

Predator odour recognition and avoidance in a songbird

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Summary

1. Although the ability to detect chemical cues is widespread in many organisms, it is surprising how little is known about the role of chemical communication in avian life histories. Nowadays, growing evidence suggests that birds can use olfaction in several contexts. However, we still do not know the role of bird olfaction in one of the most important determinants of survival, predator detection.
2. Blue tits, *Cyanistes caeruleus* L., were exposed to chemical cues of: (i) mustelid (predator), (ii) quail (odorous control); or (iii) water (odourless control) inside the nest-box where they were provisioning 8-day-old nestlings.
3. We show that blue tits were able to detect the chemical cues and showed antipredatory behaviours to cope with the risk of predation. Birds delayed their entry to the nest-box, and they perched on the hole of the nest-box and refused to enter more times when they found predator scent than control scents inside the nest-box. In addition, birds decreased the time spent inside the predator-scented nest-box when feeding nestlings.
4. The discovery of the ability of birds to use chemical cues of predators to accurately assess predation may help to understand many aspects of bird life histories that have been neglected until now.

Key-words: avian olfaction, chemical ecology, *Cyanistes caeruleus*, predation risk assessment, provisioning rates

Introduction

The importance of chemical communication in different contexts of an organism's life has been extensively studied for a great number of taxa, including invertebrates and vertebrates such as reptiles or mammals. However, in the case of birds, it has been historically neglected (Kats & Dill 1998). Nowadays, it has been shown that birds can use odours in several ecological contexts and with different functions (for a review see Roper 1999; Hagelin 2007; Hagelin & Jones 2007), such as individual recognition (Bonadonna & Nevitt 2004), discrimination of aromatic plants (Clark & Mason 1987; Petit *et al.* 2002; Menerat *et al.* 2005), orientation and navigation (Bonadonna *et al.* 2004; Wallraff 2004; Nevitt & Bonadonna 2005), and foraging (Hutchison & Wenzel 1980; Nevitt, Veit & Kareiva 1995; Marples & Roper 1996; Kelly & Marples 2004). Despite the increasing evidence of olfactory abilities in birds, the role of olfaction in one of the most important determinants of an animal survival, predator avoidance, currently remains unknown in birds (Kats & Dill 1998; Hagelin 2007).

For numerous prey species, the ability of ascertaining predators is an important component of antipredatory behaviour. The importance of chemical cues for predator recognition has been documented in a number of taxa, but not in birds (Kats & Dill 1998; Hagelin 2007). To our knowledge, only three previous studies have analysed the ability of birds to use the chemical cues of predators to assess predation risk. The first study analysed the response of domestic chickens of different ages to cat odour, finding that only 7-day-old chickens avoided the cat odour, whereas such avoidance response was not observed when chickens were 4 or 10 days old (Fluck *et al.* 1996). The recent study of Godard, Bowers & Wilson (2007) showed that eastern bluebirds (*Sialia sialis*) do not avoid building the nest in nest-boxes containing scent of two different types of predators. Hagelin, Jones & Rasmussen (2003) exposed crested auklets (*Aethia cristatella*), a burrow nesting species, to a mixture of mammalian odours and found an avoidance response. However, due to the artificial nature of the compound and the lack of other odorous control, the authors could not differentiate whether the aversion response was because birds found the scent new, unpleasant or the odour was indicative of a predator (Hagelin *et al.* 2003).

The use of chemical cues of predators for assessment of predation risk could be important in many bird species, especially in those that, during part of their lifetime use habitats in which visual detection of predators is constrained. This is the

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case of hole-nesting birds such as the blue tit *Cyanistes caeruleus* L. that use cavities for breeding, where they can encounter predators such as mustelids. For accurate assessment of predation risk before entering a cavity, chemical cues should be more efficient than visual cues, and therefore, may allow birds to avoid a risky encounter with the predator inside the cavity. Here we explore the use of chemical cues of predators in a hole-nesting songbird, the blue tit *C. caeruleus*, and present evidence for predator odour recognition and avoidance in a bird.

