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### Genetic Monogamy in Wilson's Storm-Petrel

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In socially monogamous birds, sperm competition can arise when females seek or accept copulations outside of the pairbond (extrapair copulations, EPCs). EPCs are widespread among socially monogamous birds (e.g. Birkhead 1998), although there is a wide range in the level of extrapair paternity (EPP) within that social mating system, ranging from no extrapair young (EPY), for example in Northern Fulmars (*Fulmarus glacialis*; Hunter et al. 1992) and Common Loons (*Gavia immer*; Piper et al. 1997), to over half of the chicks fathered via EPCs, for example

in Tree Swallows (*Tachycineta bicolor*; Kempnaers et al. 1999). The causes for the large interspecific variation in the level of EPP are still poorly understood. A number of studies have tried to explain those differences and have found a positive correlation between EPP and degree of sociality (Møller and Birkhead 1993, but see Westneat and Sherman 1997), divorce rate (Cezilly and Nager 1995), male advertisement (e.g. plumage brightness, Møller and Birkhead 1994), and testis size (Møller and Briskie 1995). However, there are many exceptions to those trends, which suggests that the explanation is more complex (e.g. Rodrigues 1998). As Petrie and Kempnaers

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(1998) pointed out, females control success of copulation attempts in most species of birds; thus the level of EPP will depend mainly on costs and direct or indirect benefits associated with EPP for females. If male care is essential, females might be restricted in seeking EPCs if they risk losing the investment of their partner in the brood. A dynamic programming approach on male decisions to invest in or abandon a brood (Mauck et al. 1999) suggested that tolerance of EPP decreases as adult survival increases, such that males of long-lived species should withdraw care to a current brood if any doubt of parentage exists.

Seabirds are long-lived birds and, compared with other groups, have received rather little attention in the study of EPP (Gilbert et al. 1998). Seabirds exhibit very high levels of parental investment, and male parental care is indispensable. Because that is true for all species of marine birds, exploitation of the marine environment does not seem to allow lower levels of investment. That may result not only in the general occurrence of social monogamy in seabirds (see Wittenberger and Tilson 1980), but also in a set of comparable breeding and life history parameters (e.g. colonial, long-lived, little or no sexual dimorphism). Thus, if level of paternity in a population depends on one or more of those factors, seabirds should exhibit a uniform level of EPP, and that level of EPP should be low according to the model of Mauck et al. (1999).

In this paper, we report results of a DNA fingerprint study of paternity in an Antarctic seabird, Wilson's Storm-Petrel (*Oceanites oceanicus*), and try to show whether the level of EPP is indeed uniformly low in seabirds.

*Study area and methods.*—Wilson's Storm-Petrels are the smallest and one of the most abundant Antarctic seabird species. Their breeding biology was summarized by Beck and Brown (1972). They nest in colonies in scree slopes along ice-free Antarctic and sub-Antarctic coasts, where they lay a single egg in a natural cavity. They have a life history typical of a small procellariiform seabird with a long lifespan (mean life expectancy 10.4 years), late onset of breeding (at about 4 to 5 years of age), and high mate fidelity (87%; Beck and Brown 1972, P. Quillfeldt unpubl. data). Wilson's Storm-Petrels exhibit intensive biparental care. Incubation and chick feeding are shared between sexes. Chicks remain in the nest for about 60 days and are fed during brief nightly visits. Wilson's Storm-Petrels prey mainly on Antarctic krill (*Euphausia superba*) during the breeding season, although some fish and amphipods are also taken (P. Quillfeldt unpubl. data). Coloniality and a female "pre-laying exodus" of about 10.7 days to build up reserves for egg formation (Beck and Brown 1972) might create opportunities for sperm competition to occur (see Austin and Parkin 1996).

Field work was carried out in the Tres Hermanos colony on King George Island, South Shetland Is-

lands (62°14'S, 58°40'W) in the maritime Antarctic, in the austral summers of 1995–1996, 1996–1997, and 1997–1998. Nests were marked at the beginning of the breeding season and monitored for eggs and hatching chicks.

