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S16-3 Parasite defence in birds: the role of volatiles

Helga GWINNER[1], Silke BERGER
Max-Planck-Research-Centre for Ornithology, Von-der-Tann-Str. 7, D-82346, Andechs, Germany;
[1] hgwinner@.erl.ornithol.mpg.de

Abstract European starlings (*Sturnus vulgaris*) mix fresh herbs, preferably species rich in volatile compounds, into their dry nest material. By investigating air samples from starling nest boxes, we found that nestlings and their parasites encounter volatile compounds such as sabinene, myrcene, limonene, phelandrene and ocimene, some of them with medicinal properties. We compared mite load and nestling condition in experimental nests with and without herbs. Herbs had no effect on the quantity of nest-dwelling ectoparasites, but a significantly inhibiting effect on bacterial growth in late nests. There was also a tendency for mosquitoes to visit nests containing herbs less often than nests without herbs. Chicks that fledged from herb nests with higher body mass had more red blood cells and differed in some immunological parameters from controls. Herb chicks also had more basophil leucocytes, but fewer lymphocytes than nestlings from nests without herbs. More yearlings from herb nests were identified and showed interest in nest boxes in their first year as adults. The better condition of herb chicks may have been due to plant compounds that stimulate elements of the immune system to help nestlings cope better with harmful impacts from parasites and other stressors. Apart from their effects on parasites and nestlings, volatile compounds in herbs that are carried into the nests by males may also play a role in mate attraction.

Key words *Sturnus vulgaris*, Green nest material, Parasite defence

1 Introduction

In the evolutionary arms race between host and parasite, plants have developed a defence system based on secondary plant compounds comparable to the immune system of animals. One category of such compounds are volatiles, substances that dissipate in air and can act from a distance. In plants, volatiles play several roles. They fight diseases by impairing viruses, bacteria or fungi; they prevent plants from being eaten by herbivorous animals; and they are essential for reproduction because they attract insects and vertebrates for pollination and seed dispersal.

Many bird species, especially those that reuse the same nesting sites year after year (a circumstance that may result in heavy parasite loads), incorporate fresh plants into their nests. In a survey of 137 passerine species, Clark and Mason (1985) estimated that those occupying nests repeatedly were about 6 times as likely to employ green nest material as those which used nests only once. American wood storks (*Mycteria americana*), for example, provide their nests with aromatic plants like cypress, wax myrtle or pine (Rodgers et al., 1988). European starlings (*Sturnus vulgaris*) in America prefer wild carrots, yarrow, agrimony and fleabane (Clark and Mason, 1985, 1987). Corsican blue tits (*Parus caeruleus*) use milfoil, lavender, mint and other aromatic plants (Lambrechts and Hossaert-McKey, this symposium), and South African mossies (*Passer melanurus*) also use odorous plants as nest material (Milton and Dean, 1998). Starlings in South Germany carry about 70 different plant species into their nest boxes, but preferentially select those rich in volatile compounds like milfoil (*Achillea millefolia*), hogweed (*Heracleum sphondylum*), cow weed (*Anthriscus sylvestris*) or elder (*Sambucus niger*), not just immediately available plants such as clover (*Trifolium pratense*) or dandelion (*Taraxacum officinale*). In an aviary choice experiment, males preferred volatile to nonvolatile plants as green nest material (Gwinner, 1997; Lampert, 1999).

Various hypotheses have been proposed to explain the use of green nest material, but only two make sense for the European starling (Clark, 1991). One is the courtship hypothesis which maintains that fresh, odorous plants serve as a tool to attract females in species where only males bring aromatic nest material, preferably in the presence of females; in such cases, no more plants are incorporated after egg laying (Kessel, 1957; Fauth et al., 1991; Gwinner, 1997). The other is the nest protection hypothesis, according to which aromatic nest material is used as an adaptation against parasites: birds, like herbalists, may use the intrinsic properties of plants against harmful parasites and pathogens (Widmann, 1922; Wimberger, 1984; Clark, 1991).

Apart from nest reuse, which is common in hole-nesting birds, colonial breeding and communal roosting are also conducive to infestation by parasites and pathogens because of the higher probability of transfer due to social contact (Rothschild and Clay, 1957). European starlings are thus a suitable model for investigating the significance of volatiles in parasite defence.

