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S16-4 Olfaction, volatile compounds and reproduction in birds

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Abstract Despite research in several biologically-relevant contexts, including orientation and resource acquisition, olfaction studies in free-ranging reproducing bird populations are rare. This, inter alia, hampers the quantitative study of fitness-consequences of olfaction. Here we provide a brief review of the use of avian smell in the context of reproduction, focusing on a model system, the blue tit. Corsican blue tits (*Parus caeruleus ogliastrae*) incorporate into their nests small fragments of aromatic plants that deliver high levels of volatile compounds easily perceived by a human observer. Blue tits use olfactory cues to anticipate the changes of the aromatic environment in their nests by repeatedly delivering fresh plant material with high concentrations of volatile compounds. A chemical approach will enable measurement of volatile compound concentrations, the identification of the volatile compounds involved, and ultimately the study of the functional meaning and fitness consequences of olfaction in free-living populations of birds.

Key words Olfaction, Volatile compounds, Nest maintenance

1 Introduction

Olfaction or smell is defined as the capacity to detect airborne chemical compounds at a distance from their source. Anatomical, neurophysiological and behavioral studies carried out during the last four decades show that the use of olfaction in birds is more common than originally thought. Estimates of absolute olfactory sensitivity thresholds exist for at least 15 domestic and non-domestic bird species (Roper, 1999).

In contrast to extensive work on avian smell in the laboratory, the use of olfaction in the everyday life of birds remains an understudied subject. Recent investigations indicate that avian smell may be used in a wide range of biologically-relevant contexts, including orientation and resource identification. However, its importance in reproduction remains a contentious issue, mainly because its study in free-ranging reproducing bird populations is rare.

Here we focus on the role of avian olfaction in perceiving plant volatile compounds used for nest maintenance, as illustrated by a model system, the blue tit. To introduce it, we briefly review available studies of olfaction in other reproductive contexts.

2 Olfaction in different reproductive contexts

Whether free-living birds smell, or not, can be demonstrated with experimental alterations of the odorous environment, or techniques that manipulate the olfaction system, such as transection of olfactory nerves, sealing nostrils, or chemically manipulating olfactory mucosa (Roper, 1999). Expected outcomes are modification of behavior in response to the treatment that has been carried out. All these approaches have revealed use of olfaction in different biologically important contexts, including those related to reproductive activities.

2.1 Food location

The ability to find food by smell can be placed in a reproductive framework if food located by smell contributes to forming eggs, feeding partners during courtship or incubation, or raising chicks. For instance, procellariiform seabirds (e.g. Wilson's storm-petrels, *Oceanites oceanicus*; great shearwaters, *Puffinus gravis*) have a well-developed olfactory apparatus used for detecting food on the sea in the absence of visual food cues (e.g., Grubb, 1972; Verheyden and Jouventin, 1994). Procellariiforms can distinguish between cod-liver oil and control samples (e.g., sea water), and even a single chemical compound (dimethyl sulphide), indicating that optimal prey may be identified by smell (Nevitt et al., 1995). Some studies, nevertheless, failed to find olfactory effects, perhaps because of differences among the biological needs of different species or experimentally induced artifacts (e.g., Grubb, 1972; Nevitt, 1999; Bonadonna and Bretagnolle, 2002). None of these studies demonstrated experimentally that smell increases reproductive output because it enhances foraging efficiency.

2.2 Courtship and mate recognition

The avian uropygial gland may be a potential chemical source exploited for olfactory communication. The size of the uropygial gland peaks around the reproductive period in some species of birds, and the chemical compounds produced by that gland seem to vary between species, between the sexes, and over time (e.g., Jacob et al., 1979 and references therein). Body odors thus may contain cues that help in mate or species recognition (e.g., Thibault and Holyoak, 1978; Jacob et al., 1979). The odors might also be used to select a high-quality partner, if the odors reflect health or body condition (e.g., Neerkens, this symposium; Douglas et al., 2001).

Certain aspects of sexual behavior are inhibited in ducks from which olfaction capacity has been removed, suggesting that olfaction may play a key role in courtship (Balthazart and Schoffeniels, 1979). Otherwise, experimental evidence for the use of olfaction in partner recognition or choice is rare.

2.3 Nest building

Many bird species incorporate fresh plant material into the nest to ameliorate the living conditions of parents and chicks (e.g., Gwinner, this symposium). More than a decade ago, Clark and Mason (1987) and Clark and Smeraski (1990) provided evidence that captive European starlings (*Sturnus vulgaris*) are especially sensitive to specific volatile compounds of plants and use them in defence against enemies in the nest, including disease and blood-sucking ectoparasites. However, these investigations, which provide one of the best available examples of the use of olfaction in a reproductive context, do not reveal how olfaction is used in field conditions.

