

A review of the nest protection hypothesis: does inclusion of fresh green plant material in birds' nests reduce parasite infestation?

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SUMMARY

The use of aromatic plants and their essential oils for ectoparasite treatment is a field of growing interest. Several species of birds regularly introduce aromatic herbs into their nests putatively to reduce parasites. The behaviour is most often seen in cavity nesting birds and after nest building has finished. The plants are included in a non-structural manner and are often strongly aromatic. Various different hypotheses have been proposed regarding the function of this behaviour; from the plants altering some non-living factor in the nest (crypsis, water loss and insulation hypotheses) to them being involved in mate selection (mate hypothesis) or even having a beneficial effect, direct or indirect, on chicks (drug or nest protection hypothesis, NPH). Many studies have been carried out over the years observing and experimentally testing these hypotheses. This review focuses on studies involving the most popular of these hypotheses, the NPH: that plants decrease nest parasites or pathogens, thereby conveying positive effects to the chicks, allowing the behaviour to evolve. Studies providing observational evidence towards this hypothesis and those experimentally testing it are discussed.

Key words: aromatic, blue tit, starling, nest protection, nesting material.

INTRODUCTION

Many species of birds have been shown to regularly introduce pieces of fresh green material in their nests after nest building has finished (Wimberger, 1984; Clark & Mason, 1985; Lambrechts & Dos Santos, 2000; Mennerat *et al.* 2009a). There are various hypotheses for the function of this behaviour, including the crypsis hypothesis (the plants function to hide the nest), the water loss hypothesis (plants function to reduce water loss) the shading hypothesis and the nest insulation hypothesis. The following are the three most accepted hypotheses. The nest protection hypothesis (NPH), which this review focuses on, states that plants decrease nest parasites or pathogens through their phytochemical compounds, indirectly benefitting chicks (Clark & Mason, 1985). A more appropriate name for this hypothesis might be the 'chick protection hypothesis', with protection being conveyed to chicks and not the nest itself, however for continuity this review will use the current terminology. The mate hypothesis is based on the behaviour being involved in pair formation (Fauth *et al.* 1991). Finally the drug hypothesis states the plants affect chicks in a beneficial manner directly, perhaps through potentiation of their immune system or another similar mechanism (Gwinner *et al.* 2000). The behaviour is typically seen in cavity nesting species more than

open cup nesters (Clark & Mason, 1985), and those in which the behaviour has been extensively investigated are nest-box users. This would suggest the first three hypotheses are less likely to be responsible: cavity nests are by definition shaded, have higher water content and would not be more hidden by inclusion of plants inside the cavity. The behaviour has also been shown to be more likely to occur in birds that re-use their nests year on year, rather than those that build new ones (Wimberger, 1984; Clark & Mason, 1985). One would expect these birds to face higher parasite and bacterial burdens through overwintering of parasites and contamination of the previous season's nests. The plants are often actively sought out by birds and are also typically aromatic in nature (Clark & Mason, 1985; Lambrechts & Dos Santos, 2000; Petit *et al.* 2002). These factors lend circumstantial support to the NPH, i.e. these aromatic herbs provide some beneficial effects to chicks indirectly, by decreasing nest parasites or pathogens. There is, however, also some evidence to support the drug hypothesis: that plants can have a direct beneficial effect on chicks, for instance by potentiation of their immune system, independent of pathogen numbers. The mate hypothesis – that plants brought by males are a means of attracting a female mate – is also widely supported, especially in species such as starlings, in which the plants are brought only by males. The amount of plants brought could indicate to a female the strength and therefore paternal quality of a male as well as aid bond formation (Brouwer

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& Komdeur, 2004). Gwinner *et al.* (2013) also showed that yolk testosterone concentrations in European starlings' eggs increased with more green material in the nest; they suggest that females therefore adjust testosterone levels in the eggs, optimising offspring growth, dependent on the quality of the nest.

Some bird species move site when parasite load becomes too great (Feare, 1976); however, secondary cavity nesters are less able to do this and must frequently re-use nest sites. Therefore one would expect additional behaviours to have evolved in order to counteract high parasite loads in these situations. Clark & Mason (1985) suggest three major criteria if the NPH is to stand: firstly, birds must actively introduce these aromatic plants into their nests (i.e. they are found at a higher proportion in the nest than they are in the natural surroundings); secondly, the plants must have a different volatile composition to others (as this is often where their insecticidal/antibacterial properties lie, and how birds would identify them); and, thirdly, they must be effective at controlling parasite numbers or effects. Furthermore, if the NPH is to be explained by beneficial effects on the offspring, then the parasites affected must pose a threat to fitness. If this mechanism is the true evolutionary reason for this behaviour then the detrimental effects of these parasites must compromise survival or fecundity of adults, and nest building must be a heritable trait (Clark & Mason, 1988).