A total of 63 families were sampled in three consecutive breeding seasons (1995–1996: 27; 1996–1997: 6; 1997–1998: 30). In the case of eight breeding pairs, two chicks were sampled in different seasons, whereas only one chick of all other pairs was sampled. Adults were captured and individually marked in their burrows between January and February (during incubation) as well as between February and March (during chick rearing). Adults attending burrows during either of those periods were regarded as parents. In four burrows, three adults were captured and sampled. In all of those cases, the putative parents had been captured early in the breeding season during incubation or brooding the very young chick. The third bird, which was captured later in the breeding cycle, was not regarded as a parent. Throughout the paper those birds are called "additional adults."

Blood samples (approximately 50  $\mu$ L) were taken by puncture of the brachial vein. Samples were immediately suspended in 500 or 1,000  $\mu$ L APS-buffer (Arctander 1988) or in 70% ethanol (Arctander 1988) and stored at 4°C for four to twelve weeks and thereafter at -20°C until processing.

DNA was extracted using standard procedures modified according to Miller et al. (1988; for details see Lubjuhn and Sauer 1999). For each sample, approximately 5 to 10  $\mu$ g DNA was digested with 50 U of *Hinf* I following instructions of the manufacturer. Digests were separated by agarose gel electrophoresis (gel size: 20  $\times$  40 cm, 0.8% agarose, 1–2 V/cm) for 49–50 h in 1  $\times$  TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA). After drying, gels were hybridized with <sup>32</sup>P-labelled oligonucleotide probe (GGAT)<sub>4</sub>. Detailed procedures are given in Epplen (1992). All DNA of members of a family including additional adults were run in adjacent gel lanes. Bands were scored by eye within a range from 23.1 to 3 kb and were evaluated considering the criteria proposed in Westneat (1990). Bands in nestling DNA fingerprints that were not attributable to either putative parent were scored, and band-sharing coefficients were calculated according to the formula given in Wetton et al. (1987) as  $\chi = 2 N_{AB} / (N_A + N_B)$ , where  $N_{AB}$  is number of shared fragments between individuals A and B and  $N_A$  and  $N_B$  are total numbers of fragments in individuals A and B, respectively. Background band-sharing was obtained by comparing breeding birds from different burrows in adjacent lanes.

The informativity of DNA fingerprint banding patterns for assigning parentage depends on their variability which, in turn, is determined by the restriction enzyme-oligonucleotide probe combination

used and the proportion of bands that individuals share by chance. *Hinf* I digestion of DNA followed by hybridization with the oligonucleotide (GGAT)<sub>4</sub> produced banding patterns that were sufficiently variable to be individual-specific. A mean of  $38.1 \pm 5.6$  SD ( $n = 178$ ) bands per individual was scored. An informativity index  $I$  (Krawczak and Lubjuhn 1995) was calculated to quantify the evidential power of the used enzyme-probe combination and to make this study comparable to others. The high value of  $I = 48.7$  approaches the upper end of a comparative list of informativity indices of 14 DNA fingerprint studies in birds (Lubjuhn and Sauer 1999). The probability  $P$  of misassigning parentage to one of the two or both putative parents was calculated following Burke et al. (1989) as  $P = 2.6 \times 10^{-8}$  and  $P = 7.7 \times 10^{-12}$ , respectively. We concluded, therefore, that the enzyme-probe combination used produces sufficiently variable banding patterns to assign parentage in Wilson's Storm-Petrels unambiguously.

The four additional adults were sexed using PCR amplification of a highly conserved W-linked gene according to Griffiths et al. (1996), modified for Biometra®-Thermocycler T Gradient. PCR conditions were adjusted and PCR products digested with *Hae* III overnight. Fragments were separated using agarose gel electrophoresis (gel size  $7 \times 10$  cm, 3% agarose, 9V/cm).

**Results.**—Usually, band-sharing coefficients between mates are used to assess proportion of bands that unrelated individuals share by chance (e.g. Mauck et al. 1995, Lubjuhn et al. 1999). However, in this study, the mean band-sharing coefficient between mates differed from that of breeding birds of different nest burrows (see below). Thus, the latter was assumed to reflect the mean background band-sharing and was measured as  $\chi = 0.30 \pm 0.06$  SD ( $n = 32$ ).

