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ABSTRACT

Most studies on avian olfactory communication have focused on mate choice, and the importance of olfaction in subsequent nesting stages has been poorly explored. In particular, the role of olfactory cues in egg recognition has received little attention, despite eggs potentially being spread with parental odorous secretions known to elicit individual discrimination. Here, we used behavioral choice tests to determine whether female blue petrels (*Halobaena caerulea*) can discriminate the odor of their own egg from the odor of a conspecific egg. Females preferentially approached the odor of their own egg, suggesting that blue petrels can recognize their own egg using odor cues. This finding raises the question of the adaptive value of this mechanism, and may inspire further research on odor-based egg discrimination in species suffering brood parasitism.

KEY WORDS: Olfactory communication, Smell, Birds, Nesting

INTRODUCTION

Compared with visual and acoustic cues, the role of olfactory cues in avian behavior has long been neglected. In the last decade, however, birds have been shown to use odor cues to communicate with their conspecifics (for review, see Caro et al., 2015). For instance, spotless starlings (*Sturnus unicolor*) recognize the sex of conspecifics by olfaction (Amo et al., 2012a), Antarctic prions (*Pachyptila desolata*) are attracted to their mate's odor (Bonadonna and Nevitt, 2004), and mallard (*Anas platyrhynchos*) and chicken (*Gallus gallus domesticus*) males with experimentally reduced olfaction have altered sexual behavior (Balthazart and Schoffeniels, 1979; Hirao et al., 2009). However, most studies on avian olfactory communication have focused on mate choice (e.g. Amo et al., 2012b; Caspers et al., 2015; Leclaire et al., 2014; Whittaker et al., 2013), and whether olfaction is critical in subsequent nesting stages has been poorly investigated.

Brood parasitism is widespread in diverse taxa including birds (reviewed in Rohwer and Freeman, 1989; Yom-Tov, 2001), and may have led to the evolution of hosts adopting defenses based on egg recognition and rejection of the parasite egg (Rothstein, 1990). Most studies on egg recognition have investigated the role of visual cues (Soler and Møller, 1996; Spottiswoode and Stevens, 2010), while the role of olfactory cues has received little attention. However, eggs vary in odor, which can be emitted by the developing embryo itself (Webster et al., 2015) or from substances that are transferred onto the egg surface during incubation. In birds, individual odors are thought to originate mainly from preen gland secretions (Mardon et al., 2011)

that are spread onto the plumage during preening. These secretions encode information on bird species, identity, sex and kinship (Leclaire et al., 2012; Mardon et al., 2010; Whittaker et al., 2010), and can be transferred from the parent to the egg through passive or active spreading (Martín-Vivaldi et al., 2014). Because in several avian species individuals assess preen oil odor to discriminate between conspecifics (Amo et al., 2012a; Coffin et al., 2011; Whittaker et al., 2011), they could also be able to discriminate their own eggs from other eggs using olfactory cues. This has been shown in zebra finches (*Taeniopygia guttata*), where females are able to discriminate between their own eggs and a conspecific egg based on olfactory cues alone (Golüke et al., 2016). There is also one example of interspecific odor-based egg discrimination in dark-eyed juncos (*Junco hyemalis*), where females spend less time incubating their eggs when the eggs are spread with the preen oil of a male from another passerine species (Whittaker et al., 2009). More studies examining egg odor recognition in birds may shed additional light on the potential role of olfaction in regulating parental care in birds, and indicate how widespread this ability is across the phylum.

Here, we studied egg-odor recognition in the blue petrel, *Halobaena caerulea* (Gmelin 1789), a species with a particularly good sense of smell, where individuals are known to recognize their own odor, the odor of their partner (Mardon and Bonadonna, 2009) and the odor of their nest (Bonadonna et al., 2004). As eggs are likely to smell of a mixture of the odor of the incubating parents, the nest environment and possibly the developing embryo, we expected blue petrels to also discriminate the odor of their egg from the odor of a conspecific egg.

