SHORT COMMUNICATION

Mate fidelity and parentage in Cory’s shearwater *Calonectris diomedea* – field studies and DNA fingerprinting

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Abstract

Field studies on Cory’s shearwater *Calonectris diomedea*, which were carried out in a breeding colony in the Aegean Sea between 1989 and 1993, revealed that almost all breeding mates stay together over many consecutive years. Mates usually changed when one partner disappeared (e.g. through death), whereas ‘divorce’ occurred at a rate of 2.7%. Since birds are nesting at very close quarters, the potential for extrapair copulation (EPC) and subsequent extrapair fertilization (EPF) seems to be high. Multilocus DNA fingerprints were used to determine the true parentage of 46 offspring (broods contain a single chick only) from 29 pairs (few pairs were studied in two and three successive years). There were no cases of extrapair paternity.

*Keywords: Calonectris diomedea, DNA fingerprinting, extrapair copulation, extrapair fertilization, mate fidelity, pedigree analysis*

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Introduction

Most DNA fingerprinting studies of birds indicate that extrapair fertilizations (EPFs) may occur regularly and are the rule rather than the exception. (Wetton et al. 1987; Burke et al. 1989, 1991; Birkhead et al. 1990; Gibbs et al., 1990; Morton et al. 1990; Westneat 1990; Westneat et al. 1990; Graves et al. 1992; Schulze-Hagen et al. 1993). However, EPF frequencies are not absolute and the same species can have very different degrees of EPF in different populations, e.g. pied flycatcher and swallows (Birkhead & Møller 1992). Depending on the species and its pairing strategies, extrapair fertilizations can be extremely rare, even in a colonially living bird, such as the Eleonora’s falcon (*Falco eleonorae*). (Swatschek et al. 1993).

Petrels and shearwaters are generally assumed to be socially monogamous with a high degree of mate fidelity (Warham 1990) and low or nonexistent rates of EPF might be expected. In keeping with this Hunter et al. (1990) found that there were no EPFs in a colony of fulmars (*Fulmarus glacialis*) even though 2.4% of the observed copulations in the colony were EPCs. However, Austin et al. (1993) reported in a colony of short-tailed shearwater (*Puffinus tenuirostris*) as much as 18% of the offspring may have been the result of EPF. This is in contrast with our findings in Cory’s shearwater which has a similar life history and breeding habits.

Cory’s shearwater (*Calonectris diomedea*) is a long-lived species which breeds in the Mediterranean (*C. d. diomedea*) and on some Atlantic islands (*C. d. borealis* and *C. d. edwardsii*) and winters in the southern Atlantic (Cramp & Simmons 1977). *C. diomedea* breeds colonially on islands, providing shelter for its nest, which is a simple hollow on the ground usually in rock crevices. Birds lay a single egg at the end of May, the young hatch in July and fledge in October. Neighbouring nests are often close to each other (1.0–7.0 m apart). *C. diomedea* is classified as a socially monogamous species and field data indicated a high degree of mate fidelity over successive years for birds of the Atlantic Islands (Mougin et al. 1984) and the eastern Mediterranean (Ristow et al. 1990, 1991). Since these shearwaters live so closely together, the opportunity for EPCs is high.

We have analysed mate fidelity in a breeding colony off Crete between 1985 and 1993 and checked every year whether the breeders had maintained their mates. Between 1989 and 1992 blood was taken from incubating birds and their respective offspring. In several cases we collected blood from the offspring of the same pair in two or three consecutive years. DNA was extracted from the
blood and subjected to DNA fingerprinting employing multi-locus probes to assess the parentage, i.e. whether there is evidence for any EPF, as in Puffinus tenuirostris (Austin et al. 1993).

Materials and methods

Field studies

The shearwater population studied (approx. 1000 pairs) lives off Crete on a small rocky island (300 × 900 m). We have analysed the biology and ecology of breeding birds since 1975 (Wink et al. 1982; Ristow et al. 1990, 1991). Nests are reused in successive years and are under rock crevices. We were able to capture the incubating birds on the nests and their nestlings. Between 1985 and 1993 extensive care was taken to locate and check all breeding sites within the study plot and of its vicinity. Approximately 544 pairs (c. 120/year) were monitored. In a breeding colony of C. diomedea, not only breeding birds return at night, but also nonbreeders ('bachelors') (Ristow et al. 1990, 1991). Since bachelors and unrelated birds may be seen regularly near or at the entrance of a crevice containing a nest, extreme care must be taken not to confuse these birds with the true inhabitants. In our study only those birds that were captured while actually incubating were considered to be the pair birds of the nest.

