



Aromatic plants in nests of blue tits: positive effects on nestlings

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For altricial birds, detailed studies of parental care include egg incubation, brooding and chick feeding, all of them being crucial to offspring survival. Few studies have explored nest building and maintenance from this perspective, although the nest is the first environment experienced by hatchlings. On Corsica, blue tits, *Cyanistes caeruleus*, incorporate fresh fragments of several species of aromatic plants in the nest cup throughout the nesting period, and replenish the nest with fresh fragments of the same plant species quickly after experimental removal. In this study, we experimentally tested whether aromatic plants used by blue tits affect the growth and condition of nestlings. Because we expected the effects of plants to depend on environmental conditions, we tested this hypothesis both in enlarged and in control broods. Aromatic plants positively affected chick mass gain in enlarged broods. Chicks in nests containing aromatic plants also had higher haematocrit levels. In addition, in 1 of the 2 study years, feathers developed faster in aromatic-treated nests than in nests where aromatic plants had been replaced by a neutral nest material (moss). However, no significant effect of aromatic plants on chick body size at fledging was found. We propose several hypotheses on the proximal mechanisms whereby aromatic plants affect chick growth and condition.

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Parental care is a crucial component of reproduction in higher vertebrates (Clutton-Brock 1991). In contrast to the many studies focusing on incubation, brooding and chick feeding in altricial birds, surprisingly few studies have explored nest building and nest maintenance as components of parental care, although the nest is the first environment experienced by hatchlings. Nest composition and structure may be very important to the brood, because they affect both the physical properties of the nest (e.g. thermal insulation, Lombardo et al. 1995; Hilton et al. 2004) and the nest microorganisms and invertebrate communities, including nest parasites, that nestlings encounter during growth (Heeb et al. 2000; Lucas & Heeb 2005). Therefore, in most species, especially in

hole-nesting species which may reuse the same cavity for several breeding attempts, caring for the nest should be selected for (Lack 1968).

The nest matrix of many species often consists of various materials including dry grass, moss or twigs, whereas lining materials include mammal hair or down feathers. In addition to basic nest materials, some bird species regularly add to their nests fresh plant fragments which are rich in volatile secondary compounds (Wimberger 1984; Clark & Mason 1985). These plant species, which are actively selected by the birds, often represent a small proportion of the species present in the habitat (Gwinner 1997; Gwinner et al. 2000; Lambrechts & Dos Santos 2000). Three main hypotheses may be proposed to explain this behaviour.

The first and most frequently invoked hypothesis, called the 'nest protection hypothesis', suggests that nest greenery protects the nestlings from detrimental nest ectoparasites, thereby providing benefits to the brood (Clark & Mason 1985). The second 'courtship hypothesis' suggests that the use of green plants during nest building may play a role in mate attraction, for instance when the ability to find particular plants reflects aspects related to territory and/or individual quality that may be beneficial to chicks (Fauth et al. 1991; Gwinner 1997; Brouwer & Komdeur 2004; Polo

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et al. 2004; Veiga et al. 2006). A third hypothesis, the 'drug hypothesis', proposes that the addition of aromatic plants to nests has positive effects on the growth and body condition of chicks irrespective of any effect on nest ectoparasites (Gwinner et al. 2000).

In the European starling, *Sturnus vulgaris*, the effects of nest greenery on chick phenotype have been investigated in detail. Adding green material to starling nests did not affect nest ectoparasite loads (Fauth et al. 1991; Gwinner et al. 2000; Gwinner & Berger 2005), but resulted in higher levels of blood haemoglobin in nestlings (Clark & Mason 1988) and positively affected both chick body mass and haematocrit, together with other blood components (lymphocyte and basophil counts, Gwinner et al. 2000). These effects were found especially under unfavourable environmental conditions (Gwinner & Berger 2005).

On Corsica, hole-nesting female blue tits, *Cyanistes caeruleus*, actively incorporate fresh fragments of Mediterranean-type aromatic plants to the nest cup (e.g. *Lavandula stoechas*, *Achillea ligustica*, *Helichrysum italicum*, *Mentha suaveolens*). From the end of nest construction until fledging, they add aromatic plant fragments to their nests daily and replenish them with fresh fragments of the same plant species within a day of experimental removal (Petit et al. 2002; A. Mennerat, unpublished data). The aromatic plant species found in blue tit nests represent only a small fraction of the plants available in the habitat (Petit et al. 2002). Some of the selected plant species possess *in vitro* antiseptic or fungicidal properties (Petit et al. 2002; Rossi et al. 2007) and repel blood-sucking mosquitoes in laboratory conditions (Lafuma et al. 2001). Yet they do not affect infestation by nest-dwelling ectoparasites (Mennerat et al. 2008). A noticeable difference from other greenery-using passerines (e.g. European starling, Gwinner et al. 2000) is that female blue tits keep adding aromatic plants to their nests during the whole nestling period, which strongly suggests that these plants have some fitness-related effect during this time.

