Smelling home: a good solution for burrow-finding in nocturnal petrels?

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Summary

Many burrowing petrels are able to return to their nests in complete darkness. The well-developed anatomy of their olfactory system and the attraction that food-related odour cues have for some petrel species suggest that olfaction may be used to recognize the burrow. In contrast, surface-nesting petrels may rely on visual cues to recognise their nest. We performed experiments on nine species of petrel (with different nesting habits) rendered anosmic either by plugging the nostrils or by injecting zinc sulphate onto the nasal epithelium. Compared with sham-treated control birds, we found that anosmia impaired nest recognition only in species that nest in burrows and that return home in darkness. Therefore, petrels showing nocturnal activity on land may rely on their sense of smell to find their burrows, while petrels showing diurnal activity or surface nesters may disregard olfactory cues in favour of visual guidance.

Key words: homing, orientation, olfaction, petrel, zinc sulphate, anosmia.

Introduction

The highly developed anatomy of the olfactory system of petrels (Order Procellariiformes, birds) (Bang, 1966, 1971; Bang and Wenzel, 1985) has always seemed surprising since birds are usually considered microsmatic animals. Members of this order, however, have the largest olfactory bulbs among birds, representing up to one-third of the brain volume, and show considerable expansion of the olfactory epithelium (for references, see Warham, 1996). Nevertheless, the reason why these pelagic birds have developed such apparatus and its function in their life are still unsolved questions (for a review, see Bonadonna, 2001). Several experiments have provided evidence that some species of petrels are attracted by food-related odour cues, suggesting that olfaction could have developed as an adaptation to improve foraging efficiency at sea (Nevitt, 1999, 2000; for a review, see Roper, 1999). However, despite a well-developed olfactory apparatus, some Procellariiformes families such as albatrosses (Diomedeidae) do not seem to be attracted by food-related odours. This suggests that petrels may use olfaction in alternative ways.

Most petrels nesting in burrows or crevices return to the nest at night under the cover of darkness (Warham, 1990), and the musky scent diagnostic of occupation has led researchers to suggest that these birds may use olfaction in locating or identifying their home burrow. Only a small number of studies have investigated the role of olfaction in burrow recognition and localisation. The most convincing evidence that olfaction plays a role in burrow recognition comes from studies on storm-petrels (family Hydrobatidae, Grubb, 1973, 1974, 1979;

Minguez, 1997), Cory's shearwater *Calonectris diomedea* (Benvenuti et al., 1993) and blue petrels *Halobaena caerulea* (Bonadonna et al., 2001). Birds of these species are not able to find their burrows if made anosmic. Other studies are more controversial, including those on the wedge-tailed shearwater *Puffinus pacificus* (Shallenberger, 1975), Manx shearwaters *Puffinus puffinus (*James, 1986) and snow petrel *Pagodroma nivea* (Haftorn et al., 1988) (for a discussion, see Bonadonna, 2001; Bonadonna et al., 2001).

We suggest that some of this controversy has resulted because researchers have focused on establishing the sensory abilities of birds irrespective of the adaptive forces that may shape these abilities. Birds of species that are not restricted to nocturnal activity, for example, might rely more heavily on visual cues to find their burrows. Similarly, surface-nesters may not need to rely (or rely less) on olfactory cues compared with burrow-nesters. Our aim in this study was to examine the role of olfaction in burrow recognition by nine species of petrel with different nesting habits (burrowing, crevice-nesting and surface-nesting). For one of these species, we also used two different techniques to impair olfactory ability.