In 51 of 63 chicks investigated, all bands could be attributed to the putative parents. The remaining 12 chicks showed one novel fragment each that was unattributable to either parent. Those single novel fragments were interpreted to result from mutations for several reasons. (1) The mean band-sharing coefficient between putative parents and chicks with no novel fragments in their banding patterns ( $0.65 \pm 0.06$  SD; min = 0.48; max = 0.80;  $n = 102$ ) did not differ from that between putative parents and chicks with one novel fragments in their banding patterns ( $0.65 \pm 0.08$  SD; min = 0.49; max = 0.85;  $n = 24$ ;  $t$ -test:  $t = 0.298$ ;  $df = 124$ ;  $P = 0.77$ , see also Fig. 1), but both values differed significantly from the mean band-sharing coefficient of breeding birds from different nest burrows ( $0.30 \pm 0.06$  SD; min = 0.2; max = 0.41;  $n = 32$ ;  $t$ -test vs. putative parents and chicks without novel fragments:  $t = 28.15$ ;  $df = 132$ ;  $P < 0.001$ ;  $t$ -test vs. putative parents and chicks with one novel fragments:  $t$ -test:  $t = 19.61$ ;  $df = 54$ ;  $P < 0.001$ ; see also Fig. 1). Furthermore, both distributions of

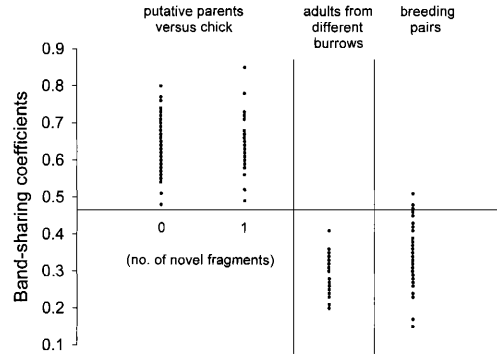


FIG. 1. Distributions of band-sharing coefficients of putative parents and chicks (separated for chicks with no [102 values] or one [24 values] novel fragment), of adults from different nest burrows (32 values), and of breeding partners (55 values).

band-sharing coefficients for chicks and putative parents did not overlap with that of birds from different nest burrows (Fig. 1). (2) For the restriction enzyme-oligonucleotide probe combination used here, mean expected number of novel fragments can be estimated as 10.2 novel fragments per chick if one putative parent was not the genetic parent (for calculation see Jeffreys et al. 1985, Burke and Bruford 1987). The calculation of that value is based on the background band-sharing found for birds from different nest burrows. To test for possible effects of philopatry, we additionally calculated mean expected number of novel fragments for the case where the genetic father is a first-degree relative of the putative father, using 0.65 as average band sharing of first-degree relatives (see above for adults vs. chicks with no novel fragments). However, even in the case where the genetic father is a first-degree relative of the putative father, an average of 2.92 novel fragments would be expected. (3) All band-sharing values between chicks and putative parents lay in an interval that is typical for first-degree relatives (e.g. 0.3 to 0.9 for Common Gulls [*Larus canus*], Bukacinska et al. 1998; 0.3 to 0.8 for Coal Tits [*Parus ater*], Lubjuhn et al. 1999). The mutation rate per fragment, estimated from our data on single novel fragments as 0.005, was also found to be within the range of other DNA fingerprint studies in birds (for calculation and a range from 0.006 to 0.002 see Burke and Bruford 1987, Kempnaers et al. 1992, Decker et al. 1993). Thus, we conclude that neither EPP nor intraspecific brood parasitism occurred in the 63 families sampled for analysis.

Mean band-sharing coefficient of mates was  $0.36 \pm 0.08$  SD (min = 0.17; max = 0.52;  $n = 55$ ) and was significantly higher than that of adults from different nest burrows ( $t$ -test:  $t = 3.90$ ;  $df = 85$ ;  $P < 0.001$ ), although distributions overlapped over a wide range

TABLE 1. Occurrence of EPY in seabirds determined by DNA fingerprint studies: Distribution of extrapair offspring (percentage EPP chicks), extrapair copulations (EPCs as percentage of total observed copulations), and mate fidelity (percentage of breeding pairs that survive and re-pair in the following breeding season) among seabird species. Only species were considered for which data on EPC or EPP are available.