In this review, the literature will be searched to assess the inclusion of fresh green plant material into birds' nests and the possible evolutionary function of this behaviour, with specific focus on the NPH. Studies providing observational and experimental evidence for and against this hypothesis will be collated and summarized, and the major factors affecting them are discussed. Which hypotheses are most plausible will also be assessed as well as what factors affect the credibility of studies and therefore how study design can affect the clarity of results are examined.

OBSERVATIONAL STUDIES OF GREEN PLANT USE IN BIRDS' NESTS

Starling *Sturnus vulgaris*

Various different species of birds have been shown to regularly introduce fresh green plants into their nests; including starlings *S. vulgaris* (Clark & Mason, 1985; Gwinner, 1997; Brouwer & Komdeur, 2004; Gwinner & Berger, 2006), tits *Paridae* (Cowie & Hinsley, 1988; Banbura *et al.* 1995; Lambrechts & Dos Santos, 2000; Petit *et al.* 2002; Mennerat, 2008; Mennerat *et al.* 2009a; Pires *et al.* 2012; Tomas *et al.* 2012), sparrows *Passer* (Sengupta, 1981; Milton & Dean, 1999), wood

storks *Mycteria americana* (Rodgers *et al.* 1988), even raptors (Wimberger, 1984; Malan *et al.* 2002; Ontiveros *et al.* 2008; Dykstra *et al.* 2009; Heinrich, 2013). The behaviour is most extensively studied in starlings and blue tits, between which there are some major differences.

In starlings the plants are brought exclusively by the males, who are at the same time singing to attract females (Clark & Mason, 1985; Gwinner, 1997). Male starlings are also often polygynous, competing for multiple females to nest with. This, combined with the fact that amount of greenery brought is a function of the length of courtship and that males carry fresh green plants to the nest in an 'eye catching manner', lends more support, at least in the case of starlings, towards the plants serving a role in bond formation or male status signalling (mate hypothesis). Clark & Mason (1985) also found that the plants preferred by starlings (i.e. found in a higher proportion in nests than in the environment) produced higher concentrations of volatile compounds, than a random subset of plants from their environment.

Later studies have shown that starlings have a seasonal variation in olfactory sensitivity, and that during the breeding season changes suspected to occur in their olfactory bulb allow them to use olfactory cues for plant detection (Clark & Smeraski, 1990; De Groof *et al.* 2010). Gwinner & Berger (2008) also showed, through the use of aviary choice experiments involving chicks reared in different scented nests, that green nesting material selected through olfaction is reliant on experience-dependant as well as 'innate' cues. Blue tits have also been shown to have olfactory abilities sensitive enough to detect small changes in their aromatic environment as a result of the plants they incorporate; either when replacing herbs removed in experiments or reacting to experimental inclusion of herbs into their nests (Petit *et al.* 2002; Mennerat *et al.* 2005; Mennerat, 2008).

Blue tit *Cyanistes caeruleus*

In blue tits the behaviour is performed mainly by females and continues throughout the egg laying and chick stages and actually increases through the breeding cycle (Lambrechts & Dos Santos, 2000). This occurs when parasite load, and therefore threat to chicks in the nest, would be highest (Tripet & Richner, 1999). Two studies have noted that fresh green plants are brought by females specifically in the evening, prior to roosting (Cowie & Hinsley, 1988; Banbura *et al.* 1995). However, in these studies the plants were either not accurately identified or were not specifically aromatic, and therefore may serve as insulation. A range of highly aromatic herbs, detectable even by human olfaction, have been recorded being brought to the

nest by blue tits, and the behaviour in this species has only been reported in Mediterranean regions of Europe; mainly Corsica (Banbura *et al.* 1995; Lambrechts & Dos Santos, 2000; Petit *et al.* 2002; Mennerat, 2008) and Portugal (Pires *et al.* 2012). Mennerat *et al.* (2009a), showed there to be significant variation in herb composition between individuals within a territory and that this variation was conserved between years, suggesting an individual preference by blue tits for different aromatics. The inclusion of these specifically aromatic herbs, and the fact that in monogynous tits there is no obvious involvement in their annual courtship ritual, have lent more support towards the NPH for these species.

EXPERIMENTAL STUDIES USING GREEN PLANTS IN BIRDS' NESTS

Experimental studies, in which plants have been artificially introduced or removed, have mainly focused on blue tit and starling nests. They are widespread and common hole-breeding species, and have both been shown to perform these behaviours. They are examples of widely studied model organisms, which may partly explain why the behaviour is often documented in them.

Starling *S. vulgaris*

Clark & Mason (1985) found that the same plants that were preferred by starlings and produced higher concentrations of volatile compounds, were also more effective at preventing *Menacanthus* (louse) eggs from hatching and at inhibiting bacterial growth *in vitro*. They also found that wild carrot *Daucus carota*, naturally used by starlings, can decrease numbers of northern fowl mite *Ornithonyssus sylviarum* in the nest and that chicks in those nests had higher haemoglobin (Loye *et al.*, 1991); a direct factor in flight fitness of fledglings (Kovach & Szasz, 1968). Along with *Erigeron philadelphicus* (Asteraceae), also preferred by starlings, it can also decrease the emergence of feeding instars of the northern fowl mite (Clark & Mason, 1988).