DNA fingerprinting

Processing of blood samples. Blood (c. 100 μL) was stored in an EDTA-SDS buffer. DNA isolation, digestion by restriction enzymes (HindII), agarose electrophoresis, capillary transfer to a nylon membrane (Biodyne B) followed standard protocols (Swatschek & Wink 1992; Swatschek et al. 1993).

Hybridization and detection. Nylon membranes were prehybridized in hybridization mixture (5 × SSPE, 0.1% SDS, 1% powdered milk, 5 × Denhardt’s solution; Sambrook et al. 1989) for 2 h at 39 °C. Then the hybridization mixture was decanted and fresh hybridization mixture containing 10 pmol/mL of the digoxigenated oligonucleotide probe (CGAT), (Fresenius) was added. Hybridization was carried out at 39 °C overnight. Membranes were washed three times with 6 × SSC for 30 min each.

Further detection employed immunological methods: DNA/DNA hybrids were detected by an antibody which was raised against digoxigenin (Boehringer). This antibody was coupled to a phosphatase which in turn produced a coloured precipitate at the sites of hybridization (Boehringer). After colour reactions were completed, nylon membranes were documented and processed using the Bioprofil system (Fröbel, Lindau).

Table 1. (a) Site tenacity of Cory’s shearwaters, whose fate was known, in consecutive years (between 1989 and 1993) and (b) mate fidelity in C. diomedea. The fate of 544 pairs which were breeding together was determined in the next following year

<table>
<thead>
<tr>
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<th>Number*</th>
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<tbody>
<tr>
<td>(a) <em>Known breeding birds</em></td>
<td></td>
</tr>
<tr>
<td>Birds in the same nest next year</td>
<td>1038 (100%)</td>
</tr>
<tr>
<td>Birds moved to a new site</td>
<td>892 (85.9%)</td>
</tr>
<tr>
<td>Birds disappeared (probably dead)</td>
<td>38 (3.7%)</td>
</tr>
<tr>
<td>(b) Pairs</td>
<td></td>
</tr>
<tr>
<td>Same pair together</td>
<td>108 (10.4%)</td>
</tr>
<tr>
<td>one mate disappeared, no new mate</td>
<td>10 (0.9%)</td>
</tr>
<tr>
<td>one mate disappeared, remaining bird with new partner</td>
<td>78 (7.3%)</td>
</tr>
<tr>
<td>Both partners disappeared</td>
<td>402 (37.3%)</td>
</tr>
<tr>
<td>Mates alive, but both with new partners (&quot;divorce&quot;)</td>
<td>37 (6.8%)</td>
</tr>
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*The same individuals were evaluated repeatedly if they bred in several years
†values may be slightly higher since the missing partner might have been breeding in an inaccessible nest

Results and discussion

Site and mate fidelity

Between 1985 and 1993 more than 120 breeding pairs of C. diomedea were monitored every year. Birds are long-lived with an annual adult mortality of less than 11% (Ristow et al. 1991; Table 1). They regularly returned to the same nest site every year, even when a mate had disappeared (Table 1). Thus, the chances that both mates meet in consecutive years are high. Since we searched all available nest sites within the study plot and also in its vicinity, we are certain that disappeared birds had died and not just moved to an adjacent nest site. We monitored the mate fidelity of 544 pairs, whose fate we could determine in the following year (Table 1): 73.9% of all pairs had maintained their mate, c. 16.5% had lost one partner. Normally, when a new mate was found at a nest, the one replaced was never recovered again, indicating that it had died in the meantime (for example, in its wintering quarters). Nine pairs had bred together in the same nest since 1985 (≥ 9 years). Thus the majority of birds maintained their mate between years. Change of partners ("divorce") is a rather rare event and took place in 15 instances (≥ 2.7%). Summarizing the field data, it is evident that C. diomedea belongs to the group of socially highly monogamous species which includes many other albatrosses, petrels and shearwaters (Cramp & Simmons 1977; Warham 1990).
Do extrapair fertilizations occur in *C. diomedea*?

Distances between nests can be as small as 1.0 m and many nonbreeding ‘bachelors’ visit the colony every night. In order to evaluate whether EPF occurs in *C. diomedea*, we analysed the pattern of band sharing for both parents and one offspring for 13 pairs, two offspring for 14 pairs and three offspring for two pairs. Blood of incubating birds was sampled in May/June and their respective young in August/September.