2.4 Nest relocation and discrimination

Olfactory navigation can be placed in the context of reproduction if returning quickly to the nest enhances efficiency in caring for mate or chicks. Birds may also use odor cues to relocate breeding grounds after migration, which could affect the probability of acquiring a mate or optimal breeding site. Studies on homing pigeons (*Columba livia*) and starlings showed that anosmic individuals returned to the colony or nest site at a significantly lower rate than controls when individuals where displaced over increasingly long distances. The results suggest that, independent of artifacts (e.g., stress), olfaction may help in orientation, especially in unfamiliar areas (e.g., Wallraff et al., 1995; Walraff, 2001).

Selection may favor olfaction in avian species that cannot use visual or acoustic cues for finding the nest. This implies that burrows or nests possess individual odor signatures, such as produced by chemical compounds in stomach oil or preen waxes (e.g., Jouventin 1977; Thibault and Holyoak, 1978; Jacob et al., 1979). In nocturnal Leach's petrels, breeding captives discriminate between own nest material and plain dirt after controlling for visual and acoustic cues (Grubb, 1974). However, captives of the same species did not seem to discriminate between stomach oil, preen gland oil and control odors during choice experiments.

Comparative analyses of nine species of petrels suggest the existence of a relationship between the development of smell and circadian activity patterns, i.e. nocturnal vs. diurnal activity (Bonadonna and Bretagnolle, 2002). Some anosmic, nocturnally-active petrels and shearwaters had difficulties in relocating their burrows at night (e.g., Grubb, 1974; Benvenuti et al., 1993; Bonadonna et al., 2001; Bonadonna and Bretagnolle, 2002; but see Shallenberger, 1975), whereas diurnally active species can relocate burrows without smell (Bonadonna and Bretagnolle, 2002). Surprisingly, some nocturnal species, such as Manx shearwaters (*Puffinus puffinus*) seem to use visual rather than odor cues to find their burrows at night, as indicated by results of field experiments manipulating odor, visual and acoustic features in and around the burrow (Brooke, 1978; James, 1986). Furthermore, some species with well-developed olfactory bulbs, such as the snow petrel (*Pagodroma nivea*), may also use visual signs to relocate the nest site (e. g., Hafthorn et al., 1988).

Chicks of British storm petrels (*Hydrobates pelagicus*) use smell to recognize the nest. Nest recognition by chicks may be useful in species where parent-offspring recognition is not well developed and adults limit care to chicks that stay in the nest (Minguez, 1997). In domestic chicks (*Gallus domesticus*), an attractive response to odor is developed around the time of egg hatching (Porter and Picard, 1998).

2.5 Offspring recognition

Olfaction may be used in offspring recognition, and therefore may influence parental care. This has been experimentally demonstrated in ring doves (*Streptopelia risoria*). Experimental body odor change in squabs (young) resulted in higher squab mortality and lower body weight (Cohen, 1981). Potential side-effects of experimental design, such as systemic toxicology induced by the odor treatment, were excluded. Manipulation of the olfactory nerves of the parents restored, to some extend, parental care for odor-manipulated squabs. Changes in parental care in response to such changes of the odor environment have not been reported in natural populations.

3 Nest maintenance, volatile compounds and olfaction: blue tits as a model system

Cavity-nesting female blue tits on Corsica add fragments of fresh plant material to the nest cup from the time of egg laying to fledging (Lambrechts and Dos Santos, 2000; Petit et al., 2002). Of over 200 plant species available in blue tit habitat, only 10 (< 5%) were recorded in the nest. Five plant species were found in at least 40% of the more than 100 nests sampled: *Achillea ligustica* 79%, *Lavendula stoechas* 52%, *Mentha suaveolens* 45%, *Pulicaria odora* 43%, *Helichrysum italicum* 39%. Some of the selected plants were not observed in blue tit breeding territories, indicating that it can be costly to acquire them. The number of plant species per nest differed between 1 and 5, perhaps due to variability in herb availability or parental experience in finding herbs.

The plants added to the nests are all highly aromatic, delivering strong-smelling aromatic odors easily perceptible by humans. Chemical analyses of the volatile compounds emitted by the five more important species were performed on three potted plants of each. Petit et al. (2002) used the headspace technique to do this. Volatile compounds were then identified using a gas chromatograph (GC) and GC/MS. These analyses revealed numerous volatile compounds, mainly monoterpenes and sesquiterpenes. The mean number of volatile compounds emitted varied between 12 (*Pulicaria odora*) and 30 (*Lavendula stoechas*) per plant species.