2 Methods

2.1 Estimation of parasite load

The main ectoparasite of starlings in a South German nest box colony is the red fowl mite (*Dermanyssus gallinae*). We scored mites from the nest boxes and the scabs on the bellies of nestlings, which are remains of the mite blood meals (Feare, 1984; Gwinner et al., 2000); we also sampled bacteria on nestling bellies with agar paddles. The paddles were incubated in the laboratory, and the attached bacteria colonies counted. Mosquitos were caught in the nest boxes as well, with sticky paper.

2.2 Nest investigation and manipulation

For one season we collected, weighed and identified the herbs that males had carried into their nests from the onset of nest building to egg deposition. To investigate the effect of green nest material on ectoparasites and nestlings, we collected data over five reproductive seasons from more than 150 nests. Natural starling nests were exchanged for artificial ones. In half of them — the “herb nests” — we mimicked the nest condition of an average starling nest in our colony. It comprised a mixture of the six most frequent green nest plants. The control nests, so called “grass nests”, consisted of the same amount of dry material supplemented only with fresh grass, a material almost never used as nest greenery (Gwinner, 1997; Gwinner et al., 2000). To identify the volatile plant compounds, the air in the nest boxes was sampled and analyzed by gas chromatography and mass spectrography. For the statistical analyses (ANOVA, *T*-test and Mann Whitney *U*-test), means per nest were used.

3 Results

3.1 Are nestlings exposed to volatile compounds in their nests?

Because no plants are carried into nests after egg laying, we investigated whether volatile compounds were still present in the nest boxes during the nestling period. We sampled head-space air from nest boxes with both experimental and natural nests inhabited by 7- to 10-day-old nestlings. In the samples from herb nests and natural nests, but not or hardly at all in grass nests, we detected and identified such volatile substances as sabinene, myrcene, limonene, phelandrene or ocimene, which are used in human medicine or parasite defence (Gwinner and Krock, unpubl.).

3.2 Herbs and parasites

Parasite load of nests can be expected to increase through the reproductive season. The main parasite of our starlings, the fowl mite, produces about one generation per week (Sikes and Chamberlain, 1954). Nestlings of one or two broods are found in nests from early May through June. If the incorporation of green nest material serves for parasite defence, starlings should enhance their efforts progressively through the season to provide later nests with more herbs. This we found, the mass and the numbers of plant species carried to the nests by males increasing in parallel with the seasonal development of mites (P=0.0001, n=53). In spite of this, no effects reducing nest-dwelling mites and other ectoparasites could be detected (Gwinner et al., 2000).

Bacteria load in the nest boxes also increased through the season. It was higher, however, in nests containing no herbs. More bacterial colonies were counted on samples from grass nests than from herb nests late in the season (P= 0.025, n=26/27; Gwinner and Berger, unpubl.).

Fewer mosquitoes were caught in herb than grass nests (P= 0.055, n=26/25). Due to weather conditions in the year of this experiment, mosquitoes were in low numbers. Hence mosquitoes then probably played no role as blood suckers, although as vectors for diseases they may still have been effective.

3.3 Herbs and nestlings

Apart from insecticidal or bactericidal effects, volatile compounds may influence the condition of nestlings as well. They could, for example, reinforce the appetite of nestlings, as spices do in seasoning. They might also soothe the itching caused by mites, fleas or other ectoparasites. Chicks may “feel” better, eat more, and gain more weight. We compared the body mass of fledglings that grew up in herb and grass nests over years. In some years, there were significant differences between them, but not in others. Overall, however, it was highly significant (P= 0.005, n= 77/77). The effect of herbs was particularly marked when environmental conditions were unfavorable, for example when mite load in nests was high, temperature low, and nutrition of the nestlings reduced due to persistent rain or high clutch size (Fig. 1). In all these contexts, herbs in nests had a significantly beneficial overall on fledgling mass, whereas for other environmental conditions, only the feeding mode of the parents had a significant additional effect” (Fig. 1c). Herb chicks also had significantly more red blood cells (hematocrit: P=0.003, n= 50/44) during three of five years of the investigation.

We ringed more than 300 nestlings individually to determine recruits for the following year. Out of 23 returned yearlings, 15 had grown up in herb nests and only 8 in grass nests. Ten birds from herb nests but only one from a grass nest showed interest in nest boxes. Four of the 10 herb birds bred and reared young.