Materials and methods

Plugged-nostrils homing experiments

Data analysed for this study were collected for several species as follows. In December 1984, and January and December 1985 at 66°40′S, 140°00′E on the Antarctic coast:

Cape petrel *Daption capense* L., a population of more than 400 pairs in small colonies of 10–15 nests spaced 1–5 m apart on scree-covered slopes; Wilson's storm petrel *Oceanites oceanicus* (Kuhl), a population of several thousand pairs in isolated crevice nests on scree-covered slopes; snow petrel *Pagodroma nivea* (Forster), a colony of approximately 80–100 crevice nests spaced 0.5–5 m apart on a scree-covered slope; Antarctic fulmar *Fulmarus glacialoides* (Smith), a colony on a cliff of approximately 50 pairs, with nest spaced 0.5–2 m apart. In November 1987 at 49°28′S, 69°57′E, Kerguelen archipelago: thin-billed prions, *Pachyptila belcheri* (Mathews) and common diving petrel *Pelecanoides urinatrix* (Gmelin), burrows on a slope in a mixed crowded colony of more than 10 000 pairs for each species. In June 1988 at 30°09′N, 15°52′W, Selvagen Islands: Cory's shearwater *Calonectris diomedea* (Scopoli) subspecies *borealis*, two colonies of approximately 50–70 burrows spaced 1–2 m apart in artificial

walls; Bulwer's petrel, *Bulweria bulwerii* (Jardine and Selby), one colony of approximately 30 burrows in a scree-covered slope; Madeiran storm petrel *Oceanodroma castro* (Harcourt), one colony of approximately 30 burrows in a scree-covered slope.

Our experimental procedure was similar to those of Grubb (1974) and Haftorn et al. (1988) in that experimental birds had their nostrils sealed with window putty. Birds were caught at the nest and kept in captivity in individual boxes for 1–3 h. Each box was then randomly assigned to a control or treatment group (see Table 1). Both control and experimental birds were handled, banded and manipulated for 2–4 min in a similar way. During manipulation, we plugged the nostrils using window putty for both experimental and control birds, but in control birds plugs were then removed. After treatment, birds were returned to their box for 1–3 h to let them recover from the stress of the manipulation. Releases at Adelie Land were

Table 1. *Homing performance and global homing success in plugged nostrils experiments*

			Day after the release							
	N/A	Treatment	0	1	2	3	Later	Lost	Total	GHS
Common diving petrel	b/n	Control	0	3	–	–	–	0	3	1
		unplugged nostrils	0	0	1	–	–	–	1	
		plugged nostrils	0	1	4	4	4	3	7	0.57*
Madeiran storm petrel	b/n	Control	2	3	3	3	3	1	4	0.83
		unplugged nostrils	1	1	2	–	–	–	2	
		plugged nostrils	0	0	0	0	0	2	2	0*
Wilson's storm petrel	b/n	Control	2	2	2	2	2	1	3	0.93
		unplugged nostrils	1	6	10	11	–	–	11	
		plugged nostrils	1	1	1	2	–	4	6	0.33**
Thin-billed prion	b/n	Control	1	2	3	3	4	0	4	1
		unplugged nostrils	0	2	2	3	–	–	3	
		plugged nostrils	0	0	0	0	0	3	3	0***
Bulwer's petrel	b/n	Control	4	5	–	–	–	0	5	1
		unplugged nostrils	12	14	15	–	–	–	15	
		plugged nostrils	3	5	6	–	–	0	6	1
Cory's shearwater subsp. *borealis*	b/d	Control	5	–	–	–	–	0	5	1
		unplugged nostrils	2	–	–	–	–	–	2	
		plugged nostrils	7	–	–	–	–	0	7	1
Cape petrel	s/d	Control	0	1	1	1	4	0	4	1
		unplugged nostrils	0	2	2	2	3	–	3	
		plugged nostrils	2	2	2	2	4	3	7	0.57
Snow petrel	b/d	Control	3	5	7	7	7	2	9	0.90
		unplugged nostrils	6	8	10	11	–	–	11	
		plugged nostrils	4	6	6	6	6	3	9	0.67
Antarctic fulmar	s/d	Control	1	2	2	3	–	0	3	1
		unplugged nostrils	0	1	3	–	–	–	3	
		plugged nostrils	1	2	2	2	4	0	4	1

N/A, nest type and activity on land; b=burrow- or crevice-nesting, s=surface nest, n=nocturnal, d=diurnal (from Warham, 1990). GHS, global homing success (one-tailed *G*-test). *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Asterisks indicate a significant difference between GHS of plugged birds with respect to pooled control and unplugged birds.