Gwinner *et al.* (2000), replaced 148 starling nests over 3 consecutive years with entire man-made nests containing a mix of fresh and dry grass (*Brachypodium silvaticum*), to mirror the natural starling nests found in their area. Some were then supplemented with a mix of six aromatic herb species in order to investigate the effects of these herbs on chicks and parasite levels. They found no decreases in parasite numbers with herbs, but did find that starling chicks from nests to which herbs were added were heavier and had higher haematocrit, providing support for the drug hypothesis. The number of basophils, eosinophils, lymphocytes and heterophils per hundred leucocytes were also

assessed, after 'Haema Schnellfaerbung' staining. They found chicks from herb nests had lower lymphocyte levels; which could suggest they faced less of an immune challenge. This could be correlated with the lower bacterial loads also found in their herb nests. However at the same time the blood smears from herb chicks also contained higher levels of basophils; another white blood cell involved in parasite defence. Gwinner and Berger continued the study and data was collected on fledgling mass for a further 2 years (N = 202), mite load scores for 1 more year (N = 138) alongside bacteria (N = 80) and mosquitoes (N = 51) for a year each. After this increase in sample size still no effect of the plants was found on mite numbers, however fewer bacteria were collected from herb nests and chicks from these nests again had a significantly higher weight (Gwinner & Berger, 2005). Fewer mosquitoes were found in herb nests, however, mosquitoes numbers were low and this result was not quite significant ($P = 0.055$, $n = 26/25$) (Gwinner & Berger, 2006).

Brouwer & Komdeur (2004) investigated the NPH and mate hypothesis by emptying half of their starling nest-boxes to provide parasite free nest-boxes, while leaving some infested nest-boxes containing old nests. They also assigned half these nests to green removal or green addition treatments; nests either had a selection of the average species found naturally in their starlings' nests introduced or had any of the fresh green material added by the starlings themselves removed. This provided a 2×2 experimental design investigating the effects of the fresh green plant material added by the starlings and the presence of an old nest, on parasite levels, scabbing caused by mites on chicks and other parameters of chick health. They found no effect of experimentally increasing fresh plants in starling nests on parasite number, scabbing, chick body mass or survival. They also found no preference by starlings for the emptied nest-boxes and that no more greenery was added to the infested nest-boxes; it is difficult to assess how high the parasite loads were that remained in the 'infested' nest-boxes and there could also have been multiple other resources, such as time and energy, gained by a starling nesting in a box with an old nest in it. Chicks from cleaned boxes did have significantly lower scab scores but there was no correlation between scabbing and green material manipulations. They did however find that nests from which herbs were experimentally removed produced fewer clutches. Fauth *et al.* (1991) showed that scab scores on starling chicks could be lowered when parasite numbers were decreased, through use of insecticide, but again failed to show any effects of experimentally introducing herbs on parasite numbers. Brouwer & Komdeur (2004) confirmed earlier findings that fresh green plants are brought to the nest only by males. Female spotless starlings

Sturnus unicolor, a similar species with the same plant bringing behaviour have been shown to start removing these green plants while the males supply them; this is unlikely to have evolved for a parasite treatment function, but could relate to extending paternal investment efforts so males do not become polygynous or to avoid signalling to other females a productive male or nest in case of intraspecific brood parasitism (Veiga & Polo, 2012). Although some of the herbs brought may also function in anti-pathogenic ways, and even be aromatic in nature; perhaps providing an olfactory cue to male quality in a dark nest environment, the most likely evolutionary mechanism for this behaviour to have occurred in starlings is probably through sexual selection.

Tree swallow *Tachycineta bicolor*

In an interesting attempt to separate the mate and nest protection hypotheses, Shutler & Campbell (2007) experimentally introduced yarrow *Achillea millefolium* into the nests of tree swallow *T. bicolor* during laying; it is a species that does not naturally add fresh greenery to its nests. They found flea numbers to be twice as high in the control nests compared with the herb nests, and with no effect on chicks' size, number or leucocyte levels, provide more support for the NPH than for the drug hypothesis. Although one would expect the reduction in parasite load to have indirect improvements on chick health, the burden may not have been high enough to cause detectable detrimental effects in the first place. However in a similar study 3 years earlier involving tree swallows, yarrow instead was associated with higher parasite numbers, but also increased hatching success and lowered reproductive failure (Dawson, 2004).