MATERIALS AND METHODS

Study site

This study was conducted on a small sub-Antarctic island (Ile Verte, 49°51′S, 70°05′E) in the Kerguelen Archipelago in October–December 2015. The blue petrel is a common burrow-nesting species in this region, and a study colony of about 80 burrows has been followed since 2001 on this island. Burrows are fitted with a closable aperture over the incubating chamber to facilitate capture. During egg incubation, partners alternate incubation shifts, relieving each other from the nest every 8–12 days (Warham, 1996). We performed the experiment on 24 females while they were brooding their single egg. Females were tested between 9 and 21 days after egg laying (mean±s.e.m.: 17±1 days). In blue petrels, egg laying is highly synchronized (Fugler et al., 1987; Jouventin et al., 1985), and the mean age difference between two eggs in a given test was 0±1 days (range: −7 to +8 days). All conspecific eggs tested (*n*=21) were brooded by their female parent at the time of the test. This study was performed according to guidelines established by IPEV and CNRS for the ethical treatment of animals and complied with current French regulations.

Y-maze experiment

We presented petrels with a binary choice in a plastic Y-maze made from standard PVC wire. The maze had three symmetrical arms

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(arm length, 60 cm; width, 12 cm; height, 11 cm). The angle between two arms was ~120 deg. One arm was used as starting point and was fitted with two trap doors (30 cm apart) to provide a temporary holding compartment for the test bird. The end of each choice arm was equipped with a separate compartment for the odor source (i.e. an egg), also accessible via a trap door to the outside. Eggs were placed onto a small cup made of aluminium foil. A second partition was positioned 20 cm from each end and equipped with a central processing unit cooling fan (Akasa®, model: DFS501012M) to provide low-noise and controlled airflow ($267 \, l^{-1} \, min^{-1}$) through each choice arm. Thus, birds did not have direct access to the egg but received scented air flowing at a constant rate. The maze was washed after each trial with 96% ethanol to remove any odor residue. The allocation of the bird's own or the conspecific egg to either choice arm changed randomly between trials to eliminate possible bias between the choice arms themselves or their relative positions with respect to the colony, shoreline or other environmental features. The observer was not the same person as the one placing the eggs in the maze so that behavior was recorded blind to treatment. Tested eggs and birds were removed from their nest just before the test and were put back into their nest just after the test, so that each egg or bird was away from the nest for less than 25 min. Once returned to the nest after the experiment, the birds immediately resumed warming the egg. Time spent in each arm of the maze was easily assessed by the noise of the bird walking in the maze. Birds that never entered either choice arm and usually sat calmly in the holding compartment were reported as no-choice birds.

Although the inside of the maze was dark, one might argue that birds could potentially see the eggs through the fans. For birds to recognize their own eggs based on color, intraspecific variation in egg coloration would be expected (Kilner, 2006). By contrast, blue petrels lay monomorphic white eggs in dark burrows. A possible impact of acoustic cues on egg recognition also seems unlikely in our experiments carried out during the first half of incubation when embryos do not appear to vocalize (Gottlieb and Vandenbergh, 1968; Rumpf and Tzschentke, 2010). We therefore believe that our experiment tested primarily olfactory recognition rather than visual or acoustic recognition.

Statistics
We used binomial tests to assess female choice as defined by the first Y-maze arm visited by the bird. We also used a paired t-test to assess the difference between the time spent in the arm containing the bird's own egg and the time spent in the arm containing the conspecific egg. We also used Kruskall–Wallis tests to determine whether female choice varied with (i) the age of her own egg and (ii) the age difference between her own egg and the conspecific egg. The effects of these two variables were also tested on the difference between the time spent in the arm containing the female's own egg and the arm containing the conspecific egg, using linear models. Tests were performed with R software (www.R-project.org/). We used two-tailed tests with a significance level set to $\alpha=0.05$.

