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Short report

Blue tits (*Cyanistes caeruleus*) respond to an experimental change in the aromatic plant odour composition of their nest

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ABSTRACT

Although the use of olfaction by birds is now widely recognised, the olfactory abilities of passerine birds remain poorly explored, for historical reasons. Several studies however suggest that passerines can perceive volatile compounds in several biologically relevant contexts. In Corsica, recent findings suggest that cavity-nesting blue tits may use volatile compounds in the context of nest building and maintenance. Although they build their nests mainly from moss, female blue tits also frequently incorporate fragments of several species of aromatic plants in the nest cup. In field experiments, breeding female blue tits altered their nest maintenance behaviour in response to experimental addition of aromatic plants in their nest. In aviary experiments, captive male blue tits could be trained to detect lavender odour from a distance. Here I report results from a field study aimed to test whether adult blue tits altered their chick-feeding behaviour after an experimental change in nest odour composition. I experimentally added fragments of aromatic plant species that differed from those brought in the nests before the start of the experiment in a set of experimental nests and added moss, the basic nest material, in a set of control nests. Both male and female blue tits hesitated significantly longer entering the nest cavity after addition of new aromatic plant fragments, as compared to moss addition. This response was especially observed during the first visit following the experimental change in nest plant composition. Nest composition treatment had no effect on the time spent in the nest. This study demonstrates that free-ranging blue tits detect changes in nest odour from outside the nest cavity.

1. Introduction

After having long been debated, the use of olfaction by birds is now widely recognised. An increasing number of studies demonstrate that birds of various orders use well-developed olfactory capacities in biologically relevant contexts (e.g. Bonadonna and Nevitt, 2004; Roper, 1999; Wallraff, 2004). In passerine birds however, the use of olfaction remains poorly explored because these birds, which have a very small relative olfactory bulb size, have historically been considered as having weak olfactory abilities (Bang and Cobb, 1968). Yet several studies suggest that passerine birds can perceive volatile compounds (e.g. Mäntilä et al., 2004; Kelly and Marples, 2004), especially passerine species that add fresh plant fragments into their nests (Clark and Mason, 1987; Petit et al., 2002). On Corsica, females of the cavity-nesting blue tit *Cyanistes caeruleus* daily add fresh fragments of aromatic plants into their nests during the whole nesting period (Lambrechts and Dos Santos, 2000). Each female blue tit brings an individual-specific set of aromatic plant species to her nest (Mennerat, unpublished data). After experimental removal of aromatic plants, they rapidly replenish their nest with fragments of the same species (Petit et al., 2002; Mennerat unpublished data). The results found by Petit et al. (2002) suggest that they are able to perceive variations in concentration of plant volatile compounds inside the nest cavity. In addition, conditioning experiments provided evidence that male blue tits use olfaction in a food-searching, non-reproductive context (Mennerat et al., 2005). Whether blue tits can detect nest odours from outside the nest cavity in the wild remains so far unknown. Here I present results from an experiment testing whether free-ranging, reproducing blue tits can detect a change in the aromatic odour composition of their nests. I predicted that blue tit parents, if sensitive to nest odour, would adopt a cautious behaviour when detecting an experimental change in the aromatic composition of their nest. This behaviour could be expressed either in a longer hesitation before entering the nest cavity and/or a shorter stay in the nest cavity. Since only females select and add aromatic plants into

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their nests (Petit et al., 2002), I expected them to be more affected than males by the experiment. Finally, I expected that birds would become accustomed to their new aromatic environment after a while, so that their response would decrease over successive visits following the experimental change in nest composition.

2. Materials and methods

2.1. Preliminary determination of the aromatic composition of nests

The study was carried out in the Muro valley in Corsica where blue tits accept nest boxes for breeding (for a description of the sites, see Lambrechts et al., 2004). From the onset of nest building onwards, I visited nests every third day and noted the presence or absence of the five aromatic plant species most frequently used by blue tits at the Muro site (*Lavandula stoechas*, *Helichrysum italicum*, *Achillea ligustica*, *Mentha suaveolens*, *Pulicaria odora*; Petit et al., 2002). These five species represent 86% of the amount of plant fragments found in nests at this study site (Mennerat, unpublished data) and can easily be identified from morphological characteristics. Since none of the nests under study naturally contained all five species prior to the experiment, manipulation of nest aromatic composition consisted in adding only those aromatic plant species that were missing among these five. These plant species have distinctive odour profiles, as revealed by gas chromatography on volatiles released by plants (Lambrechts and Hossaert-McKey, 2006; Petit, unpublished data).

