

## SHORT COMMUNICATION

## King penguins can detect two odours associated with conspecifics

Gregory B. Cunningham<sup>1,\*</sup> and Francesco Bonadonna<sup>2</sup>

## ABSTRACT

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 had a significantly greater response to the feathers and feces presentations 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*).

**KEY WORDS:** King penguin, Olfaction, Conspecific odours

## INTRODUCTION

Penguins have acute odour recognition of food-related odours and are likely to use these odours to aid in foraging (Cunningham et al., 2008; Wright et al., 2011; G.B.C. and F.B., unpublished results). Interestingly, work by Coffin et al. (2011) suggested that Humboldt penguins (*Spheniscus demersus*) could recognize kin using olfactory cues alone suggesting 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 (e.g. Bonadonna and Sanz-Aguilar, 2012) and in nest recognition (Bonadonna et al., 2003b), 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. (2003a) 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 probably 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* Miller 1778) 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 we recently demonstrated that King penguins are sensitive to a food-related odour (G.B.C. and F.B., unpublished results). 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.

## 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 to 18 January 2015 from 04:30 h to 09:00 h.

Porter et al. (1999) developed a simple technique to test the olfactory capabilities of chicken (*Gallus domesticus*) chicks. The authors found that 1- to 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. Although King penguin eggs at Ratmanoff can hatch in January, none hatched while we were there. Chicks that had hatched during January to April 2014 were too old to be tested with the Porter method in the month of January 2015 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, although the stimuli were always exchanged for a new one within 30 min. Sleeping adults were found on the beach and one of the three scents was presented by holding the rod 2–3 cm beneath their beak for 15 s. 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.

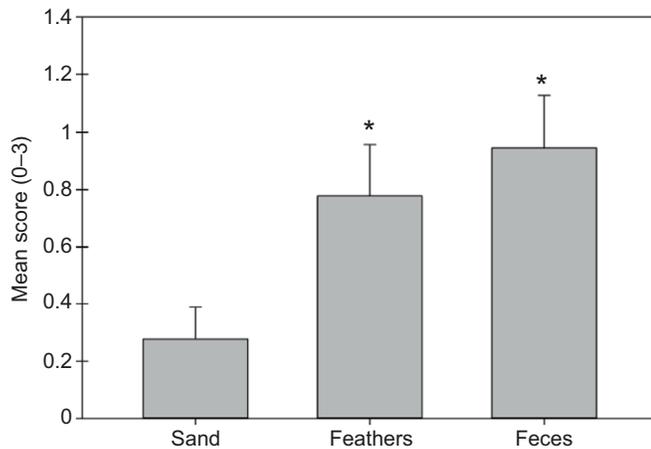
## RESULTS AND DISCUSSION

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 had a significantly greater response to the feces and feathers presentations compared with 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 ( $Z=0.89$ ,  $P=0.38$ ).

King penguin adults exhibited a reaction to the scent of adult feces and feathers. Although only a first step in showing the

<sup>1</sup>Department of Biology, St John Fisher College, 3690 East Avenue, Rochester, NY 14618, USA. <sup>2</sup>Centre d'Ecologie Fonctionnelle et Evolutive CEFE, UMR 5175, CNRS, Université de Montpellier, Université Paul-Valéry Montpellier, EPHE, 1919 Route de Mende, 34293 Montpellier Cedex 5, France.

\*Author for correspondence (gcunningham@sjfc.edu)



**Fig. 1. Responses of sleeping adult King penguins to presentations of sand, feathers and feces.** Values are means  $\pm$  s.e.m. The response to the odour of feces and feathers were significantly greater than to sand ( $*P \leq 0.05$ ).

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 case.

Firstly, 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., 2003b). The burrow odours are probably composed of feces and feathers. King penguins and Emperor penguins (*Aptenodytes 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 (G.B.C., unpublished results). Additionally, displaced King penguin chicks orienting to the colony at night are only able to successfully orient when the winds blow 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 with 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 8 m 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 that 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 14 m (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, would presumably 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 (G.B.C. and F.B., unpublished results), 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.

#### 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-Émile Victor (IPEV) for logistic support. This study was performed in accordance with IPEV and CNRS guidelines for the Ethical Treatment of Animals.