We performed our experimental study in a nest-box breeding population subjected to predation pressure by mustelids (Díaz & Carrascal 2006). To test whether birds were able to recognize the chemical cues of mustelids and to use them to assess the risk of predation, we added predator scent to nest-boxes in which birds were feeding 8-day-old nestlings. We simulated a situation where the parents return to the nest with food and find the chemical cues of the predator inside the nest-box. We added two control scents (odorous and odourless) to other nest-boxes. To assess the short-term response of parents to the predator scent, we recorded parental provisioning behaviour after adding the treatments to the nest-boxes. To examine the possible consequences of parent behaviour in relation to presence of chemical cues of the predator in the long-term, we measured body condition of nestlings at days 8 and 13 of age to examine differences in growth rate of nestlings in relation to nest scent. We hypothesized that birds were able to detect the odour of the predator and therefore they would exhibit antipredatory behaviours such as a delay in their entry to the nest-box, a reduction in the time spent feeding nestlings or a reduction in provisioning rates. As a consequence, we expected to find effects in the long term, with nestlings from predator-scented nest-boxes showing lower growth rates. An alternative hypothesis would be that, even birds detect and recognize the odour of the predator, the huge current inversion made on reproduction makes them assume some predation risk costs and therefore parents may maintain the current provisioning rate of nestlings to ensure their survival.

Material and methods

The experimental study was performed in Miraflores de la Sierra (Madrid), Central Spain (40°49'N, 03°46'W). Nests with similar hatching date and similar number of nestlings were randomly assigned to each treatment. Accordingly, there were no differences in hatching date and number of nestlings among treatments (ANOVA; $F_{2,43} = 0.11$, $P = 0.90$ and $F_{2,43} = 1.22$, $P = 0.31$, respectively). These variables were included in the models, but, as they were not significant, they were removed. We introduced papers impregnated with the scent of: (i) mustelid (predator, $n = 15$), (ii) quail (odorous control, $n = 15$); or (iii) water (odourless control, $n = 16$) in nest-boxes when nestlings were 8 days old. Each nest-box was assigned to one treatment during the study. Two absorbent papers were placed hidden between the nest and the walls of the nest-box to avoid birds visually detecting them. We prepared odourless control treatment by adding several drops of water to a clean absorbent paper. We added water to control paper because we wanted to resemble the level of humidity of the papers containing the two other treatments. Water has been widely employed as an odourless control stimuli in studies about chemical detection (e.g. Cooper & Burghardt 1990; Cooper 1998;

Amo, López & Martín 2004). We obtained odorous control by introducing absorbent papers in a cage containing three Japanese quails (*Coturnix japonica* Temminck & Schlegel). We removed faeces of quails from the papers when collecting papers from the cages. We used this species because its odour is very strong and because it is not natural or present in the study area. Therefore, the use of this odorous control allows us to compare the behaviour of birds when they find the odour of a predator inside the nest-box or a new pungent odour but without biological significance. We obtained predator scent by placing clean absorbent papers inside the cage of a male ferret (*Mustela furo* L.). We used a ferret because, even though ferrets are not natural predators of blue tits, the scent (especially that one produced by anal sac secretion that they use to mark the territory) is very similar to those of other mustelids, such as *M. nivalis* L. (Brinck, Erlinge & Sandell 1983) that include birds in their diets. This similarity in odour may explain that ferret scent is recognized and avoided by other species that are not natural prey of such mustelid (e.g. Zhang, Sun & Novotny 2007). Therefore, ferrets are good predator odour donors. We chose papers soiled with fresh urine and gland secretions associated to scent-marking behaviour, that ferrets released with urine, whereas papers containing faeces were discarded. We placed papers in the respective cages 3 days before the experiment, to ensure odour collection. When collecting papers daily for the experiment, we selected papers containing recent cues. This method of odour collection has proven successful in previous studies (e.g. Amo *et al.* 2004), including birds (Godard *et al.* 2007).

