

## Examining the development of individual recognition in a burrow-nesting procellariiform, the Leach's storm-petrel

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### SUMMARY

**Burrow-nesting petrels use their well-developed sense of smell for foraging, homing to their nest, and mate recognition. The chicks of burrow-nesting petrels can apparently learn odours associated with prey while still in the nest, but the development of individual-specific odour recognition is less well understood. We used a simple two-choice test to determine whether 4- to 6-week-old chicks of a small, burrow-nesting species, the Leach's storm-petrel (*Oceanodroma leucorhoa*), prefer the scent of their own nest material to (1) the scent of similar organic material collected from the colony or (2) the scent of a conspecific's nest material. Results suggest that chicks clearly preferred the scent of their own nest material to that of similar organic material collected from the colony (96%;  $N=24$ ; binomial test,  $P<0.001$ ). Results further suggested that birds preferred the scent of their own nest material to that of a conspecific, though the preference was statistically less robust (67%;  $N=39$ ; binomial test,  $P=0.05$ ). Because Leach's storm-petrel chicks do not normally leave their burrow prior to fledging, an ability to recognise individual or nest-specific odours is not likely to be used for homing but instead may be linked to the development of individual recognition in different contexts.**

Key words: olfaction, odour learning, individual odour, seabird, Procellariiformes.

### INTRODUCTION

Among birds, the Procellariiformes ('tube-nosed' seabirds) are known for their excellent sense of smell. Many species use olfactory cues to assist them in both locating productive areas for foraging (Nevitt, 2000) and area-restricted search of productive waters (Nevitt et al., 1995; Nevitt, 1999a; Nevitt, 1999b; Nevitt, 2000; Nevitt et al., 2004; Nevitt and Bonadonna, 2005). In addition, several burrow-nesting species use odour cues to relocate their burrow when returning to the colony for incubation shifts (Bonadonna and Bretagnolle, 2002; Bonadonna et al., 2004). It has also recently been demonstrated that another burrow-nesting procellariiform, the Antarctic prion (*Pachyptila desolata*), can recognise individual-specific odours, which may assist birds in both burrow and mate recognition (Bonadonna and Nevitt, 2004).

Most work to date has focused on adults, whereas olfactory behaviour development in chicks has received much less attention. It has been shown that, as chicks, several species of petrels are responsive to a variety of scented compounds, including prey related odours (Cunningham et al., 2003; Cunningham et al., 2006), but whether chicks recognize personal odours has not been well studied. One of the few studies that addressed personal odour recognition in chicks was carried out on European storm-petrels (*Hydrobates pelagicus*) on Benidorm Island, Western Mediterranean. Here, Minguez (Minguez, 1997) found that 1- to 2-week-old chicks required an intact sense of smell to relocate their burrow when displaced short distances (10–30 cm). Subsequent experiments showed that European storm-petrel chicks were attracted to their own body odour, even when tested against the scent of a conspecific (De Leon et al., 2003). Although European storm-petrels typically nest in burrows or among rocks in cliff faces

(Cramp et al., 1974), on Benidorm Island, they often nest communally in burrows that tend to open into a common vestibule. Because chicks stray into this area as part of their normal behavioural repertoire, De Leon et al. (De Leon et al., 2003) concluded that the function of familiar odour recognition was to facilitate homing to the correct burrow.

Although the nesting behaviour described may be somewhat unusual even for European storm-petrels, in most other species, chicks do not leave their burrow prior to fledging because of heavy predation in colonies (e.g. Priddel and Carlile, 1995; Votier et al., 2006). Moreover, many species disperse to other islands to breed, suggesting that developing or retaining a memory for the scent of the home burrow or colony would not be required for homing in pre-reproductive individuals. Because individual recognition could serve functions other than homing, our goal was to test whether a different species of storm-petrel that does not leave the burrow prior to fledging would express this same behaviour.

Leach's storm-petrels, (*Oceanodroma leucorhoa* Vieillot) are one of the most abundant burrow-nesting procellariiforms breeding in the northern hemisphere. Adults are generally faithful to both their burrow and their mate throughout their lifetime (Morse and Buchheister, 1979). Like all procellariiforms, Leach's storm-petrels lay a single egg, which they incubate for 40–50 days. Burrows are typically less than a metre deep (Huntington et al., 1996) and chicks remain in a nest cavity located at the deepest section of the burrow until they fledge to forage at sea when they are about 60 days old (Warham, 1990). The fledgling abandons the burrow and does not return to land until it is ready to breed 4–5 years later (Warham, 1996). Because Leach's storm-petrel chicks remain in their burrows prior to fledging and are not natally philopatric (Huntington et al.,

1996), we reasoned that these birds would not be adapted to learn the scent of their natal burrow for homing purposes. We explored this hypothesis using simple two-choice tests to determine whether Leach's storm-petrel chicks (1) prefer the scent of their own nest material to the scent of similar organic material collected from the colony, or (2) prefer the scent of their own nest material to the scent of nest material of a conspecific.