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

G.B.C. designed the experiment, supervised the analysis of the videos, analysed the data and drafted the manuscript. F.B. revised the manuscript prior to final submission.

#### Funding

The research was funded by an Institut Polaire Français Paul-Émile Victor (IPEV) grant (ETHOTAAF 354) to F.B. A grant to G.B.C. from St John Fisher College paid for travel expenses.

## References

- Aubin, T. and Jouventin, P.** (1998). Cocktail-party effect in King penguin colonies. *Proc. R. Soc. Lond. B Biol. Sci.* **265**, 1665-1673.
- Bonadonna, F. and Sanz-Aguilar, A.** (2012). Kin recognition and inbreeding avoidance in wild birds: the first evidence for individual kin-related odour recognition. *Anim. Behav.* **84**, 509-513.
- Bonadonna, F., Benhamou, S. and Jouventin, P.** (2003a). Orientation in "featureless" environments: the extreme case of pelagic birds. In *Avian Migration* (ed. P. Berthold, E. Gwinner and E. Sonnenschein), pp. 367-377. Berlin: Springer.
- Bonadonna, F., Cunningham, G. B., Jouventin, P., Hesters, F. and Nevitt, G. A.** (2003b). Evidence for nest-odour recognition in two species of diving petrel. *J. Exp. Biol.* **206**, 3719-3722.
- Coffin, H. R., Watters, J. V. and Mateo, J. M.** (2011). Odor-based recognition of familiar and related conspecifics: a first test conducted on captive Humboldt penguins (*Spheniscus humboldti*). *PLoS ONE* **6**, e25002.
- Cunningham, G. B., Van Buskirk, R. W., Bonadonna, F., Weimerskirch, H. and Nevitt, G. A.** (2003). A comparison of the olfactory abilities of three species of procellariiform chicks. *J. Exp. Biol.* **206**, 1615-1620.
- Cunningham, G. B., Strauss, V. and Ryan, P. G.** (2008). African penguins (*Spheniscus demersus*) can detect dimethyl sulphide, a prey-related odour. *J. Exp. Biol.* **211**, 3123-3127.
- Hackett, S. J., Kimball, R. T., Reddy, S., Bowie, R. C. K., Braun, E. L., Braun, M. J., Chojnowski, J. L., Cox, W. A., Han, K.-L., Harshman, J. et al.** (2008). A phylogenomic study of birds reveals their evolutionary history. *Science* **320**, 1763-1768.
- Jouventin, P.** (1982). *Visual and Vocal Signals in Penguins, Their Evolution and Adaptive Characters*. Berlin: Verlag Paul Parey.
- Kooyman, G. L., Chereh, Y., LeMaho, Y., Croxall, J. P., Thorson, P. H., Ridoux, V. and Kooyman, C. A.** (1992). Diving behavior and energetics during foraging cycles in king penguins. *Ecol. Monogr.* **62**, 143-163.
- Lengagne, T., Jouventin, P. and Aubin, T.** (1999). Finding one's mate in a King penguin colony: efficiency of acoustic communication. *Behavior* **136**, 833-846.
- Nesterova, A. P., Mardon, J. and Bonadonna, F.** (2009). Orientation in a crowded environment: can King Penguin (*Aptenodytes patagonicus*) chicks find their crèches after a displacement? *J. Exp. Biol.* **212**, 210-216.
- Nevitt, G.** (1999). Foraging by seabirds on an olfactory landscape. *Am. Sci.* **87**, 46-53.
- Nevitt, G. A.** (2008). Sensory ecology on the high seas: the odor world of the procellariiform seabirds. *J. Exp. Biol.* **211**, 1706-1713.
- Porter, R. H., Hepper, P. G., Bouchot, C. and Picard, M.** (1999). A simple method for testing odor detection and discrimination in chicks. *Physiol. Behav.* **67**, 459-462.
- Wallraff, H. G. and Andrae, M. O.** (2000). Spatial gradients in ratios of atmospheric trace gases: a study stimulated by experiments on bird navigation. *Tellus B* **52**, 1138-1157.
- Wright, K. L. B., Pichegru, L. and Ryan, P. G.** (2011). Penguins are attracted to dimethyl sulphide at sea. *J. Exp. Biol.* **214**, 2509-2511.