A Principal Component Analysis was performed on the relative proportions of the volatile compounds in the five species using a covariance matrix. The first axis of the PCA explained 35 % of total variance and the second component 30% (Fig.1). The first component clearly separates odors from *Mentha suaveolens* (Mint) and *Achillea ligusta* (Ac). The second component clearly separates odors from *Lavandula stoechas* (Lav). Higher loading on the two components are mainly for monoterpenes found in one or other of the studied species, such as fenchone and camphor (P36 and P48) in *Lavandula stoechas* or piperitone and piperitenone (P60 and P66) in *Mentha suaveolens*. Because the chemical profiles clearly differ across the five plant species, blue tits most probably maintain a "cocktail" of odors in the cavity nest (also Lambrechts and Dos Santos, 2000).

The high selectivity for plants brought to the nest,

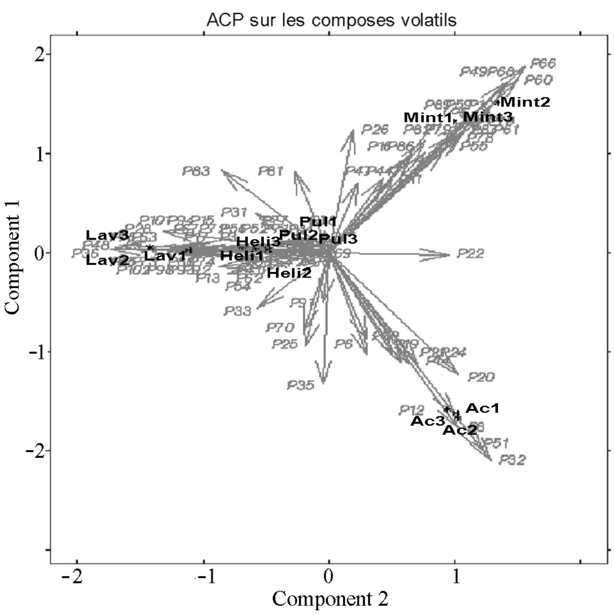

Fig. 1 Results of Principal Components Analysis on covariance performed on the percentage of volatile compounds identified by GC analyses in the bouquet of five primary herb species in nests of Corsican blue tits

The first (35% of the variance) and second (30%) principal components clearly separate all five species. Volatile compounds are indicated by arrows (multiplied by 100 to fit the scale of the graph), showing that each species has a different bouquet of volatile compounds.

their aromatic properties, and their regular replacement, suggests that olfaction plays a key role in nest maintenance in blue tits. To test whether the tits respond directly to nest odor, all aromatic plant fragments were first removed from all nests, and then hidden caches of fresh aromatic plants were replaced in half of the nests ("Herb+" treatment) while the rest were left devoid of aromatics, as controls ("Herb–" treatment). Nests were checked at intervals of 24 hours (n = 16) and 48 hours (n = 64) after the treatments to monitor for newly-added aromatic plant fragments and score the presence or absence of the five primary plant species.

Blue tits added fragments in significantly lower proportion in "H+" nests than "H–" nests over both time periods (24 hours: H+: 0% vs. H–: 87.5%; 48 hours: H+: 56% vs. H–: 94%). In other words, the tits brought more new fragments to nests without any aromatics than to those in which such fragments could be smelled but not seen. The simplest interpretation of these findings is that the blue tits are sensitive to changes in the olfactory environment of the nest. In the nests devoid of fragments, odor concentrations probably decreased quickly to levels low enough to stimulate the tits to look for fresh material, whereas in nests with hidden fragments, a sufficiently high level of odor concentration was maintained to inhibit "herb reload" behavior (Petit et al., 2002).

The nest protection hypothesis is the most probable functional explanation for herb loading by blue tits (Petit et al., 2002). The majority of the aromatic plant species used widely employed in human house cleaning or soft-medicine, possessing chemical substances known for their antibacterial, antiviral, fungicidal, insecticidal and/or insect repellent action: camphor, eucalyptol, limonene, linalool, myrcene, piperotenone, pulegone, and terpin-4-ol (unpubl. data). This suggests that blue tit olfaction helps to maintain a strongly smelling disinfected living environment. Further experiments should determine whether the tits are especially responsive to those chemical compounds that defend the birds against parasites and disease, and whether they prefer odor cocktails from different plants rather than odors of single plant species.

4 Conclusions

A fundamental, yet rarely tested, assumption in evolutionary ecology is that avian olfaction has been selected to respond to those chemical cues that maximize fitness. Some studies indicate, for instance, that birds are sensitive to plant volatiles that kill or repel nest parasites, or respond to odor cues that indicate the presence of resources essential for survival. We therefore suggest that future studies of avian olfaction should focus even more on the identification of the chemical cues that are exploited by free-living populations of birds, and at the same time on the consequences of odor cue recognition for survival and reproduction. Comparative and experimental studies will also be required to reveal genetically based adaptive differences in odor cue recognition across populations and species. It is clear that the techniques developed in chemical ecology are essential for the development of successful multi-disciplinary approaches that aim to understand better the adaptive significance of avian olfaction.

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