

King penguins can detect two odours associated with conspecifics

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Keywords:

King penguin, olfaction, conspecific odours

Recent studies on olfaction in penguins have focused on their use of odours while foraging. It has been proposed for some seabirds that an olfactory landscape shaped by odours coming from feeding areas exists. Islands and colonies, however, may also contribute to the olfactory landscape and may act as an orienting map. To test sensitivities to a colony scent we studied whether King penguins (*Aptenodytes patagonicus*) could detect the smell of sand, feathers or feces by holding presentations beneath their beaks while they naturally slept on the beach. Penguins responded to the feathers and feces presentations significantly more than to sand. Although only a first step in exploring a broader role of olfaction in this species, our results raise the possibility of olfaction being used by King penguins in three potential ways: 1) locating the colony from the water or the shore, 2) finding the rendezvous zone within the colony where a chick or partner may be found, or 3) recognizing individuals by scent, as in Humboldt penguins (*Spheniscus demersus*). Further studies must be conducted to determine how sensitivity to feathers and feces is involved in the natural history of this species.

Introduction

Penguins have acute odour recognition of food-related odours and likely use these odours to aid in foraging (Cunningham et al., 2008; Wright et al., 2011; Cunningham et al., submitted). Interestingly, work by Coffin et al. (2011) suggested that Humboldt penguins (*Spheniscus demersus*) could recognize kin using olfactory cues alone, proposing a non-foraging role for olfaction in penguins. This should not be surprising, since the penguins' closest relatives, the Procellariiforms (Hackett et al., 2008), appear to use olfaction in a social context (for example Bonadonna and Sanz-Aguilar, 2012), and in nest recognition (Bonadonna et al., 2003), in addition to foraging (reviewed in Nevitt, 2008).

Nevitt (1999) first introduced the concept of an olfactory landscape, suggesting that Procellariiform seabirds are able to locate productive areas of the ocean by orienting towards scented areas of high primary productivity. Bonadonna et al. (2003) extended this concept to include the idea that the islands where seabirds nest would likewise emit odours that birds could use to navigate from long distances. These island cues likely consist of plant and animal-based compounds. In locations where there are few physical features available for orientation, the ability to detect scents associated with a colony would be particularly beneficial.

King penguins (*Aptenodytes patagonicus*) are among the most studied seabirds in the world. Past studies of their sensory biology have focused on their use of acoustic cues in individual recognition (Jouventin, 1982) or visual cues while foraging (Kooyman et al., 1992). We know little of how these birds use their chemical environment but Cunningham et al. (submitted) recently demonstrated

that King penguins are sensitive to a food-related odour. Whether they use olfaction for colony recognition or, more generally, for orientation, is unknown. To address this, we tested adult King penguins with the scent of feathers and feces that may reflect the scent of the colony or an individual.

Results

Overall there were significant differences among our three presentations (Fig. 1.; Kruskal Wallis test statistic = 10.37, d.f. = 2, $P = 0.02$). We found that birds responded significantly greater to the feces and feathers presentations as compared to the sand (Dunn test; $Z = 3.12$, $P = 0.0018$ and $Z = 2.24$, $P = 0.025$, respectively). The responses to feathers and feces were not significantly different ($Z = 0.89$, $P = 0.38$).

Discussion

King penguin adults exhibited a reaction to the scent of adult feces and feathers. Although only a first step in showing the significance of olfaction beyond foraging in this species, the implications of sensitivity to feces and feathers are suggestive in three ways. Each of the following hypotheses must be further tested to determine if, in fact, a penguin's sensitivity to feathers and feces is adaptive in each manner.