In this study, we tested the hypothesis that fresh aromatic plant fragments have positive effects on nestling development and condition, even in the absence of nest ectoparasites (the 'drug hypothesis'). Because such effects are expected to be stronger under food-limited conditions, we also manipulated the workload of parent tits by enlarging broods, thereby increasing food constraints to nestlings. We used a two-way experimental design by (1) manipulating the aromatic plants and (2) enlarging broods. We predicted that nestlings in aromatic-treated nests, compared to control nests, should develop faster and be in better condition at fledging, and that these effects should be more pronounced in enlarged broods.

METHODS

Study Site and Field Protocols

The study was conducted in 2006 and 2007 in a Corsican population of blue tits using nestboxes for breeding ('Pirio' site, 42°31'N, 08°46'E, evergreen oakwood, *Quercus ilex*). All nestboxes were monitored throughout the breeding season to determine the onset of egg laying, clutch size, hatching date, the number of hatchlings and the number of chicks fledged (for a description of the site and field protocols, see Blondel 1985). Nestlings were ringed at day 5 posthatching. Every second day from day 5 posthatching to day 15 posthatching, we weighed them (± 0.1 g) with a Pesola spring balance and scored the development of their wing feathers according to a pictorial guide of typical feather development in blue tit chicks (P. Perret, http://www.cefe.cnrs.fr/esp/pdf/PP_SuiviRepro.pdf). Since hatching asynchrony is frequent in the study population (Caro et al. 2005), broods visited at day 5 posthatching often contained younger chicks. To distinguish between

these chicks before we ringed them, we cut their head down feathers in individually distinct patterns. Individual chick age was calculated from the age assessed when chicks were first weighed. Tarsus length, wing length and haematocrit at fledging were measured at days 14–15 posthatching. Tarsus length was measured (± 0.1 mm) with a digital calliper. Wing length was measured (± 0.5 mm) with a ruler. Chick body mass and tarsus length are often associated with the probability of offspring recruitment in the local blue tit population (Blondel et al. 1998; Heeb et al. 1999). We measured wing length because it is sensitive to the rearing environment and maternal effects (e.g. Råberg et al. 2005; Biard et al. 2007). Together with body mass, haematocrit determines chick aerobic capacity, which is related to postfledging survival in the study population (Thomas et al. 2007). We therefore used haematocrit as a measure of nestling physiological condition. For each chick, around 20 μ l of blood were collected from the brachial vein into a heparinized microcapillary tube, and centrifuged for 3 min at 13 000 rpm. The haematocrit value was defined as the percentage volume of erythrocytes in the total blood sample.

Experimental Design

Our experiments included two components: brood enlargement and addition of aromatic plants to nests in a 2×2 experimental design. The total sample was 80 nests (596 chicks), that is, 40 nests each year with 10 nests randomly assigned to each experimental treatment. In the study population, the main ectoparasites are nest-dwelling haematophagous blow fly larvae (*Protophthora* spp.). Blow fly infestation intensity is both extremely high and highly variable among nests and has severe consequences for chick growth, condition and postfledging survival (Hurtrez-Boussès et al. 1997; Simon et al. 2004; Thomas et al. 2007). Since aromatic plants do not affect blow fly infestation intensity in the study population (Mennerat et al. 2008), to minimize phenotypic variability of chicks related to variation in blow fly infestation intensity, we removed blow fly larvae from all nests under study (see below).

Brood Enlargement

We manipulated broods when chicks were 2–3 days old. Nestlings were transported in cotton bags between nests. In nests randomly assigned to the 'enlarged' treatment, we enlarged broods by adding three nestlings of the same age originating from supplementary nestboxes located near the study site. To control for effects of the external origin of chicks, we cross-fostered three chicks of equal ages in nests assigned to the 'nonenlarged' treatment. For other reasons not related to this study, we also cross-fostered three chicks in nests assigned to the 'enlarged' treatment. Mean \pm SD brood size was 6.7 ± 1.4 chicks in control broods and 9.1 ± 1.4 chicks in enlarged broods.