Figure 1 illustrates typical DNA fingerprints of 4 breeding pairs and their respective nestlings. As can be seen from Fig. 1, the multi-locus probe revealed complex patterns of DNA restriction fragments which are individual-specific. The mean (± SD) band-sharing coefficient (calculated according to Wetton et al. 1987) between unrelated birds ($n = 57$) is $0.288 ± 0.049$ (Fig. 2). The number of scorable bands per fingerprint ($53.9 ± 8.3$) was high enough to attribute DNA bands of the nestling, in all instances, to bands present in the lanes from either the male or the female. The mean band-sharing coefficient between offspring and parent birds ($n = 92$) is $0.749 ± 0.054$ which is typical for that of parent-offspring relationships (Burke et al. 1991).

Unmatched bands never amounted to more than 1 (Fig. 2). We conclude that the birds that were incubating the egg were also the genetic parents, i.e. there was no evidence for EPF. Among the pairs tested, seven were breeding together for the first time (two birds had a new partner after a ‘divorce’ and two after a mate had died; three birds were mated to a first time breeder).

![Fig. 1 DNA fingerprint of four families of *C. diomedea* and respective nestlings. M = male, F = female, 1, 2, or 3 nestlings of consecutive years. DNA bands of the male are marked on the left, those of the female at the right.](image)

![Fig. 2 Frequency distribution of band-sharing coefficients in *C. diomedea*. The following four situations are evaluated: Band-sharing coefficients between males and offspring ($n = 46$); Band-sharing coefficients between males and offspring ($n = 46$); band-sharing coefficients between breeding mates ($n = 30$); band-sharing coefficients between unrelated birds ($n = 57$).](image)

Also a careful pedigree analysis in a fulmar colony provided no evidence for EPFs although EPs occurred at a rate of 2.4% if all copulations seen were included (Hunter et al. 1990). These and our findings differ from the experimental data reported for *Puffinus tenuirostris* (Austin et al. 1993): Out of 22 nestlings belonging to 32 adults four instances were discovered where a nestling was not related to one of the nest attending adults. Austin et al. (1993) had caught the birds by setting traps into the entrance of nest burrows. These shearwaters were considered to be the parent birds. If we would have proceeded similarly with *C. diomedea* (which is of course a different species living under different conditions) we would have caught parent birds but also unrelated bachelors and nonbreeders which regularly visit burrows of breeding birds (in some nights up to 30% of all birds captured can fall into this category). To overcome the problem of ‘artificial EPFs’ we sampled only birds that were incubating.

Comparing the band-sharing coefficients between mates and between unrelated birds the coefficients are slightly higher between mates with a mean value of $0.362 ± 0.013$ ($n = 30$; range 0.20–0.47) as compared to $0.289 ± 0.049$ ($n = 57$; range 0.39–0.17) (Fig. 2). Although the data are not completely independent we used a t-test to evaluate their statistical significance. Halving the degrees of freedom (d.f. = 42), produces a t-value of 5.62, indicating a significant difference ($P < 0.001$). This result can be confirmed by using nonparametric statistics: the application of the U-test (Wilcoxon, Mann & Witney) also indicates a highly significant result ($P < 0.0001$). According to Fig. 2, about 33% of the pairs studied had a band-
sharing coefficient between 0.4 and 0.5, which is indicative for a closer genetic relationship. No band-sharing coefficient of birds from the random sample falls into this frequency class. This indicates that breeding mates are genetically related to some degree.

We have discovered recently that young shearwaters settle closely to the site of birth when they return for breeding (Wink et al. 1993). Some of the males (females display a higher dispersal) were found in the same nest in which they were born several years before and could by chance mate with their mother if she is still alive. If birds show such a high degree of philopatry than genetically related clusters should develop which could explain the slightly elevated band-sharing between mates as seen in Fig. 2.

In conclusion, C. diomedea is a colonially breeding, long-lived shearwater with strong site and mate bonds, in which EPFs seem to be absent or at least quite rare. Mechanisms seem to exist in some socially monogamous species which reduce the risk of EPF and achieve unequivocal parentage by means such as fidelity, high copulation frequencies, mate guarding or sperm competition (Gyllensten et al. 1990; Birkhead & Möller 1992; Swatschek et al. 1993).

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References


The paper is the result of the long-term collaboration between D. Ristow and M. Wink (Head of Department) on the ecology and biology of Cory’s shearwater. Ingrid Swatschek is presently preparing her doctoral thesis on the application of DNA fingerprinting for studying colonially nesting birds. This work represents one contribution from current studies of this laboratory in which we are employing molecular methods for studies in ecology and evolution.