathrm{a}} + N_{\mathrm{b}})$, where N_{ab} is the number of shared bands, and N_{a} and N_{b} are the number of bands in individuals 'a' and 'b', respectively (Wetton *et al*. 1987). For all broods, band-sharing coefficients were calculated between offspring and putative fathers, offspring and putative mothers, and between offspring in the same brood. As a measure of the background level of band-sharing, band-sharing coefficients were determined for assumed nonrelatives, i.e. offspring and substituted 'fake' fathers. As a measure of the level of band-sharing for first-order relatives, band-sharing coefficients were calculated between each member of families known to be related (from the exclusion analysis). The band-sharing values analysed were the weighted means of the probe combinations for each dyad.

To exclude parentage in families where one parent only was sampled, one-tailed 95% confidence limits (mean $\pm t \times \mathrm{SD}$) were calculated using arcsine-transformed band-sharing coefficients for known relatives and assumed nonrelatives. Offspring/putative parent dyads with band-sharing coefficients below the lowest 95% confidence limit for their category were considered to indicate EPP.

Results are presented as mean ± standard deviation (SD).

Results

Blood was taken from 30 broods involving 28 males, 24 females and 70 offspring. Due to blood degradation and poor-quality fingerprints for some samples, fingerprints were obtained from 22 families comprising 19 males, 14 females and 33 chicks. Probe 33.15 produced 25.20 ± 6.00 bands and probe 33.6 produced 21.70 ± 5.20 bands per fingerprint.

Exclusion analyses using unattributable bands were carried out on 10 families (13 chicks) and nine 'fake' families (12 chicks) probed with 33.15, and 11 families (16 chicks) and 10 'fake' families (16 chicks) probed with 33.6. There was almost no overlap in the distribution of the frequency of unattributable bands between putative families and 'fake' families, and there was no significant difference between the observed frequency of unattributable bands in putative families and that expected from a Poisson distribution (probe 33.15: $\chi^2 = 0.84$, d.f. = 1, not significant (NS); probe 33.6: $\chi^2 = 3.49$, d.f. = 1, NS), strongly suggesting that all putative parents used in this analysis were true genetic parents (see Fig. 1).

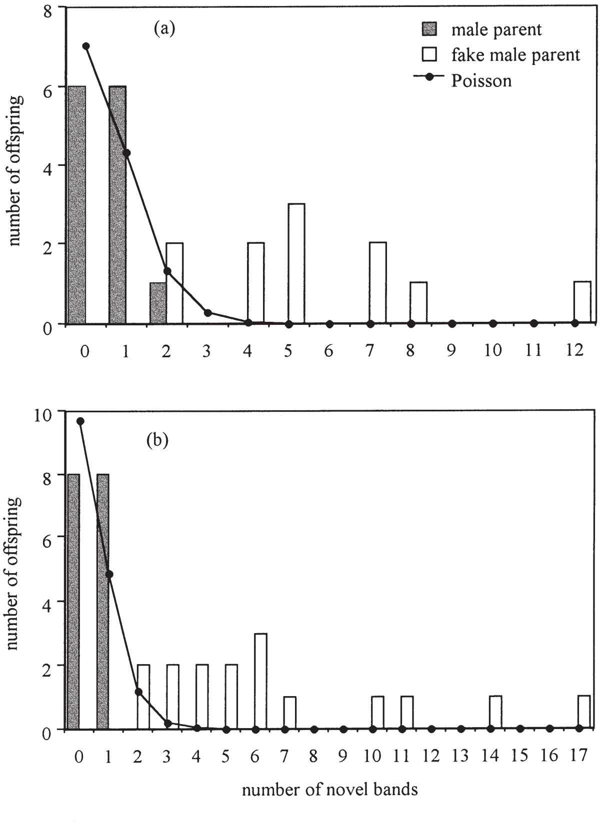

Fig. 1 The frequency distribution of unattributable bands (bars) in offspring from true families and from 'fake' families (i.e. unrelated birds, where the putative father was substituted for the closest other male on the gel). (a) Probe 33.15; (b) Probe 33.6. Also shown (lines) are the Poisson distributions, which did not differ from the observed distributions (Probe 33.15: $\chi^2 = 0.84$, d.f. = 1, NS; Probe 33.6: $\chi^2 = 3.49$, d.f. = 1, NS).

Band-sharing coefficients between chicks and putative fathers averaged 0.60 ± 0.09 and between assumed nonrelatives averaged 0.34 ± 0.11. Chick/putative mother dyads averaged 0.62 ± 0.09 and putative siblings averaged 0.59 ± 0.10. The one-tailed 95% confidence limit for the chick/putative father distribution was 0.45 (lower limit) and for chick/'fake' father it was 0.52 (upper limit). Frequency distributions of band-sharing coefficients are shown in Fig. 2. No family dyad had a band-sharing coefficient below the lower 95% confidence limit for its respective category, so no EPP was concluded.

