Chemical kin label in seabirds

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Chemical signals yield critical socio-ecological information in many animals, such as species, identity, social status or sex, but have been poorly investigated in birds. Recent results showed that chemical signals are used to recognize their nest and partner by some petrel seabirds whose olfactory anatomy is well developed and which possess a life-history propitious to olfactory-mediated behaviours. Here, we investigate whether blue petrels (Halobaena caerulea) produce some chemical labels potentially involved in kin recognition and inbreeding avoidance. To overcome methodological constraints of chemical analysis and field experimental experiments, we used an indirect behavioural approach, based on mice olfactory abilities in discriminating odours. We showed that mice (i) can detect odour differences between individual petrels, (ii) perceive a high odour similarity between a chick and its parents, and (iii) perceive this similarity only before fledging but not during the nestling developmental stage. Our results confirm the existence of an individual olfactory signature in blue petrels and show for the first time, to our knowledge, that birds may exhibit an olfactory kin label, which may have strong implications for inbreeding avoidance.

1. INTRODUCTION

Birds exhibit a wide array of communication mechanisms (e.g. colours, song and calls) but rarely display obvious olfactory-driven behaviours. This is probably why, despite three decades of physiological and behavioural studies establishing the existence of avian olfactory functions [1], chemical communication has been essentially ignored. Recent research, however, suggests that chemosignals may contribute to avian social behaviours [2–6], thus challenging our understanding of birds’ ecology. Petrel seabirds, which possess a developed olfactory system [7], a noticeable musky scent and a life history favourable to olfactory-mediated behaviours [8], are appropriate model systems to investigate these questions [9].

Burrow-nesting petrels, such as blue petrels (Halobaena caerulea, Gmelin 1789), form socially and genetically (very low extra-pair paternity rate [10,11]) lifelong monogamous pairs, breed in dense colonies on remote islands and most species are philopatric [8]. These traits suggest that kin recognition may be critical for discriminating between potential mates and avoiding inbreeding. Previous behavioural results have shown that some petrel species recognize and prefer their mate’s odour, but avoid their own, preferring the odour of a conspecific bird [12,13]. This preference for non-personal scents was first reported in rodents and implicated as a mechanism for assessing relatedness [14]. In mice, such behaviour is related to major histocompatibility complex (MHC) genes, kin recognition and inbreeding avoidance [15]. This might also be the case in petrels [9].

Compared with other organisms, information on the production and the social use of self-produced odours in birds has received relatively little attention. Recent findings show that the chemical profile of individual petrels is consistently similar from year to year, and different from that of other birds, thus suggesting that it may constitute an olfactory signature on bird feathers. More precisely, a detailed chemical examination of the uropygial secrections and feathers of Antarctic prions (Pachyptila desolata) and blue petrels (by gas chromatography) has shown that these two petrel species exhibit critical socio-ecological information, such as species, gender and individual identity, which could be involved in olfactory behaviours observed in the field (i.e. individual recognition, self-odour avoidance) [16–18].

Although very advanced, the previous chemical and behavioural studies on procellariiforms did not actually investigate whether these birds could produce and recognize some chemical signals relative to kin, which could be implicated in inbreeding avoidance. This lack of data is mainly owing to methodological difficulties. The ecology of petrels makes it difficult to collect enough samples of kin-related birds to have sufficient statistical power in gas chromatography—mass spectrometry analysis [17]. Petrels have only one chick per year, and the age of first breeding is often at 7–9 years old [8]. In addition, molecular tools to assess relatedness are missing in these species. To bypass these main methodological difficulties, we used an indirect behavioural approach, using mice as ’noses’, in order to investigate whether blue petrels’ chemical profile may contain kin labels that may play a role in kin recognition in a natural population. More precisely, we used the ’biological olfactometer’ previously developed and validated by our team [19] to assess the degree of similarity between petrel odours. If individual odours contain a kin label in blue petrels, then individual odour patterns, as perceived by mice, will show higher similarity between kin than between non-kin petrels. Thus, mice habituated to a chick odour should investigate the odour of a non-related individual more often than expected by chance when presented with the odour of one of the parents.

2. MATERIAL AND METHODS

(a) Biological model, odour collection and preparation of odour samples

Odour samples were collected from blue petrels (H. caerulea) in December–January 2007–2008, 2008–2009 and 2009–2010 on île Verte (49°51’ S, 70°08’ E), Kerguelen Archipelago, southern Indian Ocean. Odours were collected from a total of 46 breeding pairs, and when possible, from their unique chicks. Birds of unknown sex were genetically sexed [20]. We sampled chicks at two different stages: in 2008–2009, one-month-old chicks with down (nestlings; n = 11), and in 2009–2010, chicks with feathers 2–3 days before fledging (fledglings; n = 3). Nestling chicks do not exhibit preening behaviour, and the greased down feathers tuft around the preen gland is absent
Table 1. Experimental design, distribution of birds odour samples and mice sample size. (Details in the electronic supplementary material.)