2.2. Experimental manipulation of aromatic plants in nests

The experiment was carried out in a sample of 23 nests. In each nest, two treatments were applied in a random order in two consecutive mornings (days 9 and 10 post-hatching). The "aromatic" treatment consisted in adding a total amount of one gram of fresh leaves of the aromatic plant species previously missing in nests (see above), in equal proportions. The amount of aromatic plants added in nests was therefore constant across nests. This amount lies within the natural range of fresh aromatic plant fragments daily added by blue tits (see Mennerat et al., 2008). Fresh leaves were chopped into fragments of the same size than those naturally added into nests by blue tits (approximately 1 cm × 1 cm, personal observation). Plant fragments were hidden between the nest itself and the wall of the nestbox, so that birds could not see them. One gram of fresh moss (main nest material) was added in control "moss-treated" nests. The moss species used in this experiment was collected at one unique place, therefore can be considered as a homogeneous category. A great care was taken in using the same procedure in control moss-treated nests as in aromatic-treated nests. Both treatments were preceded by observation from a distance (>20 m). To reduce disturbance to the birds, nestboxes were opened to add aromatic plants or moss only after both parents had left the nest and flied away.

2.3. Behavioural observations

All observations were performed at more than 20 m from the nestboxes. During observation, the observer (A. Mennerat or P.-A. Bernier) was hidden under a camouflage tarpaulin or, when available, behind natural shelters (e.g. rocks, tree trunks). "Hesitation time" was defined as the time spent between the first physical contact of a bird with the nestbox and the time it entered the cavity to feed the chicks (i.e. totally disappeared inside the nestbox). With a stopwatch, the observer recorded both the hesitation time and the time spent in the nest cavity for the first three visits of each parent after treatment. Parents were ringed in previous years (males on the right tarsus and females on the left tarsus), which allowed individual recognition during the experiment. No bird was captured less than 6 months before the experiment. Sex was subsequently confirmed when birds were trapped at the end of the breeding season (for a description of field protocols, see e.g. Blondel, 1985). The observers (A.M. or P.-A.B.) were both non-smokers and did not use perfumes. At the start of the experiment, they made several observations together, to ensure that their observations were concordant. At each particular nestbox, observations for both treatments were performed by the same person (either A.M. or P.-A.B.), who was therefore not blind regarding the treatment. After each observation, all experimentally added materials (plants or moss) were removed from nests.

2.4. Statistical analyses

Hesitation time and time spent in the nest were square-root transformed prior to analyses to meet assumptions of normality. Since male and female behaviours in a pair are not independent from each other, mixed-effects models were performed with nest as random factor. Treatment, sex and visit order (first, second or third visit to the nest) were included as fixed factors. Since behaviour may differ according to sex or visit order, I also tested the 'treatment × sex' and 'treatment × visit order' interactions. Models were fit by maximum log-likelihood with the R version 2.6.0 software.

3. Results and discussion

Birds hesitated significantly longer to enter the nest cavity under the aromatic treatment than under the moss control treatment ($P < 0.05$). Hesitation time rapidly decreased over successive visits ($P < 0.0001$) (Fig. 1). Time spent into the nest was not affected by treatment ($P = 0.78$), but was longer at the first visit ($P < 0.05$). Altogether, this experiment provides evidence that free-living adult blue tits detect aromatic compounds from outside the nest cavity, as expressed in a longer time spent before entering the nestbox when new aromatic plants were added.

Females spent longer time spans in the nest than males ($P < 0.0001$) (Table 1). This is consistent with other studies of parental behaviour in the blue tit, showing that females – not males – perform nest sanitation in addition to chick feeding during their stay in the nest (e.g. Hurtrez-Boussès et al., 2000).