To assess the short-term response of parents to the predator scent, we recorded parental provisioning behaviour immediately after adding the treatments to the nest-boxes by filming nest-boxes for 45 min with a video camera placed several meters away. An observer blind to treatments analysed video tapes and recorded the time elapsed from the onset of filming until one of the parents entered the nest-box for the first time. We also recorded the number of times birds perched on the hole of the nest-box but refused to enter before entering for the first time. We recorded the number of provisioning events of both parents and the time spent by both parents inside the nest-box. To determine possible changes through time in the responses, we divided each 45-min period into nine consecutive periods of 5 min each. Therefore, variables were: time from the beginning of filming until one of the parents entered the nest-box, number of times parents perched on the hole and refused to enter the nest-box and number of provisioning events as well as time spent by parents inside the nest-box in each 5 min time sequence (0–5, 6–10 min, etc).

To examine the possible consequences of parent behaviour in relation to presence of chemical cues of the predator in the long term we refreshed the odours 2 days later. We weighed and measured tarsus length of nestlings at days 8 and 13 of age to examine differences in growth rate of nestlings in relation to nest scent. Within-brood means were employed in analyses. We used body mass/tarsus length as a measure of nestling condition.

Data were log-transformed to ensure normality. We used one way ANOVA to test for differences in time to enter the nest-box for the first time and number of times parents perched on the hole and refused to enter the nest-box among treatments. We used repeated measures ANOVA to test for differences in the time spent inside the nest-box between treatments and among the nine time sequences of each nest (within-subjects factor). We included the interaction in the model to test whether responses to the different treatments changed in the course of time. We used repeated measures ANOVA to test for differences in the body condition of nestlings among treatments and between nestling ages (8 vs. 13, within-subjects factor). We included the interaction in the model.

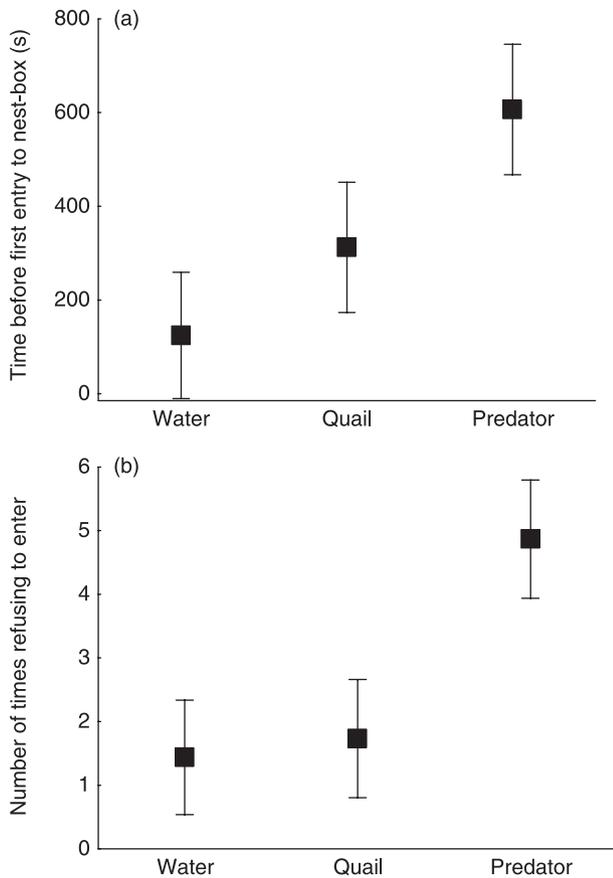


Fig. 1. Evidence for predator odour detection in blue tits. Each figure shows the behaviour of parent birds before entering the nest-box where they were feeding 8-day-old nestlings. The nest-box contained the scent of water ($n = 16$), quail ($n = 15$) or predator ($n = 15$). Mean \pm SE of (a) time elapsed from the beginning of filming until the first parent entered the nest-box for the first time (s); (b) number of times parents perched on the hole of the nest-box but refused to enter from the beginning of the filming period until the first parent entered the nest-box for the first time. Figures show non-transformed data.