Blue tit *C. caeruleus*

Mennerat *et al.* (2009c) experimentally enlarged blue tit broods, by moving chicks between nests at 2–3 days old, as well as repeatedly adding five species of aromatics post hatching. They showed that body mass was significantly increased by the aromatics in enlarged groups only, suggesting a possible condition-dependent positive effect. Feather development was also significantly increased by the presence of the aromatics, while decreased in the enlarged broods. In this experiment they removed all nidicolous parasites, by removing and microwaving nests, in order to show that the effect of aromatics occurred independently of them. Both of these studies provide strong support towards either the 'drug hypothesis' or a non-parasite related NPH, involving inhibition of other nest pathogens.

Tomas *et al.* (2012), found that experimental introduction of French lavender *Lavandula stoechas*

and cotton lavender *Santolina chamaecyparissus* into blue tit nests, two herbs commonly added by blue tits in nature, significantly decreased the abundance of parasites in the nests of yearling females only. The authors suggested that this could be because older females reduce parasites through other techniques such as preening and nest sanitation; behaviours which have been shown to increase in more heavily parasitized blue tit nests (Hurtrez-Boussès *et al.* 2000). Yearling females also had significantly higher numbers of blackfly and biting midges in their nests, but these parasites were unaffected by herbs. The plants were introduced every 3 days, so as to maintain the aromatic environment in the nest, but ceased at egg laying. It has been argued that this lack of any fresh plants for over 2 weeks during the hatchling stages and when parasite load is highest (Tripet & Richner, 1999), could partly explain the apparent ineffectiveness of the herbs. However, it has been shown that the amount of volatiles in the head space air of starling nests increases from the incubation to hatchling stages, despite no more fresh plants being added after laying (Gwinner, 2013). Suggestions were made that this is due to the increased humidity and movement of hatchlings breaking up the plants.

Lafuma *et al.* (2001) showed the repellent, and possibly also masking, effects of a mixture of these commonly used aromatic herbs against mosquitoes *Culex pipiens*. They showed that a mix of herbs (as well as *Lavandula* species by themselves) had significant repellent effects on mosquitoes. They also had a repellent, or possibly masking, effect when mosquitoes were introduced to live chicks in a choice experiment. Over a 14 h period mosquitoes had access to two boxes, one containing a live chick and the following four aromatic herbs; yarrow *Achillea ligustica*, curry plant *Helichrysum italicum*, French lavender *Lavandula stoechas* and pink rock-rose *Cistus Creticus*, and one simply containing a chick. This shows the possibility of overlooking the effects of flying insects, parasites that are rarely measured in these studies, instead focussing on the more easily detected nidicolous parasites. Bacteria are also often overlooked; Mennerat *et al.* (2008, 2009b), when introducing two herbs commonly found in blue tit nests, failed to show any effect on blowfly *Protocalliphora* numbers, but found decreases in bacterial richness and diversity.

Table 1 displays all the studies found by literature review, which experimentally tested the NPH in the field, by artificially adding different aromatic herbs to birds' nests and assessing parasite or pathogen loads as well as various parameters of chick health.

Limitations of studies

There is great heterogeneity in the results of experimental tests of the NPH, and there are multiple

Table 1. Studies investigating the NPH through artificial manipulation of aromatic herbs in wild birds' nests

Authors	Clark and Mason	Fauth <i>et al</i>	Gwinner <i>et al</i>	Brouwer and Komdeur	Dawson
Year published	1985	1988	1991	2004	2004
Host species	European starling <i>S. vulgaris</i>	European starling <i>S. vulgaris</i>	European starling <i>S. vulgaris</i>	European starling <i>S. vulgaris</i>	Tree swallow <i>T. bicolor</i>
Sample size (N)	N/A	12	100	n/a	39
Geographic location	USA	USA	USA	The Netherlands	Canada
Plants introduced	Mixed subset of listed plants ^a	Wild carrot <i>D. carota</i>	Plant removal only	Average species found in their nest boxes; not listed	Mixed subset of listed plants ^a
Parasites and pathogens measured	lice <i>Menacanthus</i> fowl mites <i>O. sylvitarum</i> bacteria (<i>Streptococcus aurealis</i> , <i>Staph epidermis</i> , <i>Pseudomonas aeruginosa</i> , and <i>Escherichia coli</i>) – tested in vitro	Fowl mites <i>O. sylvitarum</i>	Fowl mites <i>O. sylvitarum</i>	Red mites <i>Dermanyssus gallinae</i> , Mallophaga (biting lice), Hen fleas <i>Ceratophyllus gallinae</i>	All nidicolous ectoparasites
Effects seen	Plants preferred by starlings and decreased lice hatching and inhibited growth of <i>S. aurealis</i> , <i>S. epidermis</i> and <i>P. aeruginosa</i>	Herb nests contained fewer mites and chicks had higher haematocrit	No decrease in nest parasites	No effect of green material on scab score (caused by mites), body mass or survival to fledging	Increased fleas with herb treatment. But also increased hatching success and decreased reproductive failure
Hypothesis support for most:	NPH	NPH	Mate hypothesis	Mate hypothesis	Drug hypothesis
Authors	Gwinner and Berger	Shutler and Campbell	Mennerat <i>et al</i>	Mennerat <i>et al</i>	Tomas <i>et al</i>
Year published	2005	2007	2009 (a)	2009 (c)	2012
Host species	European starling <i>S. vulgaris</i>	Tree swallow <i>T. bicolor</i>	Blue tit <i>C. caeruleus</i>	Blue tit <i>C. caeruleus</i>	Blue tit <i>C. caeruleus</i>
Sample size (N)	Combined with data from previous 3 years (Gwinner <i>et al.</i> 2000); fledgling mass (N = 202), mite load (N = 138), bacteria (N = 80)	67	40	80	59
Geographic location	Germany	Canada	Corsica	Corsica	Spain
Plants introduced	Mixed subset of listed plants ^a	yarrow <i>A. millefolium</i>	Mixed subset of listed plants ^a	Mixed subset of listed plants ^a	Mixed subset of listed plants ^a