Herb effects were more clearly expressed in adverse environmental conditions. Malnutrition, cold weather, and parasites can cause stress; and stress affects the immune system. Some plants, *Echinacea* for example, are known to boost the immune system, especially phagocytosis (Wagner and Jurcic, 1991; Steinegger and Haensel, 1988). We therefore hypothesized that nest herbs may act like a drug stimulating the defence system. We investigated young of 25 nests for leucocyte expression and found that nestlings from grass nests had more lymphocytes than herb chicks (P= 0.01). This may be related to a higher bacterial load in grass nests.

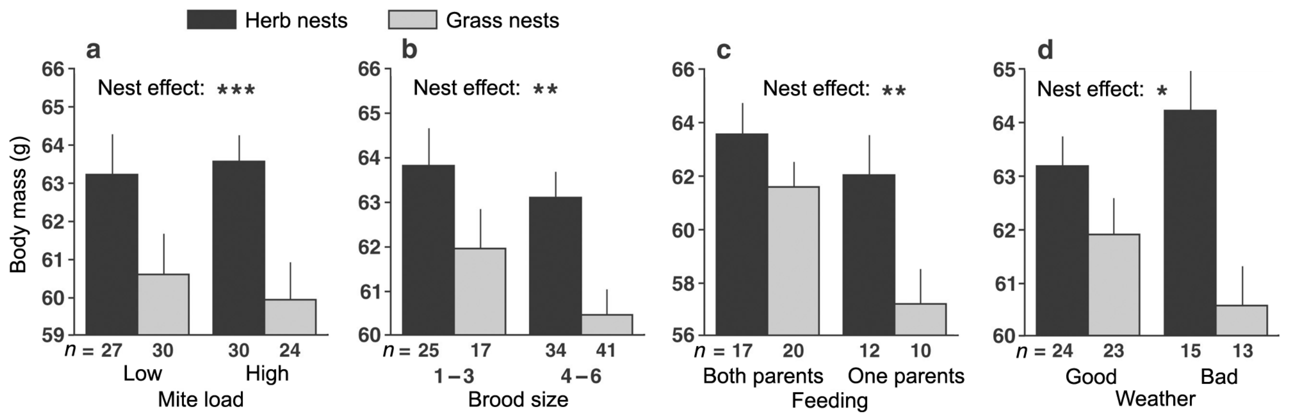

Fig.1 Body mass of wild starling nestlings in experimental nests at day 18, close to fledging
Black columns = with herbs, pale columns = without herbs. Left two columns in each graph = favorable conditions: (a) low mite load, (b) small brood size, (c) both parents feeding, (d) good weather during first week of nestling life (mean max T 19°C and 2 days with 2 l rain/m). Right two columns in each graph = unfavorable conditions: (a) high mite load, (b) high brood size, (c) little or no feeding by males, (d) bad weather (mean maximal T °C and 7 days with 6 l rain/m).

Another white blood cell, the basophil, was seen more often on blood smears of herb chicks than of grass chicks (P=0.01, n=12/13; Gwinner et al., 2000). Basophils are reported to play a role in parasite defence, wound healing processes, and in coping with extreme temperatures and malnutrition, all situations to which nestlings can be exposed (Roitt et al., 1996; Maxwell and Robertson, 1995). We therefore consider it likely that volatile compounds of herbs strengthen elements of the immune system, allowing the nestlings to cope better with detrimental conditions so that their growth can proceed undisturbed.

3.4 Do volatiles play a role in the sexual life of starlings?

It is the male that provides the nests with herbs. Males often display green plants in an obvious manner to surrounding females. Plant incorporation peaks 4–5 days before the first egg is laid, when females decide on a male, and it stops almost completely with laying. Starlings can discriminate odors, and do so especially well during the reproductive season (Clark and Mason, 1987; Clark and Smeraski, 1990). In an aviary experiment, we tested whether hand-raised yearling males without courtship experience prefer plants scented with milfoil (the most preferred plant under natural conditions) or vanilla (unknown in the natural environment of starlings) over non-scented plants of the same species. Males significantly preferred milfoil-scented plants to non-scented or vanilla-scented plants for presentation to females. Hence we consider that male starlings discriminate herbs by olfactory cues, with preference for an odor associated with the most preferred nest plant in nature (Gwinner and Berger, unpubl.).