Results

There were significant differences between treatments in time elapsed from the beginning of the filming until the first parent entered for the first time into the nest-box (ANOVA, $F_{2,43} = 4.67$, $P = 0.01$, Fig. 1a). When the nest-box contained the odour of a predator, birds delayed the time to enter for the first time, compared to water (unequal N HSD test, $P = 0.01$). There were no significant differences between water and quail treatments ($P = 0.17$), and between quail and predator odour ($P = 0.50$). During this time, birds approached the nest-box several times, perching on the entry hole and later flying away. There were significant differences between treatments in the number of times that birds perched on the hole and refused to enter the nest-box for the first time (ANOVA, $F_{2,43} = 5.68$, $P = 0.006$, Fig. 1b). Birds perched on the hole without entering more times when they perceived the odour of a predator than when the nest-box contained water (unequal N HSD test, $P = 0.007$) or quail odour ($P = 0.04$). There were no significant differences between water and quail odour ($P = 0.83$).

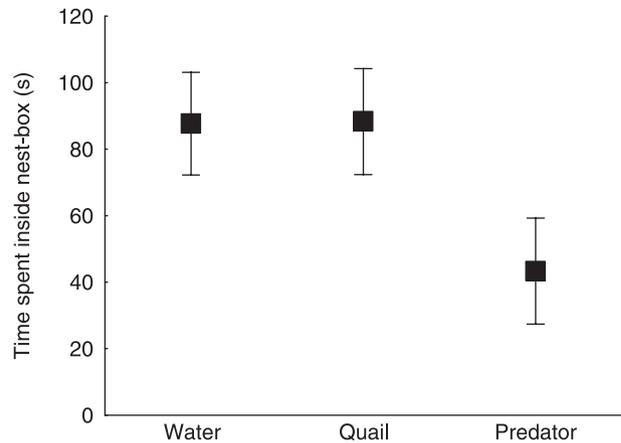


Fig. 2. Mean \pm SE of total time spent inside the nest-box (s) by blue tit parents feeding 8-day-old nestlings in a nest-box containing the scent of water ($n = 16$), quail ($n = 15$) or predator ($n = 15$) during 45 min of filming. Figure shows non-transformed data.

Time that birds spent inside the nest-box differed between treatments (ANOVA, $F_{2,43} = 4.88$, $P = 0.01$, Fig. 2). Birds spent less time inside the nest-box when it contained predator odour than when it had water (unequal N HSD test, $P = 0.02$) or quail odour ($P = 0.048$). There were no significant differences between water and quail odour ($P = 0.90$). There were significant differences in relation to the time sequences ($F_{8,344} = 8.97$, $P = 0.001$). Birds spent less time inside the nest-box during the first minutes than they did later in the filming period. Therefore, there were significant differences between the first 5 min and the time sequences from minute 15 to the end of the recording period ($P < 0.04$ in all cases). The interaction between treatment and time sequence was not significant ($F_{16,344} = 0.69$, $P = 0.80$).

There were no significant differences in provisioning rates in relation to the treatment (ANOVA, $F_{2,43} = 0.81$, $P = 0.45$), but there were slight differences in provisioning rates in relation to the time sequences ($F_{8,344} = 0.68$, $P = 0.047$). However, *post hoc* comparisons did not show significant differences (unequal N HSD test, $P > 0.05$ in all cases). The interaction between treatment and time sequences was not significant ($F_{16,344} = 0.78$, $P = 0.71$).

Nestling body condition improved from day 8 to day 13 (ANOVA, $F_{1,37} = 97.12$, $P < 0.0001$). There were no significant differences in the body condition of nestlings between nest-boxes with different odours ($F_{2,37} = 0.24$, $P = 0.78$), and the interaction between treatment and age was also not significant ($F_{2,37} = 0.12$, $P = 0.89$). Therefore, the increase in body condition of nestlings in the course of time did not differ between treatments.