First, penguins may use the scent of feathers and feces to locate the colony or the island from a distance. Many Procellariiform seabirds are able to home to their burrow using olfactory cues. Using Y-maze experiments it has been shown that

many Procellariiforms significantly prefer an arm of a Y-maze associated with their own burrow scent over that of a conspecific (for example Bonadonna et al., 2003). The burrow odours likely are composed of feces and feathers. King penguins and Emperor penguins (*A. forsteri*), however, are unique amongst birds in that they do not have a nest. Instead, these penguins incubate and brood their young chick on their feet. Visual cues are known to be important in short distance orientation (Nesterova et al., 2009), but it is unknown how these birds locate the colony from a distance. Odours from feces and feathers, composing the overall odour of the colony, could be used by penguins searching for the colony either from land or from the sea. Since penguins are flightless, they have limited height from which to search for the colony, and scent offers a long distance cue whereby the colony can be located. Supporting this idea, at Ratmanoff, King penguin adults returning to the beach to provision young commonly arrive downwind of the colony and then walk into the wind (Cunningham pers. obs.). Additionally, displaced King penguin chicks orienting to the colony at night were only able to successfully orient when the winds blew from the direction of the colony (Nesterova et al., 2009). Using odours to locate a colony or a position in a colony may be more beneficial at some beaches compared to others. Penguins returning to Ratmanoff, which lacks any obvious topographic cues, may rely more on odours than penguins returning to La Baie du Marin on Possession Island in the Crozet archipelago, where the penguin colony is found at the base of a tall valley. Clearly, future studies investigating colony detection at a large scale should be conducted.

Secondly, King penguins may use the scent of feces and feathers to orient themselves to groups of birds within the colony. It is well established that King penguins use acoustic cues to identify their mates and their offspring at the time of provisioning (Jouventin, 1982; Lengagne et al., 1999). In general, an adult returning from the sea arrives on the beach, and makes its way to a “rendezvous zone,” near where the bird last saw its partner or chick. Returning birds begin to call within 8m of the rendezvous zone (Lengagne et al., 1999), the partner or chick replies, and the returning bird uses this reply to find its partner or chick. How a returning bird is able to find the rendezvous zone is unknown. Finding the general area of the partner or chick may be complicated because when a King penguin arrives on the beach it encounters thousands of birds of similar height which visually obstruct the zone it is trying to locate. Identifying the zone acoustically is also difficult, since the birds standing between the returning individual and the zone would both attenuate the signal, and jam it with their own vocalizations. A penguin’s specific voice is all but unrecognizable from the background noise beyond a distance of 14m (Aubin and Jouventin, 1998). Since birds tend to stay in similar groups through the life cycle on the beach, returning birds could use the odours associated with the group of birds to locate the rendezvous zone, before switching to acoustic cues to locate their partner or chick. These odours, presumably, would be made up of the scent of feces and/or feathers. In support of this, pigeons (*Columba livia*) use olfactory cues to find the general area of their loft, before switching to visual cues to identify their specific loft (Wallraff and Andreae, 2000).

Finally, sensitivity to feathers and feces may underlie the ability to recognize individuals. Coffin et al. (2011) found that Humboldt penguins were able to differentiate between kin and non-kin using odours. Other studies on Procellariiforms suggest similar abilities in the penguin's closest relatives. For example, Bonadonna and Sanz-Aguilar (2012) found that two species of petrels were able to recognize the scent of their mates, conspecifics and themselves. A sensitivity to feces and feathers in King penguins suggests that these birds, too, may be able to recognize individuals by scent. Jouventin (1982), however, reported that when King penguin adults had their bills taped closed, thus preventing them from vocalizing, they walked past their partner in the colony, highlighting the importance of acoustic cues in individual recognition. A closed bill, however, may impede a penguin's ability to smell. Clearly much remains to be studied in the area of individual recognition in this species.

While it is possible that a King penguin would respond similarly to the scent of the feces or feathers of any species of bird, this study represents a first step in understanding how a King penguin might identify elements of the colony or other conspecifics. Along with other research we have conducted (Cunnngham et al., in prep) which investigates where penguins arrive on the beach and their orientation, we are beginning to appreciate the complex olfactory world that King penguins occupy and how these sensitivities may relate to conspecific identification.

Materials and Methods

We tested 108 adult King penguins at Cape Ratmanoff, Kerguelen Island (70°33'13"E, 49°14'09"S). Here, a large colony in excess of 100,000 pairs of birds breeds during the austral summer along a flat beach. The experiments were carried out from 11 - 18 January 2015 from 0430 – 0900.