## MATERIALS AND METHODS

### Study sites

Experiment 1 was performed in August 2001 at the Bowdoin College Biological Station on Kent Island, New Brunswick, Canada (44°35'N, 66°45'W) where at least 2000 breeding pairs of Leach's storm-petrels have been convincingly documented (Cannell and Maddox, 1983). Experiment 2 was performed in September 2006 on Bon Portage Island, Nova Scotia, Canada (43°26'N, 65°45'W) where there are approximately 50 000 breeding pairs of Leach's storm-petrels (Oxley, 1999). At both locations, experiments were performed during daylight hours (between 08:00 and 17:00 h). To control for environmental conditions, trials were performed inside, in well-ventilated, darkened field laboratories situated approximately 15 min walking distance from the petrel colonies. All procedures were carried out in adherence to guidelines provided by the University of California, Davis Institutional Animal Care and Use Committee.

### Experiment 1

We tested 24, 4- to 6-week-old chicks (3–5 weeks from fledging). Chicks were tested one at a time using identical procedures, as follows. The chick was first removed from its burrow and placed in a cotton 'bird bag'. Then, approximately 200 ml of organic material was removed from the nest chamber directly beneath where the chick had been sitting. This material was placed in a clean Ziplock® (S C Johnson, Racine, WI, USA) plastic bag. Nest material consisted mainly of leaf litter and other plant debris. Similar organic material was also collected from within the petrel colony at a distance of ~3 m from any nest, and placed in a different clean Ziplock® plastic bag. To guard against the possibility that chicks could use visual cues to recognise their burrow material, care was taken to ensure that the constitution of material collected from within the colony closely matched that of the nest material and that there were no discernible visual differences between the two samples. The chick and its nest material were then transported to the laboratory. To avoid cross-contamination between burrow odours, fresh latex gloves were worn while handling each sample of nest material. In addition, gloves and plastic bags were used only once and then discarded.

### Experiment 1 set-up

Artificial nest chambers were constructed from round plastic storage containers (12 cm diameter by 8 cm high). Each container was cut with a '∩' shaped access hole such that when the container was turned over on a flat surface, a chick could easily walk into it. Because preliminary trials suggested that chicks were negatively phototactic, chambers were lined inside and out with black duct tape.

For an experimental trial, two chambers were placed side by side with the openings situated at a 90° angle to each other such that a chick positioned in front of them could easily investigate either one of the chambers by moving its head from side to side. The chambers were placed on plastic coated lab paper that was changed after each trial. We used a disposable Dixie® (Georgia-Pacific, Atlanta, GA, USA) cup to measure approximately 90 ml of the test-chick's nest

material, which was then placed on the plastic-coated lab paper within one of the chambers. A similar amount of the material collected from the colony was measured and placed inside the other chamber using the same procedure. To avoid a directional bias, a coin toss determined the position (right or left) of each type of nest material. To avoid pseudoreplication, we used multiple chambers and rotated their position (right or left) between trials. Nest material was thoroughly mixed and all experiments were performed in the dark to reduce visual cues. Once a trial was completed, the chambers and all surrounding areas were wiped clean with diluted methanol (~10%).

To begin a trial, the chick was placed at a 'start' position facing the chambers, approximately 6 cm from the midpoint between the entrances. From this position, the chick needed to take a few steps to reach the entrances, and could easily extend its neck to probe each chamber with its bill before making a choice. A choice was called if the chick entered a chamber and remained inside for 2 min. The time taken to choose was recorded. If the chick did not make a choice by 15 min, 'no choice' was called and the trial was terminated. Any chick that either darted into a chamber in under 15 s or defecated during a trial was considered too stressed to perform the experiment and was immediately returned to its burrow. Once the trial was completed, the chick was weighed to the nearest gram with a 100 g Pesola® spring scale (Baar, Switzerland); tarsus and flattened wing chord were measured, and the chick was then immediately returned to its burrow along with its nest material. Each chick was absent from its burrow for no longer than 30 min and each bird was tested only once.