Interestingly, the significant effect of aromatic odour manipulation was only detected during the first visit following the experimental treatment (Fig. 1), which indicates that blue tits

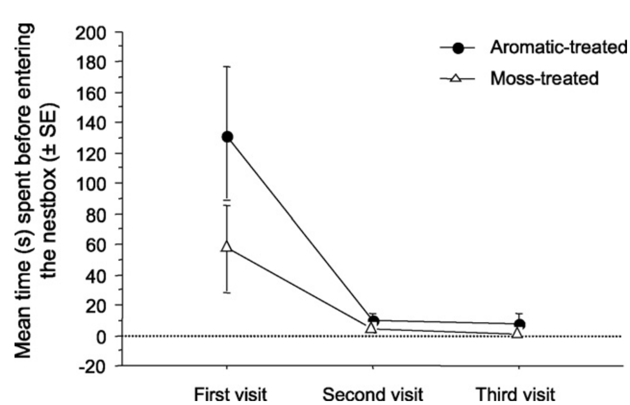

Fig. 1. Mean time (±S.E.) spent by adult blue tits before entering their nestbox in aromatic-treated (addition of new aromatic plant species) and moss-treated control nests (addition of moss), over successive visits after manipulation.

Table 1
Effect of addition of new aromatic plants in nests on the time spent before entering the nestbox (hesitation time) and the time spent in nest, as tested by mixed-effects models (see Section 2)

	d.f.	Hesitation time		Time spent in nest	
		LR	P	LR	P
Treatment	1	4.84	0.03	0.08	0.78
Sex	1	0.86	0.35	46.79	<0.0001
Visit order	2	30.54	<0.0001	9.00	0.01
Treatment × sex	1	0.29	0.59	0.16	0.69
Treatment × visit order	2	3.23	0.20	1.78	0.41

d.f.: degrees of freedom; LR: likelihood ratio.

quickly habituated to the new aromatic odour of their nests. This is useful in interpreting previous studies which failed to demonstrate a significant effect of experimentally added fresh plants on nestling growth or body condition (e.g. Clark and Mason, 1988; Dawson, 2004; Mennerat et al., 2008). Although one cannot exclude that other factors, such as favourable environmental conditions, may have masked positive effects of plants on chick growth or condition in these studies (Mennerat et al., 2008), at least it is likely that experimental addition of fresh plants in nests has no durable impact on parental feeding behaviour.

Amo et al. (2008) found that adult blue tits feeding chicks delayed their entry in the nestbox after detecting a predator scent in it. This study shows that blue tits respond in a similar way after detecting a change in the aromatic plant composition of their nest, which suggests that aromatic nest odour composition is significant to blue tits. The biological meaning of nest aromatic odour, however, remains hypothetical. Contrary to my predictions, both sexes responded in a similar way to the experiment, although only females naturally add aromatic plants to their nests. This supports former observations in captive blue tits that males can perceive aromatic odours at a distance from their source (Mennerat et al., 2005). Blue tits probably mainly use vision to orientate and it seems unlikely that they find their nests by smell, as demonstrated in other bird species (e.g. Bonadonna et al., 2003, 2004). Recent findings however indicate that the aromatic plant species composition of nests in this population is both variable across nests and strongly consistent over time for each individual female (Mennerat et al., submitted). Therefore we do not exclude that aromatic plants may be used as an olfactory signature of the nesting cavity, giving information about the identity of its occupants to other individuals in the population.

The blue tit is the only tit species in our nestbox study sites that exploits fresh plants during reproduction. Neither great tits *Parus major* nor coal tits *P. ater* have been found to add such plants in their nests (personal observation). To our knowledge, the sensitivity of other non-greenery using passerines to nest odour remains so far unstudied. Similar experiments in other bird species breeding in nestboxes (e.g. the great tit) would be useful in addressing this issue. Obviously, further studies are needed to investigate how precisely passerines discriminate the volatile compounds emanating from their nests and what biological meaning it may have to these birds.

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