For each day values represent numbers of birds present in the nest (cumulative frequency).

performed on the coastline at 6–10 km from the colony or from a boat at 20–40 km from the colony. Releases at Kerguelen and Selvagen Grande were performed on the coastline at 50–500 m from the colony.

The morning after release, and for subsequent days, the burrows were monitored for 6 days on the Antarctica coast and for up to 10 days at Kerguelen and Selvagen Islands. Each time a treated bird homed, we noted whether the 'mastic' was still in place on both nostrils.

Zinc-sulphate homing experiments

Since occlusion of the nostrils does not completely eliminate the perception of odour stimuli (Wallraff, 1988), we performed an additional experiment using a different technique: zinc-sulphate-induced anosmia (Benvenuti et al., 1992). The experiment took place in Mayes Island (49°28′S, 69°57′E), Kerguelen Archipelago (December, 2000 and January, 2001) on 34 thin-billed prions *Pachyptila belcheri*. 24 birds were deprived of their sense of smell using a solution of zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) in distilled water at two different concentrations (12 birds with 2% $ZnSO_4$, and 12 birds with 4% $ZnSO_4$). The 2% concentration was used with reference to the study of Benvenuti et al. (1993) on Cory's shearwaters. It allowed us to assess whether a lower concentration than the 4% used by Bonadonna et al. (2001) on blue petrels was sufficient to make birds anosmic. The homing abilities (homing performance and homing success) of treated birds were compared with those of 10 sham-manipulated birds acting as controls. Treatments and release procedures were carried out using the methods described by Bonadonna et al. (2001).

In the morning, birds were captured, weighed and injected with 4 ml of zinc sulphate solution (experimental birds) or physiological saline solution (control birds) into each nostril, using a curved needle. We injected the solution directly into the nostrils because preliminary tests (performed on naturally dead prions found in the colony) with Methylene Blue solution showed that this method is the most efficient way of reaching the olfactory mucosa (see Bonadonna et al., 2001). Birds were then returned to their own burrows. They were released during the night following the treatment, 1 h after complete darkness at approximately 100–200 m from the colony. The morning after release, and on subsequent days, the burrows were

checked for the presence of birds. All burrows were monitored for at least 1 week after the last release.

Statistical procedures

For each homing experiment, we performed a Mann–Whitney *U*-test assigning a rank to each bird and taking into account the number of days elapsed between the release and homing dates (homing performance; the highest rank was assigned to lost birds). The numbers of birds homed and lost in each treatment (global homing success) were compared using a one-tailed *G*-test (predicting lower homing success in anosmic birds; Sokal and Rohlf, 1981). An additional one-tailed *G*-test was performed to compare homing success the morning after release (immediate homing success). This last test was performed only for the zinc-sulphate homing experiments because the sample sizes involved in plugged-nostrils homing experiments were too small.

Results
Plugged-nostrils homing experiments

Some of the petrels with plugged nostrils homed after having lost their plugs (hereafter referred to as unplugged birds; Table 1). We therefore compared homing performances across two groups (Table 1): control birds and birds homed with plugs in place (hereafter referred to as plugged birds). Statistical analysis was performed by pooling birds released on the sea coast and at sea, since the sample sizes were too small to consider them separately. A Mann–Whitney *U*-test revealed differences between control and plugged birds in thin-billed prions ($U=0.0$, $N_1=4$, $N_2=3$, $P<0.05$), and in common diving petrels ($U=1.5$, $N_1=3$, $N_2=7$, $P<0.05$).