Addition of Aromatic Plants to Nests

In nests randomly assigned to the 'aromatic' treatment, we added fresh fragments of five locally abundant aromatic plant species which are frequently found in blue tit nests in this site (*L. stoechas*, *H. italicum*, *Calamintha nepeta*, *A. ligustica*, *Pulicaria odora*, personal observation). Leaves of these five aromatic species were added in equal proportions (0.2 g of each species, i.e. a total amount of 1 g), every second day from day 5 posthatching to day 14–15 posthatching. Before adding aromatic plants, we carefully removed all visible aromatic plant fragments added by blue tits or by the experimenter on previous days (see below). Therefore the quantity of fresh plant fragments in nests remained constant, both through time and across nests. Nests assigned to the control 'moss-treated' group received the same treatment as 'aromatic-treated'

nests, but fresh moss (1 g) was added instead of aromatic plants. After drying, 1 g of the aromatic plants used in this experiment weighed approximately 0.3 g. This amount lies within the range of natural variation of fresh aromatic plant fragments added to nests within 24 h, a fortiori within 48 h, by Corsican blue tits (0.03–0.31 g dry mass/nest per day, A. Mennerat, unpublished data).

Removal of Nest-dwelling Ectoparasites

To remove both the aromatic plants added by blue tits during each 2-day interval and nest-dwelling ectoparasites, we needed to collect nests. However, we also had to avoid switching nests between nestboxes, because this may have affected parental behaviour (Mennerat 2008). We therefore needed to make two nests out of each original nest and subsequently switch them every second day. For each nest, we proceeded as follows. At the beginning of the experiment (at brood manipulation), we collected half of the nest material. To minimize the potential impact of this reduction in nest volume on nestlings, we added a sufficient amount of fresh, microwaved moss at the bottom of the nestbox to restore the initial volume of the nest. We then carefully re-formed the cup shape of the nest with the remaining material from the original nest, including lining material at the top of it. This manipulation took no more than 2–3 min, during which nestlings were held in a cotton bag. We finally replaced all nestlings in the nest and checked that they were well installed in the nest cup before leaving. Back in the laboratory, we killed nest-dwelling ectoparasites by microwaving half-nests for 2 min and removed all detectable aromatic plant fragments from them. At the following visit (2 days after brood manipulation), we collected the nest materials previously left in the nestbox (microwaved moss and original nest materials). We then added some new, microwaved moss at the bottom of the nestbox and replaced the original nest materials (from which we had collected aromatic plant fragments and killed ectoparasites) on it before re-forming the cup of the nest as described above. At each subsequent visit to the nest, that is, every second day until day 14–15 posthatching, we switched the two nests and, back in the laboratory, removed both ectoparasites and aromatic plant fragments from the collected nests.

Statistical Analysis

We tested the effects of brood enlargement and addition of aromatic plants on repeated measures of chick mass and chick feather development using mixed-effects models with nest and individual (nested within nest) as random factors.

We used the first axis of a principal components analysis (PCA) from the correlation matrix of nestling mass, tarsus length and wing length at day 14–15 posthatching as an estimate of nestling body size at fledging. The first axis (PC1) accounted for 59% of the total variance. We tested the effects of brood enlargement and addition of aromatic plants on nestling body size and haematocrit at fledging using mixed-effects models with nest as a random factor. Because models considering tarsus length only (instead of PC1) gave similar results, we give only the results for PC1 scores here.

In all analyses, year, chick origin (same nest, other nest from the same site or nest from another nearby site), brood manipulation and plant treatment were included as fixed factors. To account for continuous mass gain and feather growth, we included both chick age and time of day at measure as covariates. Brood size was also included as a covariate. Because the effects of plants and brood enlargement were expected to differ according to environmental conditions, we also tested the 'brood manipulation*plant manipulation', 'year*plant manipulation' and

'year*brood manipulation' interaction terms. Whenever these interactions were statistically significant, we also performed separate models for 2006 and 2007 (respectively, for control and enlarged broods). Removing nonsignificant interactions from the models did not change the results. Therefore, for clarity and consistency, we kept all three interaction terms in all models. All analyses were done using the 'mixed' procedure in SAS 9.1 software (SAS Institute, Cary, NC, U.S.A.).

Ethical Note

Brood enlargement was aimed at increasing food constraints to nestlings, which indeed negatively affected their body size at fledging compared to control broods (see Results). After brood manipulation, however, no more nestlings died in enlarged than in control broods (nine nestlings died in control broods versus 10 in enlarged broods; binomial test: $P = 0.18$). In addition, we removed all ectoparasitic blow fly larvae from nests. This liberated both the chicks and their parents from the high constraints imposed by these parasites under natural conditions (Hurtrez-Boussès et al. 1997). As a consequence, the mean haematocrit of nestlings in experimental broods was much higher than in unmanipulated, naturally infested broods (unmanipulated broods: $\bar{X} \pm SE = 43.7 \pm 0.5\%$; experimental broods: $51.3 \pm 0.3\%$; t test: $t_{752} = 3.96$, $P < 0.0001$). In addition, mean body mass of nestlings in experimental broods was not significantly lower than that of nestlings in unmanipulated broods (unmanipulated broods: $\bar{X} \pm SE = 9.94 \pm 0.04$ g; experimental broods: 9.89 ± 0.04 g; t test: $t_{752} = 1.01$, $P = 0.31$). We are therefore confident that brood enlargement did not affect the chicks more than ectoparasites would have naturally done, had we not removed them. All manipulations were performed under the authorization of the Ministère Français de l'Environnement et du Développement Durable.