Porter et al. (1999) developed a simple technique to test the olfactory capabilities of chicken (*Gallus domesticus*) chicks. The authors found that 1-2 day old chicks, held near an incandescent lightbulb, would quickly fall into a “sleep-like” state. Odours could then be puffed onto the bird’s beaks and their responses were scored on a 0 (no response) to 3 (waking up) scale. As King penguin eggs hatch at Ratmanoff from January – April, chicks at Ratmanoff beach were too old to be tested with the Porter method in the month of January when the study was started. However, throughout the day numerous adult birds can be found naturally sleeping with the tip of their beaks beneath their wings. We thus used a modified Porter method on these sleeping birds.

To test sleeping birds we prepared three different metal rods with 30 cm of duct tape with the sticky side facing out in the following ways: 1) tape covered in beach sand that was taken from a few cm below the surface, N = 36; 2) tape rolled in feathers that had recently been moulted from nearby King penguin adults, N = 36, and; 3) tape rolled in a freshly excreted sample of adult King penguin excrement, N = 36. Similar to other studies (Cunningham et al., 2003; 2008), each odour was used to test more than one adult, though the stimuli were always exchanged for a new one within 30 min. Sleeping adults were found on the beach and one of the three

scent was presented by holding the rod 2 – 3 cm beneath their beak for 15 sec. Each bird was only tested once with one presentation. Birds were scored as follows: 0) no response; 1) slight head movements or beak claps; 2) larger twitches; 3) waking up. Responses were recorded on a Sony HDR-CX330 camcorder and later scored by an observer blind to the nature of the experiment. We used a Kruskal-Wallis test to compare overall differences in the mean score to each stimulus. We then compared the responses of each odour to another using a Dunn test.

Acknowledgements

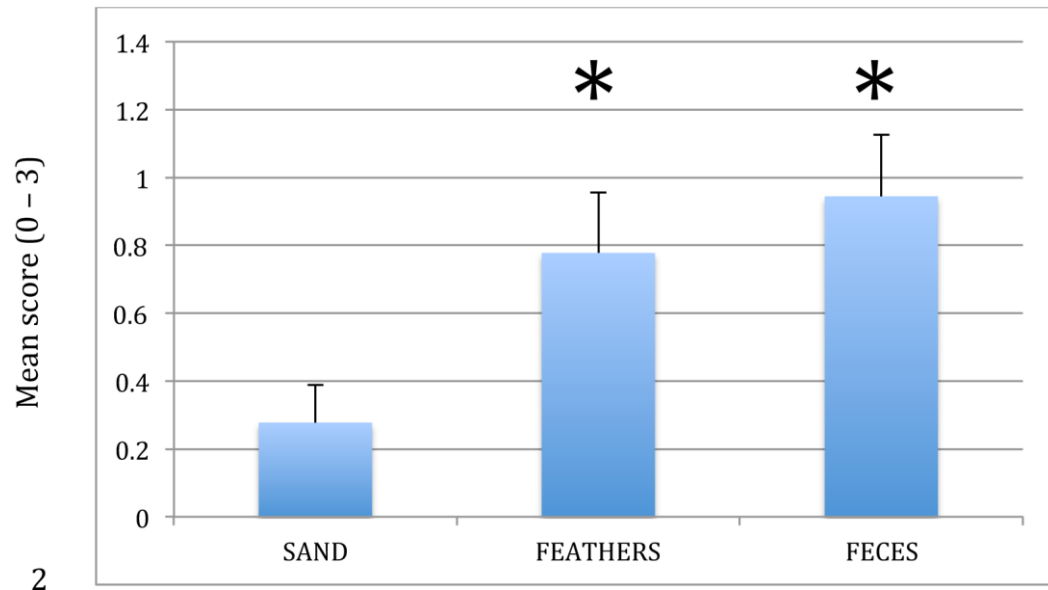
We thank Dr. I. Keddar for assistance in the field, and Dr. F.S. Dobson and Dr. K. Bonner for reviewing the manuscript. We also thank the Institut Polaire Français Paul-Emile VICTOR (IPEV) for logistic support. This study was performed in accordance with IPEV and CNRS guidelines for the Ethical Treatment of Animals. The research was funded by an IPEV grant (ETHOTAAF 354) to F.B.

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Fig. 1. Mean responses (with S.E.M.) of sleeping adult King penguins to presentations of sand, feathers and feces. The response to the odour of feces and feathers were significantly greater than to sand (* $P \leq 0.05$)