RESULTS AND DISCUSSION
When incubating females were given the choice between their own and a foreign egg, 17 out of 24 (71%) made a choice. Among them, 14 females approached their own egg first, while three females approached the foreign egg first (binomial test: $\chi^2=7.12$, $P=0.008$; Fig. 1). The average time spent in the arm containing the female's own egg was higher than the time spent in the arm containing the foreign egg (mean±s.e.m.: 5.7±0.9 versus 2.0±0.5 min, $t_{1,16}=3.11$, $P=0.007$; Fig. 2).
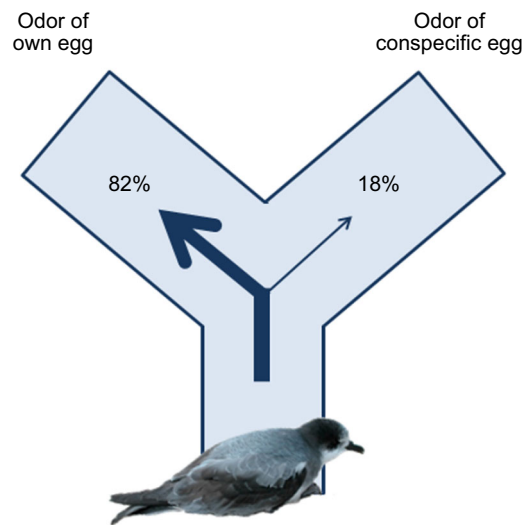
Fig. 1. Odor discrimination of eggs. Blue petrel females (*n*=17) were challenged to choose either the odor of their own egg or the odor of a conspecific egg in Y-maze tests. The percentage of females that chose the arm containing the odor of their own egg or that of the conspecific egg first is shown. Binomial test: $\chi^2=7.12$, $P=0.008$.

Recent evidence shows that egg odor can vary with embryo sex, fertility and development (Costanzo et al., 2016; Webster et al., 2015). However, the ability of birds to assess information encoded by egg odor has scarcely ever been investigated. We provide, therefore, among the first evidence that a bird species can discriminate between the odor of their egg and the odor of a conspecific egg (see Golüke et al., 2016, for evidence in zebra finches).

This finding raises the question of the adaptive benefits of egg odor discrimination in blue petrels. In several bird species, females lay
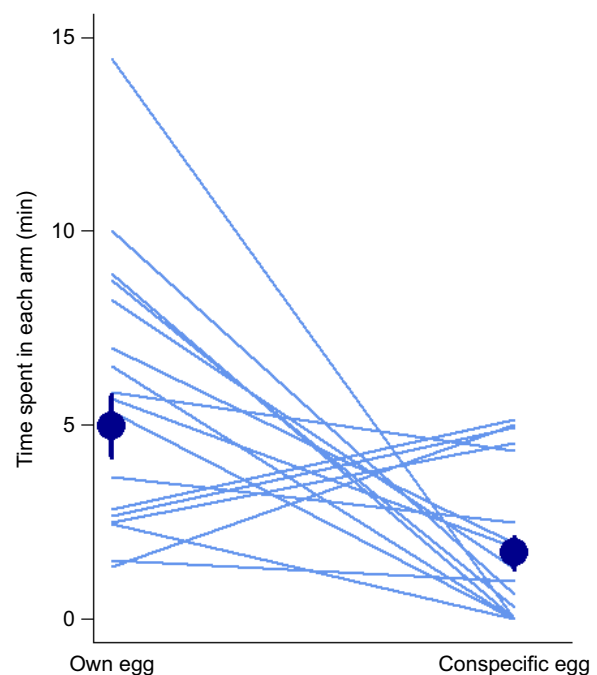
Fig. 2. Results of odor choice test. The time spent by each blue petrel female (*n*=17) in the arm containing the odor of their egg or the odor of a conspecific egg. Data are means±s.e.m., t-test: $t_{1,16}=3.11$, $P=0.007$.

eggs in the nest of conspecific individuals which then care for the eggs and the offspring (Rohwer and Freeman, 1989). Because incubation and parental care are costly, hosts may have undergone selection to develop egg recognition and rejection to avoid providing care to foreign eggs (Lyon and Eadie, 2008). Accordingly, in a several species, including American coots (*Fulica Americana*; Lyon, 2003), house sparrows (*Passer domesticus*; López-de-Hierro and Moreno-Rueda, 2010), royal terns (*Sterna maxima maxima*; Buckley and Buckley, 1972) and ostriches (*Struthio camelus*; Bertram, 1979), parents recognize and reject parasitic conspecific eggs.