The upper 95% confidence limits of unrelated birds overlapped slightly with the lower 95% confidence limits of family members. However, in no cases did the same offspring/putative father dyad occur in overlapping regions of both probes 33.15 and 33.6, which suggests the conclusion that there was no EPP.

Using standard *t*-tables in Sokal & Rohlf (1981) for $n = 22$ broods the upper 95% confidence limit for zero EPP is 13%.

Discussion

Multilocus DNA fingerprinting of 33 broods from 22 chicks revealed zero (± 13%) EPP.

Extra-pair paternity in other seabirds

Paternity studies on seabirds are sparse compared to the large number on passerines, in which some species have high EPP levels in chicks (e.g. 62% in splendid fairy wrens *Malurus splendens*: Brooker *et al*. 1990; 55% in reed buntings *Emberiza cineracea*: Dixon *et al*. 1994; 40% in American redstarts *Setophaga ruticilla*: Perreault *et al*. 1997). In stark contrast, studies on seabirds consistently find very low EPP levels. For example, there is 0% EPP in northern fulmars *Fulmarus glacialis* (Hunter *et al*. 1992), Cory's shearwaters *Calonectris diomedea* (Swatchek *et al*. 1994), herring gulls *Larus argentatus* and Caspian terns *Sterna caspia* (J. Quinn, personal communication in Birkhead & Møller 1992), and 9.3% in European shags *Phalacrocorax aristotelis* (Graves *et al*. 1993).

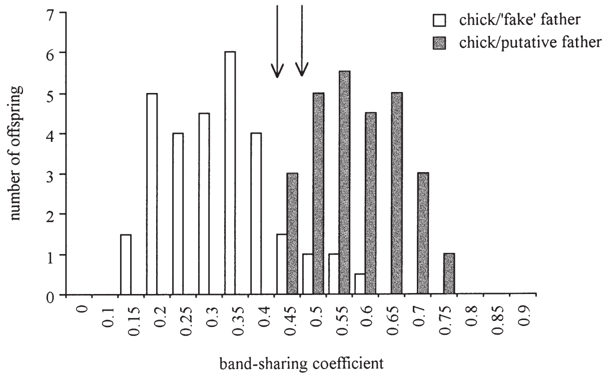

Fig. 2 Frequency distribution of band-sharing coefficients for probes 33.15 and 33.6 combined. Offspring are compared with unrelated birds and putative fathers. The left arrow indicates the upper 95% confidence limit for nonrelatives and the right arrow indicates the lower 95% confidence limit for offspring/father dyads.

Birkhead & Møller (1996) pointed out that high levels of EPP correlate positively with male plumage brightness, song complexity, size of testis and divorce rate, and negatively with the level of paternal care. Seabirds share several characteristics that suggest, from the above correlations, a low EPP: they are monomorphic, have long-term pair bonds with low divorce rates, have very high paternal investment (e.g. Ashmole 1971; Hunt 1980; Furness & Monaghan 1987), and relatively small testes (Gilbert 1996).

Comparison of EPP with EPC

The proportion of females participating in behaviourally successful EPCs during their fertile period was 23% (18/77; Gilbert 1996), which is obviously greater than the 0% extra-pair broods observed. This suggests that females may participate in EPCs for nongenetic benefits, or that paternity assurance strategies such as frequent pair copulation and mate guarding override female strategies. The mean percentage of behaviourally successful copulations that are extra-pair during a female western gull's fertile period is 2.25% (Gilbert 1996), which is close to the zero EPP found. It does appear, therefore, that males may protect their paternity with frequent pair copulations. This raises the question of why females should participate in successful EPCs if they gain no genetic benefit. Females may gain nongenetic benefits, such as the appraisal of potential future mates (Colwell & Oring 1989; Wagner 1991; Heg *et al*. 1993), or to increase the likelihood of pairing with the extra-pair male in future seasons. Further work on seabirds is needed to investigate these possibilities.

An interesting source of bias may exist in species where many chicks die before blood sampling occurs. On SEFI there is intense conspecific predation (e.g. Spear *et al*. 1987), and some chicks were predated before sampling. Poor-quality parents may be more likely to lose chicks if they are inept at protecting them, and may also be more likely to have extra-pair chicks if females choose higher-quality males than their partners for EPCs (e.g. Kempenaers *et al*. 1997). Therefore, the zero EPP found may be an underestimate. However, despite the possible bias, it remains clear that the level of EPP in western gulls on SEFI is very low.

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