### Experiment 2

We tested 43 chicks from the same age group as Experiment 1. For this experiment, chicks were collected two at a time. Each chick was removed from its burrow and immediately transferred to a 'bird bag'. Nest material was collected and stored in clean Ziplock® plastic bags following the procedures outlined for Experiment 1. The distance between the nests of experimental pairs was measured to the nearest 0.1 m with a 30 m plastic measuring tape.

### Experiment 2 set-up

The experimental set-up for Experiment 2 was the same as in Experiment 1 except that the second chamber contained approximately 90 ml of the nest material of a conspecific rather than organic material collected from within the colony. Each chick, along with its nest material, was returned to the appropriate burrow immediately after the completion of both trials. Chicks were absent from their burrows for approximately 1 h.

### Statistical analysis

Odour preferences were examined using a binomial test (Zar, 1999). For Experiment 2, logistic regressions were used to assess whether odour preferences were influenced by body condition, the age (using wing chord length as a reference), or the distance between burrows. A body condition index (BCI) was calculated for each chick as the residual score from a regression of body mass on tarsus length.

## RESULTS

When placed at the 'start' position, a chick would typically extend its neck and move its head in a broad, sweeping semi-circle around the front half of the body with the bill touching the substrate. Subtle head-bobbing or biting movements were also anecdotally observed which seemed to be associated with sampling or sniffing. After

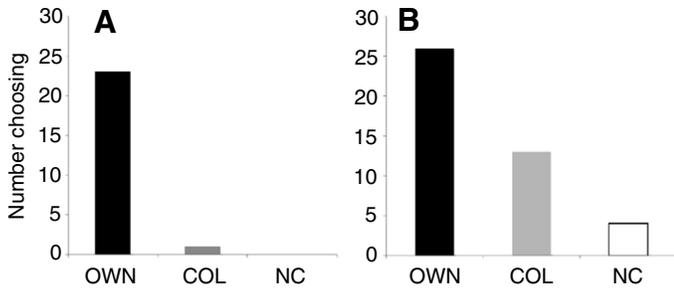


Fig. 1. Odour preferences of Leach's storm-petrel (*Oceanodroma leucorhoa*) chicks. (A) Experiment 1 (own burrow material *versus* similar colony material); OWN, own burrow material; COL, colony material; NC, no choice ( $N=24$ , binomial test,  $P<0.001$ ). (B) Experiment 2 (own burrow material *versus* conspecific's burrow material); OWN, own burrow material; CON, the burrow material of a conspecific; NC, no choice ( $N=39$ , binomial test,  $P=0.05$ ).

approximately 1–2 min, each chick tended to take a few steps forward and probed each chamber by inserting its head into each of the test containers for at least 15 s to up to several minutes. After both nests had been probed, the chick typically walked into one of them and either sat quietly or initiated digging movements.

**Experiment 1**

When given a choice between their own nest material and similar material collected from the colony, 23 out of 24 chicks (96%) chose to enter the chamber containing their own nest material (Fig. 1, binomial test,  $P<0.001$ ).

**Experiment 2**

When given a choice between their own nest material and the nest material of a conspecific, 26 chicks (67%) chose their own nest material compared to 13 chicks (33%) who chose the nest material of a conspecific (Fig. 1, binomial test,  $P=0.05$ ). Four birds failed to choose and were excluded from the analysis. Because the effect was less pronounced in this second experiment, we wanted to know whether the age (as reflected by wing length), body condition, or the distances between burrow pairs influenced whether birds chose their own scent over a conspecific's scent (Table 1). None of these parameters significantly influenced the odour preferences of chicks (logistic regressions: wing length;  $\chi^2=0.10$ ,  $P=0.75$ , body condition index;  $\chi^2=0.11$ ,  $P=0.74$ , and proximity to test-pair's nest;  $\chi^2=0.82$ ,  $P=0.37$ ).

**DISCUSSION**

When given a choice between their own nest material and similar material collected from within the colony, nearly all chicks (96%) preferred their own nest material, suggesting an ability to recognise petrel odour. Contrary to our expectations, chicks also preferred their own nest material to the nest material of a conspecific. Because these experiments were conducted under conditions

designed to reduce visual cues, the results suggest that Leach's storm-petrel chicks can distinguish the familiar odour of their burrow material from other petrel odours.