Discussion

Birds detected the chemical cues of the predator and exhibited antipredatory behaviours to decrease predation risk. Even before entering the nest-boxes, birds were able to detect the odour and modify their behaviour according to the risk of predation. When the cavity contained the odour of a predator,

birds delayed their entry for the first time to the nest-box (Fig. 1a). Birds also perched on the hole of the nest-box and refused to enter more times when the nest-box contained predator odour than when it contained water or quail scent (Fig. 1b), looking around and trying to look inside without entering, to identify the odour source and the predator presence. This antipredatory response may be crucial for survival.

Birds also decreased the time inside predator-scented nest-boxes when provisioning nestlings, compared to both controls (Fig. 2). When birds perceived an unknown odour, as was the quail odour, they did not decrease the time spent inside the nest-box. Birds are known to exhibit aversive responses to unknown odours (Jones, Facchin & McCorquodale 2002; reviewed in Roper 1999), and birds are also known to show aversive responses to ammonia (Kristensen *et al.* 2000), an end product of nitrogen metabolism excreted in the urine of birds (Goldstein & Skadhauge 2000). Despite these facts, we did not find any effect of quail scent on time spent inside the nest-box. Therefore, this result suggests that the decrease in time spent inside the predator-scented nest-box was due to the detection and avoidance to a predator scent, and not only to a new scent. Birds decreased the time spent inside the nest-box, but they did not decrease the number of feeding events. Therefore, birds solved the trade-off between avoiding predation and reproduction by maintaining constant feeding rates but decreasing time exposed to predation when entering the nest-box to feed the nestlings. In that way, birds minimized the risk of predation when provisioning nestlings inside a predator-scented nest-box and they also ensured that nestling growth was not impaired. The maintenance of feeding rates regardless of the level of predation risk inside the nest-box may explain the lack of differences in nestling growth between treatments.

Previous studies have shown antipredatory behaviours in invertebrates (e.g. Beckerman, Wieski & Baird 2007) and vertebrates such as fishes (e.g. Ylönen *et al.* 2007), amphibians (e.g. Ireland, Wirsing & Murray 2007), reptiles (e.g. Amo *et al.* 2004) or mammals (review in Apfelbach *et al.* 2005) when exposed to predator chemical cues (for a general review see Kats & Dill 1998). This is the first evidence that birds are able to detect and discriminate the chemical cues of predators and use them to assess the level of predation risk in an ecologically relevant context.

However, this should be only the first step in an emerging research line and further research is needed to clarify several points that could not be elucidated in this study. For example, here we have used scent-marked papers with mustelid urine and glandular secretions, and it should be worthy of further research to study which compounds are used by birds to discriminate the predator scent, as well as to examine whether birds are responding to the presence of chemical compounds or to their concentration. Future studies are also needed to examine the chemical senses involved in such discrimination. Throughout the manuscript, we have referred mainly to olfaction capacities of birds for chemical cues detection. However, trigeminal chemoreceptive receptors also detect volatile compounds (Mason & Clark 2000; Hagelin 2007) and could also

be involved in the detection of chemical cues of predators. The response of the nestlings to predator chemical cues inside the nest-box is also worthy of more research and could also have an effect on the observed behaviour of the parents.

Furthermore, chemical cues of predators may provide an early warning of the possible presence of a predator, but given that the odour can persist in the area once the predator has left, chemical assessment of predation risk may lead to an overestimation of predation risk if prey exhibit an antipredatory response despite the absence of the predator (Kats & Dill 1998; Amo, López & Martín 2005). Although we observed differences in the behaviour of parents in the course of time, with birds spending less time inside the nest-box during the first minutes than afterwards, these differences cannot be attributed to an adaptation to the decrease in the risk of predation in the course of time of exposure to predator chemical cues because the interaction between treatment and time was not significant. This behaviour was probably more related to the disturbance that the experimenter caused on birds when adding the treatment to the nest-boxes. So subsequent experiments are needed to examine whether birds are able to assess the decrease in predation risk associated with predator odour exposure in the course of time.

The discovery of the ability of birds to use the chemical cues of predators to accurately assess the risk of predation opens a new and promising research area to understand many aspects of bird behaviour that have been neglected until now, and points out the need of examining in depth the ecological and evolutionary role of chemical detection of predators in avian life histories.

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