Since unplugged birds recovered their olfactory capabilities during the experiment, we pooled them with the control birds in global homing success analysis. A *G*-test (one-tailed) indicated a lower global homing success of plugged birds with respect to pooled control and unplugged birds in common diving petrel ($P<0.05$), Madeiran storm petrel ($P<0.05$), Wilson's storm petrel ($P<0.01$) and thin-billed prions ($P<0.001$).

Zinc-sulphate homing experiments

Five experimental birds abandoned the burrow during the

Table 2. *Thin-billed prion homing performance, immediate and global homing success after zinc sulphate treatment*

| Treatment | Day after the release | | | | | Lost | Total | IHS | GHS |
	0	1	2	3	Later				
Saline	6	7	7	9	10	0	10	0.60	1
$ZnSO_4$ (2%)	1	3	5	6	7	2	9	0.14**	0.78*
$ZnSO_4$ (4%)	0	2	5	5	6	4	10	0***	0.60**

IHS, immediate homing success; GHS, global homing success (one-tailed *G*-test). *$P<0.05$, **$P<0.01$, ***$P<0.001$. Asterisks indicate a significant difference of either $ZnSO_4$ group from the control (saline) value.

For each day values represent numbers of birds present in the nest (cumulative frequency).

first part of the night before the release. The other birds were found incubating their egg, exhibiting normal behaviour indistinguishable from that of unmanipulated birds. Nine 2% zinc sulphate birds, ten 4% zinc sulphate birds and ten control birds were found in their burrows and released.

The homing performance and immediate and global homing successes of the three groups are given in Table 2. Homing performances were significantly lower in zinc sulphate birds for the 4% treatment (Mann–Whitney test: $U=16$, $N_1=10$, $N_2=10$, $P<0.01$), and tended to be marginally lower in the 2% treatment (Mann–Whitney test: $U=22$, $N_1=9$, $N_2=10$, $P=0.053$).

The G-test (one-tailed) indicated a lower immediate homing success of both groups of experimental birds with respect to control birds (2% $ZnSO_4$, $P<0.01$; 4% $ZnSO_4$, $P<0.001$), and a lower global homing success in 4% zinc sulphate birds ($P<0.01$) and in 2% zinc sulphate birds ($P<0.05$) with respect to control birds. One 4% anosmic prion (counted in the lost group, Table 2) was found 4 days after the release, during the night, in the wrong burrow approximately 12 m from its own nest. The following morning, the bird was not in the colony and was never found again.

Discussion

The role of olfaction in burrow recognition was addressed by performing a comparative study based on natural history. In a first preliminary analysis, we considered a larger number of burrowing, crevice-nesting and surface-nesting species by using a simple method to make them anosmic. Occlusion of the nostrils, as used in pigeon (*Columba livia*) homing experiments, reduces odour stimulus intensity to approximately 20–30% of the level existing under unimpeded condition of breathing and smelling. Odours are probably still detectable since birds can perceive odours through their choanae (Wallraff, 1988). Despite the differences in olfactory system anatomy between petrels and homing pigeons, we cannot exclude a similar ability in petrels. Consequently, the application of this method alone may not produce conclusive results. However, the use of this easy technique in homing experiments provided some preliminary information on a comparative basis (assuming that methodological bias remains constant across species) and allows inferences regarding the importance of olfaction in these different species. Despite the small numbers of birds tested, the results showed some interesting trends. Birds with plugged nostrils had lower homing performances and/or success in burrowing/crevice-nesting species showing nocturnal activity on land (hereafter referred to as nocturnal birds). The only exception is Bulwer's petrels, which were apparently not impaired in finding their nest. The strong smell, with respect to other species, issuing from the burrows of this species (Warham, 1990; V. Bretagnolle, personal observations) suggests that a partial reduction in olfactory capacity may not be sufficient to prevent these birds from smelling the nest.