Parasites and pathogens measured	Red mite <i>D. gallinae</i> and bacteria (non-specific)	Hen fleas <i>C. gallinae</i>	Blowfly <i>protocalliphora</i> bacterial richness and diversity	Removed all blowflies <i>protocalliphora</i> and nest parasites, carried out brood manipulation	Biting midges <i>Culicoides</i> blackflies (Simuliidae) mites <i>D. gallinoides</i> Hen fleas <i>Ceratophyllus gallinae</i> blowflies <i>protocalliphora</i>
Effects seen	Herb nests had less bacterial colonies, higher chick mass and haematocrit. No effect on mites were seen	Fleas double in control nests, but no effect on chick size, fledging number or leukocyte levels	Decreased bacterial richness and diversity, no effect on blowfly numbers	Increased chick mass, haematocrit and feather growth	Plants decreased nidicolous parasites, but not flies, in young females' nests only
Hypothesis study provides support for most	Drug and nest protection hypotheses	NPH	NPH	Drug and NPH	NPH

^a Plant species include: *Daucus carota*, *Achillea millefolia*, *Lavandula stoechas*, *Helichrysum italicum*, *Santolina chamaecyparissus*, *Calamintha nepeta*, *Achillea ligustica*, *Pulicaria odora*, *Bronnus inermis*, *Aegopodium podagraria*, *Heracleum sphondylium*, *Sambucus niger*, *Anthriscus sylvestris*, *Salix alba*, *Lamium purpureum*, *Agrimonia parviflora*, *Solidago rugosa*, *Solidago ulmifolia*, *Conium maculatum*, *Polemonium reptans*, *Glechoma hederacea*, *Geum virginianum*, *Geum canadense*, *Taraxacum officinale*, *Lonicera japonica*, *Alliaria officinalis*, *Senecio obovatus*

potential reasons for this. Firstly the NPH itself is fairly broad and various nest ‘pathogens’ have been shown to be affected by herbs, including fleas, mites, lice, mosquitoes and bacteria. Therefore studies may fail to show any effect by not accounting for the pathogen affected by the plant in the bird species used in their study. There is also the possibility that the effects of the plants are condition-dependent and are only measurable when the health of the birds is compromised, either due to pathogen load or environmental conditions. Food provisioning and begging have been shown to increase in chicks affected by parasites; in profitable years this increased feeding by parents could compensate any negative effects of pathogens on young, transferring negative effects to parents instead, and making any positive effect of the herbs on chicks undetectable (Christe *et al.* 1996; Tripet & Richner, 1997). Goodenough *et al.* (2011) suggests that birds nesting in man-made nest boxes, which are cleaned out annually between seasons, typically have to deal with lower parasite loads compared with those in natural cavities, and are therefore perhaps less likely to produce measurable effects on pathogen load.

Sample size of studies also varies widely and could account for some of the variation in results. Several studies which failed to detect a change in nest pathogens have had relatively small sample sizes: Gwinner *et al.* (2000) had 7 herb and 8 control nests, Dawson (2004) had 8 herb and 5 control nests. Studies that did produce a measurable effect of the herbs generally had much greater sample sizes: Tomas *et al.* (2012) had 32 herb and 27 control nests, Mennerat *et al.* (2009b) had 20 of each, and Gwinner & Berger (2005) had 79 nests in total.

With so many confounding variables, which are often impossible to eliminate in field trials like these, sample size is likely to be a limiting factor. If for instance parasite or pathogen load in the nest is to be assessed, studies have already shown that this can be confounded by variables such as fledging date (Goodenough *et al.* 2011), brood size (James F. Scott-Baumann, unpublished 2013), between season cleaning of nest-boxes (Rendell & Verbeek, 1996) and the exact species of bird nesting (Goodenough & Stallwood, 2012). As more variables are measured and included in statistical models in order to control them, the sample size must also increase. Many different methods can be used to try to control for these factors; type of nest-box and specifically hole diameter can be altered and used to attract certain species of birds, brood manipulation could be performed to control for varying clutch size, trials could also be repeated across years or nest box sites to increase sample size, however, this adds yet more confounders. All these alterations are labour intensive and can be invasive for the birds, and with a very large sample

size, studies like this become impractical. In the south west of the UK it is hard to find single sites at all with 100+ nest-boxes, and even once all the nest-boxes are recruited for a study, birds may not choose to nest in all of them.