These results are thus similar to results obtained with European storm petrels (*Hydrobates pelagicus*) (De Leon et al., 2003), although we were able to use a more straight forward methodology in the present study because we were fortunate to have access to larger numbers of birds. For example, in De Leon's studies, chicks were trained to perform an experimental task (walking through a PVC pipe) and were asked to perform this task several times before actual tests were conducted. Chicks were also used repeatedly. By contrast, we tested birds against the scent of nest materials, each bird was tested only once, and our experimental design did not require birds to be trained or repeatedly handled. Not surprisingly, in the European storm-petrel study, chicks failed to choose in increasing numbers in subsequent tests, whereas we found that nearly every Leach's storm-petrel chick was motivated to make a choice.

Because birds were not enclosed in tubes, we were also able to observe their behaviour as they were making a choice. For example, we found that chicks spent most of the time before making a choice probing each of the chambers, suggesting that they thoroughly investigated both odours before committing themselves to an artificial burrow. We also found that chicks tended to take more time (219 *versus* 127 s) to choose when they were presented with two types of nest material (Experiment 2) than when they were presented with a choice between nest material and colony material. This difference is probably not age related, since birds performing Experiment 2 were slightly older (as determined by wing chord length) and were subsequently likely to be more mobile than birds used in Experiment 1 (Table 1). More likely explanations are that petrel-related odours are harder for some birds to tell apart, or that Leach's storm-petrel chicks are curious about unfamiliar odours (for example, see Cunningham, 2005).

Parental odours as well as the chick's personal odour contribute to the odour signature of the nest. Thus, in our study, it may be that chicks were attracted to the scent of their parents, which they may have learned through association with the nest material. In another procellariiform, the Antarctic prion (*Pachyptila desolata*), it has been shown that adults prefer the odour of their partner to their own personal odour, a behaviour that must also be learned through association, most likely in the nest where birds have regular intimate contact (Bonadonna and Nevitt, 2004). It follows that chicks, too, may have an opportunity to learn familial odours during their early life in a burrow impregnated with the scent of their parents, and that this memory may be important later in life in the context of kin recognition and mate choice (Blaustein, 1983).

An alternative explanation for our results is that Leach's storm-petrel chicks may be able to use odour cues to discriminate sexes. The sex of chicks tested was not known but, interestingly, the proportion of chicks that preferred their own nest material was consistent with what would be found if they were making a choice

Table 1. Characteristics of Leach's storm-petrel chicks used in odour preference trials

| Experiment | Body mass (g) | Tarsus length (mm) | Wing length (mm) | Latency to choose (s) | Distance between nests (m) |
|------------|---------------|--------------------|------------------|-----------------------|----------------------------|
| 1          | 59.9±2.2      | 23.7±0.3           | 57±3             | 127±12                | N/A                        |
| 2          | 65.2±1.5      | 24.3±0.2           | 83±4             | 219±35                | 5.7±0.4                    |

Experiment 1: own burrow material *versus* closely matched material collected from the colony ( $N=24$ ). Experiment 2: own burrow material *versus* conspecific's burrow material ( $N=39$ ).

Values are means ± s.e.m. N/A, not applicable.

on the basis of a sex-specific odour. Because the burrow is occupied by the chick as well as by each of its parents more or less equally, the sex-specific odour of the burrow is likely to be enhanced by the chick's sex. If this were the case, we would expect 100% of birds to choose their own nest material when presented with a choice between their own nest material and the material from a burrow occupied by a chick of the opposite sex, and at random (50%) when choosing between their own nest material and the material from a burrow occupied by a chick of the same sex. Thus, overall, we would expect 75% of birds to correctly choose their own nest material, which is consistent with what we found (67%; binomial test,  $P=0.27$ ). This alternative possibility is intriguing because, although odour-based sex discrimination has been demonstrated in mammals (e.g. Keller et al., 2006; Woodley et al., 2004), it has never been shown in a petrel, or to our knowledge, in any bird. Whether Leach's storm-petrel chicks have the ability to discriminate sex using odour cues, therefore, warrants further investigation.

In conclusion, we have shown that Leach's storm-petrel chicks can recognise familiar odours and that they prefer the odour of their own nest material to either non-petrel-specific odours or odours associated with other petrel burrows in the colony. These results suggest that the burrow environment, where chicks have close contact with their parents, may give Leach's storm-petrel chicks an opportunity to learn familial odours prior to fledging. Rather than serving a function in homing, we speculate that this ability may be linked to the development of individual recognition and that a memory for familial odours may play a role later in life in the context of kin recognition and mate choice. Alternatively our results present the intriguing possibility that Leach's storm-petrel chicks can recognise sex-specific odours, an ability that has never been demonstrated in a bird. Clearly the development of the olfactory abilities in procellariiform chicks promises to be a fruitful topic for further investigation.

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