Species	% EPP chicks	% EPCs	Mate fidelity	References
<b>Sphenisciformes</b>				
<i>Spheniscus humboldti</i>	0	17.9%		Schwartz et al. 1999
<i>Eudyptes schlegeli</i>	3.8%			St Clair et al. 1995
<b>Procellariiformes</b>				
<i>Diomedea exulans</i>		4.6%	100%	Tomkins 1983
<i>Calonectris diomedea</i>	0		97%	Swatschek et al. 1994
<i>Puffinus tenuirostris</i>	9–13% <sup>a</sup>		83%	Austin and Parkin 1996
<i>Fulmarus glacialis</i>	0	2.4%	96%	Hunter et al. 1992
<i>Oceanites oceanicus</i>	0		87%	thin study
<i>Oceanodroma leucorhoa</i>	0		95%	Mauck et al. 1995
<b>Pelecaniformes</b>				
<i>Phalacrocorax carbo</i>		10%		Gregersen in Møller and Birkhead 1993
<i>P. aristotelis</i> (1987)	18%	14.1% <sup>b</sup>	64%	Graves et al. 1992
<i>P. aristotelis</i> (1989)	14%			Graves et al. 1993
<i>P. aristotelis</i> (1990, 1991)	almost 0			Graves et al. 1993
<b>Charadriiformes (Laridae, Alcidae only)</b>				
<i>Catharacta lonnbergi</i>	0			Miller et al. 1994
<i>Larus occidentalis</i>	0	2.3%		Gilbert et al. 1998
<i>Larus canus</i>	3.6%	15.7% <sup>b</sup>		Bukacinska et al. 1998
<i>Larus fuscus</i>		0		Chardine 1986
<i>Rissa tridactyla</i>		0		Brown 1967
<i>Sterna caspia</i>	0 <sup>c</sup>		50%	Quinn pers. comm.; Cluthbert 1985
<i>Alca torda</i>		3.1%		Wagner 1992
<i>Uria aalge</i>		5.3%	81%	Hatchwell 1988

<sup>a</sup> From two colonies.

<sup>b</sup> This figure includes forced EPCs or invasions of the territory by the extrapair male.

<sup>c</sup> Pilot study of 7 families.

(Fig. 1). We conclude, therefore, that breeding mates were slightly more closely related to each other than randomly compared breeding birds in the population. It has to be noted, however, that the difference of 0.06 was very small.

Four apparently non-parental adults were found in breeding burrows containing chicks (see study area and methods). The mean band-sharing coefficient of those individuals with the attending breeding mates was  $0.29 \pm 0.08$  SD (min = 0.21; max = 0.43;  $n = 8$ ) and did not differ from that of adults from different nest burrows ( $t$ -test:  $t = -0.43$ ;  $df = 38$ ;  $P = 0.637$ ), but did differ from that of breeding mates ( $t$ -test:  $t = 2.44$ ;  $df = 61$ ;  $P < 0.02$ ) and from that of first-degree relatives (values from chicks with no or one novel fragments combined:  $0.65 \pm 0.07$  SD,  $n = 126$ ;  $t$ -test:  $t = 14.74$ ;  $df = 132$ ;  $P < 0.001$ ). We conclude, therefore, that those additional adults were unrelated to the attending breeding mates. A molecular analysis of sex revealed that all four birds were males.

**Discussion.**—Our results show that in a sample of 63 nestlings of Wilson's Storm-Petrels from three years, no EPP and no brood parasitism occurred. Thus, Wilson's Storm-Petrels appear socially and ge-

netically monogamous. The probability that we have overlooked EPPs on the basis of our sample size can be calculated following Mauck et al. (1995). If the true level of EPPs was 5, 10, or 15%, we would have missed EPPs in our sample of  $n = 55$  (individuals investigated in more than one year included only once) with a probability of 0.06, 0.003, and 0.0001, respectively.

Our results agree with other studies of seabirds. As shown in Table 1, EPCs are not uncommon among seabirds, but do not usually constitute a high percentage of the total copulations, and extrapair fertilizations are either low or absent. If breeding systems or life histories of seabirds influence EPP, we expected a uniform level of EPP among species, which was supported by the data (Table 1 on EPP  $\approx 0$ ). Strict genetic monogamy is found in only a small proportion of bird populations, and thus a discussion of the special features of life history leading to strict monogamy may help explain the evolution of EPP patterns within birds. Seabirds generally have a high probability of survival to the next breeding season, and thus offspring of a single breeding season represent a smaller proportion of the potential lifetime reproductive success than do offspring in short-lived

species. Any reduction of adult survival by investment in current offspring has a much larger influence on their lifetime reproductive success than in short-lived species. Therefore, long-lived birds such as seabirds should not invest in broods of questionable paternity, as suggested by theory (Mauck et al. 1999) and supported by our data.