Given these complexities, it might be efficient to test putative anti-parasitic properties of phytochemicals such as essential oils using *in vitro* using bioassays, to complement field studies of nest protection. Gwinner & Berger (2006) identified candidate bioactive compounds in the field by sampling headspace air from starling nests; they found that although no more plants were added to the nest after egg laying, volatile substances including sabinene, myrcene, limonene, phellandrene and ocimene, which are all cyclic or mono-terpenes, were all still present during the hatchling period. Methods for testing the activity of such compounds against ectoparasites are well established (Perrucci *et al.* 1995; Yang *et al.* 2003; Kim *et al.* 2004; Bakkali *et al.* 2008; George *et al.* 2008), and could be allied to field studies in future.

CONCLUDING REMARKS

The behaviour of birds regularly seeking out and incorporating fresh aromatic herbs, of known beneficial value, into their nests is an interesting one, which could show a clear link between the negative impact of parasitism and the evolution of a self-medication strategy if more definitive evidence could be produced. Clear species differences have been shown to exist for this behaviour; male starlings supply green nesting material until egg-laying begins and it appears to have a function in mate bonding, while in blue tits, females perform the behaviour throughout the hatchling period and appear to use a more distinctly aromatic subset of herbs. Birds have been shown to replace these herbs after removal and to have the olfactory abilities to be able to detect their presence. Some studies have shown reductions in parasite or bacterial numbers after introducing the plants, some have shown apparent increases in chick health or nest success, but few have shown both. Investigations involving European starling nests in particular appear disparate with studies conducted in the United States showing clear effects on nidicolous parasites (Clark & Mason, 1985) while European studies show no effect on parasites (Gwinner *et al.* 2000; Brouwer & Komdeur, 2004) but clear increases in chick weight (Gwinner & Berger, 2005). It is possible that variations in experimental procedure have caused this, with plants incorporated during incubation and hatchling periods in the USA, but ceasing after laying in the European studies. It is also possible that a different mite species is present in the two colonies and that a different host–parasite interaction may have evolved in the USA since the introduction

of European starlings around 1900 (Mirsky, 2008). All studies highlight behaviour indicative of an involvement in mate selection. It is possible therefore that for this species plants could be involved in mating as well as providing protection to chicks later on, adding to the evolutionary development of this behaviour.

There is clearly insufficient evidence shown in this review, despite spanning 30 years, to definitely explain the evolutionary cause for birds adding fresh green aromatic herbs to their nests. It is a behaviour into which further research is required, for instance the presence of fresh herbs being regularly brought to nests could affect the incubation temperature of eggs in the nest, which could have impacts on embryo development and the future fitness of the chicks. Several studies have also shown the presence of predatory mites *Androlaelaps casalis* in starling nests (Wolfs *et al.* 2012), which have been shown to negatively correlated with poultry red mites on which they prey (Lesna *et al.* 2009). Investigation of these interactions could provide a further understanding of the hypotheses already discussed and the evolutionary reason for the inclusion of fresh green plants in nests by birds.

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REFERENCES

- Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008). Biological effects of essential oils—a review. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* **46**, 446–475.
- Banbura, J., Blondel, J., Wilde-Lambrechts, H. and Perret, P. (1995). Why do female blue tits (*Parus caeruleus*) bring fresh plants to their nests? *Journal Für Ornithologie* **136**, 217–221.
- Brouwer, L. and Komdeur, J. (2004). Green nesting material has a function in mate attraction in the European starling. *Animal Behaviour* **67**, 539–548.
- Christe, P., Richner, H. and Oppliger, A. (1996). Begging, food provisioning, and nestling competition in great tit broods infested with ectoparasites. *Behavioral Ecology* **7**, 127–131.
- Clark, L. and Mason, J. (1985). Use of nest material as insecticidal and anti-pathogenic agents by the European starling. *Oecologia* **67**, 169–176.
- Clark, L. and Mason, J. (1988). Effect of biologically-active plants used as nest material and the derived benefit to starling nestlings. *Oecologia* **77**, 174–180.
- Clark, L. and Smeraski, C. A. (1990). Seasonal shifts in odor acuity by starlings. *Journal of Experimental Zoology* **255**, 22–29.
- Cowie, R. J. and Hinsley, S. A. (1988). Timing of return with green vegetation by nesting blue tits *Parus caeruleus*. *Ibis* **130**, 553–555.
- Dawson, R. D. (2004). Does fresh vegetation protect avian nests from ectoparasites? An experiment with tree swallows. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **82**, 1005–1010.
- Dykstra, C. R., Hays, J. L. and Simon, M. M. (2009). Selection of fresh vegetation for nest lining by red-shouldered hawks. *The Wilson Journal of Ornithology* **121**, 207–210.