All the other species in which control and experimental birds showed the same capacity to home exhibit diurnal

activity on land (hereafter referred to as diurnal birds), and most nest on the surface (Warham, 1990). Their different homing abilities under the experimental conditions thus may be due to the birds' habits. Diurnal birds can rely on visual cues while nocturnal birds do not have eyes adapted for night vision (for references, see Warham, 1996). With regard to this explanation, it is worth noting the different results obtained previously for Cory's shearwaters (Benvenuti et al., 1993). The low homing performance of their anosmic birds is probably due to the nocturnality of the subspecies tested (*Calonectris diomedea diomedea*). Conversely, the subspecies *borealis*, on which we performed the present experiments, exhibits diurnal activity on land (Bretagnolle, 1990) and does not show impaired performance in returning to the nesting crevices.

Besides the possible incomplete impairment of the olfactory capabilities obtained by plugging the nostrils with mastic (Wallraff, 1988), this method causes a physical impairment that forces the birds to breathe through the mouth. This might have caused a non-specific stress response in the treated birds. Experiments using zinc-sulphate-induced anosmia (for references, see Bonadonna et al., 2001) were performed on thin-billed prions, a species in which the plugged-nostrils experiment revealed significantly impaired homing abilities. The results confirmed that this species shows an impaired homing performance if made anosmic. This technique also revealed that different concentrations of zinc sulphate solution have different effects on the birds' homing ability: birds treated with 4% $ZnSO_4$ solution had lower homing performances than birds treated with 2% solution. This could be due to incomplete anosmia using the gentler treatment (2% concentration) and/or to more rapid recovery of olfactory capacity (Cancalon, 1982).

Thin-billed prions seem to be more capable of compensating for anosmia than blue petrels (Bonadonna et al., 2001). Indeed, none of the anosmic blue petrels came back in the first 3 days after the release, while 50% of the treated prions (same 4% $ZnSO_4$ solution concentration) had returned on the third morning (Table 2). There are two possible explanations for this result. Prions struggled continuously during the injection of the solution, making the manipulation more difficult. Despite the efficiency of the method demonstrated by preliminary tests, a more difficult manipulation could have resulted in a less efficient treatment in some birds: these birds could have quickly recovered their olfactory capability, and homed earlier than fully treated birds. The other possibility is that thin-billed prions are able to shift to an alternative mechanism to find the burrow entrance. Considering the ecological similarities between the two species (Warham, 1996), we are inclined to accept the first possibility. The fortunate observation of an anosmic bird in the proximity of its burrow and its evident inability to find its own burrow suggest that olfactory cues are necessary to find the nest entrance. We therefore suggest injecting more that 4 ml of solution per nostril to obtain a more extended effect of the solution on the olfactory mucosa (Cancalon, 1982) in future studies.

Our results imply that nocturnal petrels may rely mainly on their sense of smell to find their nests, while diurnal petrels may disregard olfactory cues for visual guidance or shift to vision when olfactory cues are not available. Visual recognition of the burrow entrance is suggested by the experiments of James (1986) and Brooke (1978) on Manx shearwaters. The results obtained on Cory's shearwaters show that the same species rely mainly on olfactory cues if nocturnal and probably more on visual ones if diurnal. Consequently, the capacity to 'smell' the nest may be present even in diurnal species and is probably used if available.

Our findings further suggest the importance of the olfactory sense as an aid to nocturnal homing petrels. These birds may use a strong generic 'petrel odour' issuing from the colony to localise the colony position (Grubb, 1973). However, considering our results and the precision with which most petrels are able to land in the proximity of the nest (F. Bonadonna personal observations), we propose a second olfactory step: an individual may follow an odour plume issuing from its own nest using osmotaxis, as proposed previously for blue petrels (Bonadonna et al., 2001). We cannot confirm, however, that petrels are able to distinguish the odour of their own nest from that of the nest of conspecific birds, for which two-way choice experiments are needed.

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