A further result of our study was a slightly greater genetic relatedness between breeding partners as compared to nonmates. Similar results were found for Cory's Shearwaters (*Calonectris diomedea*) (Swatschek et al. 1994). Those results were interpreted as high philopatry by male Cory's Shearwaters, which could mate with their relatives by chance. In that way, philopatry may lead to clusters of broods with greater genetic relatedness within a colony. But mating with closer relatives might not occur simply by chance. Japanese Quail (*Coturnix japonica*; Bateson 1980) as well as Pika (*Ochotona princeps*; Peacock and Smith 1997), for example, mate preferentially with closer relatives and are thought to practice "optimal outbreeding." However, there are no data available on natal philopatry or on effects of inbreeding or outbreeding on fitness for Wilson's Storm-Petrels.

Additional adults visiting burrows in the chick rearing period are most probably prebreeding birds or failed breeders inspecting burrows for future breeding activities. Because they are not related to the attending breeding mates and because none of the birds has been found breeding in the visited burrows in consecutive seasons, we conclude that those birds are neither primary (e.g. as found in Florida Scrub Jays [*Aphelocoma coerulescens*]; Quinn et al. 1999) nor secondary (e.g. as found in Pied Kingfishers [*Ceryle rudis*]; Reyer 1980) helpers-at-the-nest. The visitors appear not have any functional relation to the breeding mates. That explanation is supported by the high proportion of prebreeders visiting the study colony, which can be as high as 80% of birds sampled by mistnetting (Quillfeldt et al. 2000), and by the fact that all four birds under investigation were males, the sex that is assumed to choose the breeding site in the prelaying period (Beck and Brown 1972).

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## Adenylate Kinase Intron 5: A New Nuclear Locus for Avian Systematics

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The explosion of use of the polymerase chain reaction (PCR) and direct DNA sequencing in recent years has provided a wealth of new data for avian systematists. Nearly all these sequence data, however, have come from mitochondrial genes, which are inherited as a single unit, typically exhibit strictly matrilineal inheritance, and have other unusual properties (Avice 1991). Although mitochondrial DNA (mtDNA) has well-established advantages for phylogenetic inference (Moore 1995), avian molecular systematists are eager to identify nuclear genes that could provide independent phylogenetic estimates (e.g. Pritchko and Moore 1997, Omland 1999). Most nuclear loci explored to date are slowly evolving protein coding genes and have been useful primarily for resolving deep relationships (e.g. relationships among taxa with divergence times more than 50 Ma ago; Graybeal 1994, but see Lovette and Bermingham 2000). It would clearly be helpful to have at our disposal nuclear DNA sequences that exhibit a faster rate of evolution, making them phylogenetically useful at intermediate and shallow taxonomic levels (e.g. Palumbi 1996, Pritchko and Moore 1997). Nuclear introns, for example, which have recently received attention for their potential utility in population-level studies (e.g. Friesen et al. 1997, Heslewood et al. 1998), may also be very useful for inferring phylogenetic relationships among species. Although these introns typically evolve more quickly

than nuclear protein coding regions, they can nevertheless evolve significantly more slowly than protein coding mtDNA, and might therefore be especially helpful in resolving nodes at depths for which the signal from mtDNA is diminished due to saturation.

Despite their potential utility, nuclear genes have so far been little used in sequence-based avian systematics. This is in large part because of a lack of appropriate PCR primers that work well across a broad spectrum of avian groups and that amplify sequences evolving at rates suitable for addressing a range of phylogenetic questions. In the course of a phylogenetic investigation of the pitohuis (J. Dumbacher unpubl. data), we explored the usefulness of a nuclear gene not previously employed for phylogenetic analysis. In this note, we report primers we have designed to amplify this new nuclear marker, intron 5 of the nuclear gene cytosolic adenylate kinase (AK1), and we document both the potential phylogenetic utility of this intron and the very broad taxonomic utility of these primers.

*Methods.*—PCR primers located in the conserved exon regions flanking AK1 intron 5 (in exon 5 and exon 6) were designed by comparing complete AK1 DNA sequences published for *Gallus* (Suminami et al. 1988) and humans (Matsuura et al. 1989), as well as amino acid sequences for several other taxa. Primer sequences and positions are given in Table 1. The other known members of the well-studied AK gene family differ in sequence so substantially from AK1, including the regions of primer annealing, that we are confident the primers reported here will amplify only AK1 (barring a recent gene duplication within

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