- Fauth, P., Kremetz, D. and Hines, J. (1991). Ectoparasitism and the role of green nesting material in the European starling. *Oecologia* **88**, 22–29.
- Feare, C. J. (1976). Desertion and abnormal development in a colony of sooty terns *Sterna fuscata* infested by virus-infected ticks. *Ibis* **118**, 112–115.
- De Groof, G., Gwinner, H., Steiger, S., Kempnaers, B. and Van der Linden, A. (2010). Neural correlates of behavioural olfactory sensitivity changes seasonally in European starlings. *PLoS ONE* **5** (12).
- George, D. R., Callaghan, K., Guy, J. H. and Sparagano, O. A. E. (2008). Lack of prolonged activity of lavender essential oils as acaricides against the poultry red mite (*Dermanyssus gallinae*) under laboratory conditions. *Research in Veterinary Science* **85**, 540–542.
- Goodenough, A. E., Elliot, S. L. and Hart, A. G. (2011). Do orientation-based differences in nestbox temperature cause differential ectoparasite load and explain patterns of nest-site selection and offspring condition in great tits? *International Journal of Zoology* **2011**. Retrieved from <http://dx.doi.org/10.1155/2011/514913>.
- Goodenough, A. and Stallwood, B. (2012). Differences in culturable microbial communities in bird nestboxes according to orientation and influences on offspring quality in great tits (parus Major). *Microbial Ecology* **63**, 986–995.
- Gwinner, H. (1997). The function of green plants in nests of European starlings (*Sturnus vulgaris*). *Behaviour* **134**, 337–351.
- Gwinner, H. (2013). Volatile Compounds in Nest Herbs. In *Chemical Signals in Vertebrates 12*. (ed. East, M. L., Dehnhard, M.), p. 356. Springer, Berlin, Germany.
- Gwinner, H. and Berger, S. (2005). European starlings: nestling condition, parasites and green nest material during the breeding season. *Journal of Ornithology* **146**, 365–371.
- Gwinner, H. and Berger, S. (2006). Parasite defence in birds: the role of volatiles. *Acta Zoologica Sinica* **52**(Supplement), 280–283.
- Gwinner, H. and Berger, S. (2008). Starling males select green nest material by olfaction using experience-independent and experience-dependent cues. *Animal Behaviour* **75**, 971–976.
- Gwinner, H., Oltrogge, M., Trost, L. and Nienaber, U. (2000). Green plants in starling nests: effects on nestlings. *Animal Behaviour* **59**, 301–309.
- Gwinner, H., Yohannes, E., Schwabl, H. (2013). Nest composition and yolk hormones: do female European starlings adjust yolk androgens to nest quality? *Avian Biology Research* **6**, 307–312. <http://dx.doi.org/10.3184/175815513X13823680612392>
- Heinrich, B. (2013). Why does a Hawk build with green nesting material? *Northeastern Naturalist* **20**, 209–218.
- Hurtrez-Boussès, S., Renaud, F., Blondel, J., Perret, P. and Galan, M.-J. (2000). Effects of ectoparasites of young on parents' behaviour in a Mediterranean population of Blue Tits. *Journal of Avian Biology* **31**, 266–269.
- Kim, S.-I., Yi, J.-H., Tak, J.-H. and Ahn, Y.-J. (2004). Acaricidal activity of plant essential oils against *Dermanyssus gallinae* (Acari: Dermanyssidae). *Veterinary Parasitology* **120**, 297–304.
- Kovach, A. G., Szasz, E. (1968). Survival of a pigeon after graded haemorrhage. *Acta Physiol Acad Sci Hung* **34**, 301–309.
- Lafuma, L., Lambrechts, M. M. and Raymond, M. (2001). Aromatic plants in bird nests as a protection against blood-sucking flying insects? *Behavioural Processes* **56**, 113–120.
- Lambrechts, M. M. and Dos Santos, A. (2000). Aromatic herbs in corsican blue tit nests: the “potpourri” hypothesis. *Acta Oecologica* **21**, 175–178.
- Lesna, I., Wolfs, P., Faraji, F., Roy, L., Komdeur, J., and Sabelis, M. W. (2009). Candidate predators for biological control of the poultry red mite *dermanyssus gallinae*. *Experimental and Applied Acarology* **48**, 63–80.
- Loye, J. E. and Zuk, M. (eds.), Clark, L. (contributor). (1991). The nest protection hypothesis: the adaptive use of plant secondary compounds by European starlings. In *Bird-Parasite Interactions-Ecology, Evolution and Behaviour*. Oxford University Press, Oxford, pp. 204–221.
- Malan, G., Parasram, W. A. and Marshall, D. J. (2002). Putative function of green lining in black sparrowhawk nests: mite-repellent role? *South African Journal of Science* **98**, 358–360.