<table>
<thead>
<tr>
<th>phase of behavioural task</th>
<th>habituation trial 1 + trial 2</th>
<th>discrimination</th>
<th>number of mice noses used in statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>duration (min)</td>
<td>10 + 10 min</td>
<td>5 min</td>
<td></td>
</tr>
<tr>
<td>experiment 1: IDENTITY label</td>
<td>referent odour R (adult female; n = 6)</td>
<td>first hole: odour A; unfamiliar adult female (n = 7)</td>
<td>second hole: odour B; referent odour; n = 25</td>
</tr>
<tr>
<td>experiment 2: KIN label; N. nestlings chicks</td>
<td>referent odour R (chick with down; n = 4)</td>
<td>first hole: odour A; unrelated adult male (n = 2) or female (n = 3)</td>
<td>second hole: odour B; father (n = 4) or mother (n = 4) of the referent chick; n = 29</td>
</tr>
<tr>
<td>experiment 2: KIN label; F. fledglings chicks</td>
<td>referent odour R (chick with feather; n = 3)</td>
<td>first hole: odour A; unrelated adult male (n = 7) or female (n = 7)</td>
<td>second hole: odour B; father (n = 3) or mother (n = 3) of the referent chick; n = 24</td>
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(b) Biological olfactometer procedure
The subjects used as ‘noses’ in our ‘biological olfactometer’ were 131 naive adult male Swiss mice (two months old; 28–32 g).

Odores were collected using special cotton swabs produced in Hungary [22], employed by the scientific police service in an ‘odor-ology protocol’ (Service Central d’Identité Judiciaire of Lyon police, France, validated by INTERPOL). Birds taken from their burrow had their feathers gently rubbed (back, under wings, rump) with the special cotton swab for 3–4 min (experimenter wearing disposable Nitrile gloves). The cotton swab was sealed in a glass jar, kept at 5–7 °C for three to four weeks in the field hut and then frozen at −20 °C until odour samples were prepared for the experiments with ‘nose’ mice. These experiments were performed using the odours of 40 individual adult and 14 individual chick blue petrels (see the electronic supplementary material for details).

(c) Statistical analysis
Data were analysed using either an exact permutation test for paired samples when \( n < 30 \), or a Student’s \( t \)-test for paired samples when \( n \geq 30 \). For habituation data analysis, see the electronic supplementary material. For the discrimination data analysis, the percentage of sniff bouts of the unrelated odour (odour A) was compared with the percentage expected by chance (50%): the null hypothesis \( H_0 \) being the percentage of sniff bouts of the unrelated odour at the chance level (50%); the alternative hypothesis \( H_1 \) being the percentage of sniff bouts of the unrelated odour above the chance level (electronic supplementary material). Fourteen mice were excluded because they did not investigate the two holes at least once during the discrimination.

3. RESULTS
In all experiments, during the habituation phase, mice exhibited a significant decline in interest for the referent odour between trial 1 and trial 2, showing that subjects progressively became familiar with referent petrel odours R (figure 1a,b and the electronic supplementary material).

In the discrimination phase of experiment 1, mice (\( n = 25 \)) investigated the unfamiliar odours significantly more than expected by chance (55.9 ± 2.8%; \( p = 0.35 \); figure 2d), suggesting that blue petrels have different individual body odours. In experiment 2N, mice (\( n = 29 \)) did not investigate the unrelated odours more than expected by chance (51.1 ± 3.1%; \( p = 0.35 \); figure 2d), suggesting that nestling chick body odour is not similar to that of their parents. By contrast, in experiment 2F, mice (\( n = 24 \)) investigated the unrelated odours significantly more than expected by chance (55.9 ± 2.8%; \( p = 0.03 \); figure 2c), suggesting that fledgling chick body odour is more similar to parents odour than to any other unrelated adult.

4. DISCUSSION
Using mice olfactory abilities in discriminating odours, we showed that: (i) mice can detect odour differences between individuals of the same sex and age-class in a non-mammal species. In spite of a lower
discrimination rate with respect to previous studies [19] (electronic supplementary material), our behavioural procedure could be considered as a new widely usable tool, complementary to chemical analysis, for the study of chemical communication in animals; and (ii) mice perceive a higher odour similarity between a chick and its parents when compared with an unrelated petrel, at the fledgling developmental stage but not at the nestling developmental stage (experiment 2N).

Results from experiment 1 clearly suggest the existence of an individual olfactory signature in blue petrels. This is consistent with both chemical studies [17,23] and behavioural studies on partner-odour recognition [13]. The originality of our study is experiment 2, where, for the first time to our knowledge, we demonstrate that birds may exhibit an olfactory kin label. This kin label seems to be expressed by chicks just before fledging, suggesting: (i) that the kin label is produced by the individual rather than acquired by impregnation with parents/nest odours in the nest during parental care, and (ii) that the olfactory label is expressed in chicks with definitive feathers and thus when the uropygial gland is probably fully functional. Although our main result is obtained with small sample sizes, owing to field constraints, possible biases are offset by the clear-cut outcome of finding it only in fledglings, and by its consistency with physiological reality (active uropygial gland). Further investigations are, however, needed to confirm these pioneering results and determine whether kin label relies on a genetic cue or is owing to an environmental effect such as diet.

The demonstration of the existence of such olfactory cues, potentially signalling relatedness among individuals, may have strong implications regarding mate choice in petrels. Indeed, the particular life-history traits of this group (long-lived, monogamous, genetically faithful and philopatric to their native island) should have led to the evolution of mating preferences promoting genetic compatibility and
quality of a potential partner, since a suboptimal mate choice would dramatically reduce fitness over a lifetime. The MHC may be involved in these processes, and mating preferences for particular MHC profiles based on chemical assessment have already been observed in fishes and mammals [24]. Our results suggesting that the same chemical assessment may exist in birds may be explained by previous reports [12,13], and shed light on the possible implication of avian chemosignals in individual recognition and genetically based mate choice. Indeed, the self-odour avoidance behaviour observed in some petrel species may actually be explained by an MHC-based mate choice and inbreeding avoidance mediated by olfaction.

All aspects of the study were performed according to guidelines established by the IPEV and the CNRS for the Ethical Treatment of Animals and complied with current French regulations.

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