Conspecific brood parasitism in blue petrels has never been studied in detail. However, every year, a few blue petrel nests (ca. 1% of nests; S.L., V.B. and F.B., personal observations) are occupied by two breeding pairs, the two pairs each laying an egg usually a few days apart while the other pair is foraging at sea, neglecting its egg. Only one individual at a time occupies the nesting burrow, and incubates only one of the eggs. Detailed observation of two 2-egg nests have shown that blue petrel parents do not incubate their own egg exclusively, but rather seem to incubate one randomly, leading to the hatching failure of all eggs of the clutch (S.L., V.B. and F.B., personal observations). As parasitic eggs apparently fail to hatch, conspecific brood parasitism does not appear to have evolved as a viable strategy to reduce investment in parental care in blue petrels. The few cases of apparent parasitism may instead come from squatter parents that did not manage to secure their own burrow. As brood parasitism is infrequent in blue petrels, its costs may be insufficient at the population level to select for egg rejection (Rothstein, 1990).

After a foraging trip, blue petrels return to the colony at night, locating their burrow using olfaction (Bonadonna et al., 2001). The burrow-derived odor cues that drive blue petrels towards their nest are unknown, although individual olfactory signatures of nest partners may play a role (Bonadonna et al., 2004). However, egg neglect is common in blue petrels (in 46% of the observed changeovers; Chaurand and Weimerskirch, 1994), and returning parents then need to locate an empty burrow occupied by the egg only. Egg-odor recognition may, therefore, have evolved to increase burrow localization efficiency in this nocturnal species. Alternatively, as part of egg odor probably originates from the nest environment, egg-odor recognition could be a by-product of burrow-odor recognition. Chemical analyses of egg odor in blue petrels are warranted to determine whether egg odor mainly originates from the embryo itself or from transfer of odorous substances by brooding parents and the nest.

Apparent failure to recognize a foreign egg in a natural context despite the ability to do so in an experimental setting may result from differences in egg age. Egg volatiles are known to vary with embryo developmental stage (Webster et al., 2015). While zebra finches discriminate the odor of their own egg from the odor of a conspecific egg when eggs are 10 days old, they do not do so when eggs are 3 days old (Golüke et al., 2016). Egg odors have therefore been suggested to be insufficiently developed shortly after laying to trigger odor-based recognition (Golüke et al., 2016). In blue petrels, when a resident female arrives in a nest just after a squatter female has laid an egg, the odor of the newly laid eggs may not yet have sufficient intensity or characteristic compounds to trigger egg discrimination. Afterwards, odorants from the parents and the environment may be transferred to the natural and foreign eggs, which then smell alike, leading to parents failing to recognize their own egg. Parents may then learn the developing odor of the two eggs concurrently so that they are not able to recognize their own egg after odor development. These hypotheses are congruent with

zebra finches not being able to recognize as foreign a conspecific newly laid egg that has been placed in their nest for 10 days (Golüke and Caspers, 2016). In our experiments, age differences between the two test eggs did not affect qualitative female choices (χ^2=1.20, P=0.55) or the time spent in the arm containing the female's own versus the conspecific egg (F=0.19, P=0.66).

As a side note, a delay after the female laid her own egg (i.e. egg age) did not affect the time spent in the arm containing her own versus the conspecific egg either (F=0.27, P=0.61), while it seemed to have a marginally non-significant effect on female choice (χ^2=5.71, P=0.058). This experiment was not designed to address this specific question, however, and hence the sample was inadequate to confidently assess the effect of embryo development stage on odor recognition. Choice experiments carried out over a broader range of times after laying will be necessary to clarify whether egg recognition by females varies with embryo age.

In conclusion, we found that blue petrel females discriminate the odor of their egg from that of a conspecific egg. Although this ability does not seem to be used in the context of conspecific brood parasitism in this species, this remains to be confirmed by a more systematic analysis of nests harboring two different breeding pairs. This report may also inspire further studies on the role of olfaction in egg rejection in species suffering brood parasitism.

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