- Mennerat, A. (2008). Blue tits *Cyanistes caeruleus* respond to an experimental change in the aromatic plant odour composition of their nest. *Behavioural Processes* **79**, 189–191.
- Mennerat, A., Bonadonna, F., Perret, P. and Lambrechts, M. M. (2005). Olfactory conditioning experiments in a food-searching passerine bird in semi-natural conditions. *Behavioural Processes* **70**, 264–270.
- Mennerat, A., Perret, P., Caro, S. P., Heeb, P. and Lambrechts, M. M. (2008). Aromatic plants in blue tit *Cyanistes caeruleus* nests: no negative effect on blood-sucking *Protocalliphora* blow fly larvae. *Journal of Avian Biology* **39**, 127–132.
- Mennerat, A., Perret, P. and Lambrechts, M. M. (2009a). Local individual preferences for nest materials in a passerine bird. *Plos ONE* **4**, e5104.
- Mennerat, A., Mirleau, P., Blondel, J., Perret, P., Lambrechts, M. M. and Heeb, P. (2009b). Aromatic plants in nests of the blue tit *Cyanistes caeruleus* protect chicks from bacteria. *Oecologia* **161**, 849–855.
- Mennerat, A., Perret, P., Bourgault, P., Blondel, J., Gimenez, O., Thomas, D. W., Lambrechts, M. M. (2009c). Aromatic plants in nests of blue tits: positive effects on nestlings. *Animal Behaviour* **77**, 569–574.
- Milton, S. and Dean, R. (1999). Nesting thyme - the use of aromatic plants in cape sparrow nests. *Africa Birds and Birding* **1**, 37–39.
- Mirsky, S. (2008). Shakespeare to blame for introduction of European starlings to US. *Scientific American* Date accessed: 25/11/14 Available: <http://www.scientificamerican.com/article/call-of-the-reviled/>
- Ontiveros, D., Caro, J. and Pleguezuelos, J. M. (2008). Green plant material versus ectoparasites in nests of bonelli's eagle. *Journal of Zoology* **274**, 99–104.
- Perrucci, S., Macchioni, G., Cioni, P. L., Flamini, G. and Morelli, I. (1995). Structure/activity relationship of some natural monoterpenes as acaricides against *Psoroptes cuniculi*. *Journal of Natural Products* **58**, 1261–1264.
- Petit, C., Hossaert-McKey, M., Perret, P., Blondel, J. and Lambrechts, M. M. (2002). Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. *Ecology Letters* **5**, 585–589.
- Pires, B. A., Belo, A. F. and Rabaca, J. E. (2012). Aromatic plants in eurasian blue tit nests: the “nest protection hypothesis” revisited. *Wilson Journal of Ornithology* **124**, 162–165.
- Rendell, W. B. and Verbeek, N. A. M. (1996). Old nest material in nestboxes of tree swallows: effects on reproductive success. *The Condor* **98**, 142–152.
- Rodgers, J. A. J., Wenner, A. S. and Schwikert, S. T. (1988). The use function of green nest material by wood storks. *Wilson Bulletin* **100**, 411–423.
- Sengupta, S. (1981). Adaptive significance of the use of margosa leaves in nests of house sparrows *Passer Domesticus*. *Emu* **81**, 114–115.
- Shutler, D. and Campbell, A. A. (2007). Experimental addition of greenery reduces flea loads in nests of a non-greenery using species, the tree swallow *Tachycineta bicolor*. *Journal of Avian Biology* **38**, 7–12.
- Tomas, G., Merino, S., Martinez-de la Puente, J., Moreno, J., Morales, J., Lobato, E., del Cerro, S. (2012). Interacting effects of aromatic plants and female age on nest-dwelling ectoparasites and blood-sucking flies in avian nests. *Behavioural Processes* **90**, 246–253.
- Tripet, F. and Richner, H. (1997). Host responses to ectoparasites: food compensation by parent blue tits. *Oikos* **78**, 557–561.
- Tripet, F. and Richner, H. (1999). Dynamics of Hen Flea *Ceratophyllus gallinae* subpopulations in blue tit nests. *Journal of Insect Behavior* **12**, 159–174.
- Veiga, J. and Polo, V. (2012). Female spotless starlings *Sturnus unicolor* remove green plants from their nests. *Journal of Ornithology* **153**, 291–296.
- Wimberger, P. (1984). The use of green plant-material in bird nests to avoid ectoparasites. *Auk* **101**, 615–618.
- Wolfs, P. H. J., Lesna, I. K., Sabelis, M. W., Komdeur, J. (2012). Trophic structure of arthropods in starling nests matter to blood parasites and thereby to nestling development. *Journal of Ornithology* **153**, 913–919.
- Yang, Y.-C., Lee, S.-H., Lee, W.-J., Choi, D.-H. and Ahn, Y.-J. (2003). Ovicidal and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. *Journal of Agricultural and Food Chemistry* **51**, 4884–4888.