4 Conclusions

Volatile plant compounds seem to play much the same roles in starling nests as in plants, where they evolved to counteract the injurious activities of parasites and pathogens and for reproductive purposes. Nevertheless, studies of the role of nest greenery have produced controversial results. A study of European starlings in north America found that some plants, such as wild carrots, depressed the population growth of northern fowl mites in nests (Clark and Mason, 1985, 1988; Clark, 1991). Another American study by Fauth et al. (1991) could not confirm such effects. Moreover, the use of aromatic nest material did not impair the main parasite (dermestid larvae) of wood storks (Rodgers et al., 1988). The fowl mite, which can affect nestlings of European starlings adversely, was not inhibited by herb treatment in our starling population. It is thus conceivable that plant compounds have effects other than that of simply suppressing parasites. Volatiles could, for example, reduce hunger for blood in the parasites, for the benefit of the host. The higher hematocrit levels in herb chicks could reflect this; but it could also just express good health. Under laboratory conditions (Clark and Mason, 1988) and in our field study, nest herbs suppressed the development of bacteria.

Odors of herbal nest material may disorient mosquitoes by masking the cues they need to find their host (Lambrechts and Dos Santos, 2000; our study). Nestlings from herb nests were fitter than nestlings in nests without herbs. This could reflect immunostimulating properties or other direct effects of the herbs on the nestlings (Gwinner et al., 2000).

For more than 4000 years, the Chinese have used certain plants to strengthen the “shield “, as they called it, which protects against disease. We interpret volatiles evaporating from nest greenery as such a shield, protecting nestlings from pathogens, the harmful actions of parasites, and other stress-inducing environmental factors. Volatile compounds may also play a role in mate attraction in which males prefer volatile herbs as “show objects” during courtship display. Whether females, however, can assess male quality from his perfume remains to be investigated.

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References

Clark L, Smeraski CA, 1990. Seasonal shifts in odor acuity by starlings. J. Exp. Zool. 255: 22–29.

Clark L, 1991. The nest protection hypothesis: the adaptive use of plant secondary compounds by European starlings. In: Loye JE, Zuk M ed. Bird Parasite Interaction. Oxford: Oxford University Press.

Clark L, Mason JR, 1985. Use of nest material as insecticidal and anti-pathogenic agents by the European starling. Oecologia 67: 169–176.

Clark L, Mason JR, 1987. Olfactory discrimination of plant volatiles by the European starling. Anim. Behav. 35: 227–235.

Clark L, Mason JR, 1988. Effect of biologically active plants used as nest material and the derived benefit to starling nestlings. Oecologia 77: 174–180.

Fauth PT, Krementz DG, Hines JE, 1991. Ectoparasitism and the role of green nesting material in the European starling. Oecologia 88: 22–29.

Feare CJ, 1984. The Starling. Oxford: Oxford University Press.

Gwinner H, 1997. The function of green plants in nests of European starlings *Sturnus vulgaris*. Behaviour 134: 337–351.

Gwinner H, Oltrogge M, Trost L, Nienaber U, 2000. Green plants in starling nests: effects on nestlings. Anim. Behav. 59: 1–9.

Kessel B, 1957. A study of the breeding biology of the European starling (*Sturnus vulgaris* L.) in North America. Am. Midl. Nat. 58: 257–331.

Lambert K, 1997. Eintrag von Gruenem Nistmaterial Beim Star, *Sturnus vulgaris*. Diplomarbeit. Wuerzburg: Universitaet Wuerzburg.

Maxwell MH, Robertson GW, 1995. The avian basophilic leucocyte. a review. World Poultry Sci. J. 51: 307–325.

Milton SJ, Dean RJ, 1998. The use of green aromatic plants in Karoo bird nests. Ostrich 69: 329.

Rodgers JA Jr, Wenner AS, Schwikert ST, 1988. The use and function of green nest material by wood storks. Wilson Bull. 103: 411–423.

Rothschild M, Clay T, 1957. Fleas, Flukes and Cuckoos. London: Collins.

Roitt IM, Brostoff J, Male DK, 1996. Immunology. London: Mosby.

Sikes RK, Chamberlain RW, 1954. Laboratory observations on three species of bird mites. J. Parasitol. 40: 491–497.

Steinegger E, Haensel R, 1988. Lehrbuch der Pharmakognosie und Phytopharmazie. Berlin, Heidelberg: Springer Verlag.

Wagner H, Jurcic K, 1991. Immunologische Untersuchungen von pflanzlichen Kombinationspraeparaten. Drug Res. 41: 1 072–1 076.

Widmann O,1922. Extracts from the diary of Otto Widmann. Trans. Acad. Sci. St. Louis 24: 1–77.

Wimberger PH, 1984. The use of green plant material in bird nests to avoid ectoparasites. Auk 101: 615–618.