RESULTS

Body Mass Gain

Brood enlargement strongly reduced chick mass gain (Table 1), especially in 2006 (2006: $F_{1,893} = 31.68$, $P < 0.0001$; 2007: $F_{1,1047} = 0.82$, $P = 0.36$). Aromatic plants had a significant positive effect on body mass gain in enlarged broods ($F_{1,1046} = 5.82$, $P = 0.02$) but not in control broods ($F_{1,895} = 1.40$, $P = 0.24$; Fig. 1, Table 1).

Feather Development

Feather development of chicks was faster in 2007 than in 2006 (Table 1). Brood enlargement had a strong negative effect on chick feather development (Table 1), especially in 2006 (2006: $F_{1,893} = 8.24$, $P = 0.004$; 2007: $F_{1,1047} = 0.74$, $P = 0.39$). Aromatic plants had a significant positive effect on chick feather development in 2007, in both nonenlarged and enlarged broods ($F_{1,1047} = 4.20$, $P = 0.04$), but not in 2006 ($F_{1,893} = 0.35$, $P = 0.55$; Fig. 2). Feather development also differed according to chick origin (Table 1).

Body Size at Fledging

Chicks were larger in 2007 than in 2006 and size depended on chick origin (Table 1). In addition, chick body size was strongly reduced by brood enlargement, but was not significantly affected by the experimental addition of aromatic plants (Table 1).

Table 1

Effects of brood size manipulation (brood enlargement) and nest plant manipulation (addition of aromatic plants to nests) on phenotypic parameters of chicks

| | df | Body mass (total df=1944) | | Feather development (total df=1943) | | Body size (PC1) (total df=477) | | Haematocrit (total df=492) | |
|---------------------------------------|----|------------------------------|---------|--|---------|-----------------------------------|---------|-------------------------------|---------|
| | | F | P | F | P | F | P | F | P |
| Year | 1 | 2.11 | 0.15 | 4.07 | 0.04 | 19.47 | <0.0001 | 9.67 | 0.002 |
| Origin | 2 | 0.97 | 0.38 | 7.56 | <0.001 | 12.92 | <0.0001 | 0.27 | 0.77 |
| Time at measure | 1 | 548.00 | <0.0001 | 26.13 | <0.0001 | 0.87 | 0.35 | 9.86 | 0.002 |
| Chick age | 1 | 6063.58 | <0.0001 | 71939.0 | <0.0001 | 52.57 | <0.0001 | 21.19 | <0.0001 |
| Brood size | 1 | 0.72 | 0.40 | 0.98 | 0.32 | 0.18 | 0.67 | 1.17 | 0.28 |
| Brood manipulation | 1 | 21.41 | <0.0001 | 1.24 | 0.27 | 13.44 | <0.001 | 5.27 | 0.02 |
| Plant manipulation | 1 | 1.15 | 0.28 | 1.32 | 0.25 | 0.96 | 0.33 | 4.45 | 0.04 |
| Brood manipulation*plant manipulation | 1 | 5.29 | 0.02* | 0.64 | 0.43 | 0.01 | 0.93 | 0.15 | 0.70 |
| Year*plant manipulation | 1 | 0.05 | 0.82 | 3.42 | 0.06‡ | 1.15 | 0.28 | 0.57 | 0.45 |
| Year*brood manipulation | 1 | 4.38 | 0.04† | 6.60 | 0.01‡ | 0.10 | 0.75 | 0.21 | 0.64 |

Effects on body mass and feather development during growth (repeated measures from day 5 to day 15 posthatching) were tested using mixed-effects models with nest and individual (nested within nest) as random factors. Effects on body size (PC1 from a principal components analysis on body mass, tarsus length and wing length measured at day 14–15 posthatching) and haematocrit were tested using mixed-effects models with nest as random factor.

* In enlarged broods only.

† In 2006 only.

‡ In 2007 only.

Haematocrit

Chick haematocrit was higher in 2007 than in 2006 (Table 1). It was positively correlated with chick age but negatively correlated with time of day (Table 1). It was also higher in enlarged than in control broods and was positively affected by addition of aromatic plants (Fig. 3, Table 1).

DISCUSSION

The incorporation of fresh fragments of aromatic plants in their nests by blue tits is an unusual behaviour which had not been reported in previous studies (e.g. Perrins 1979). Our initial hypothesis, based on the 'nest protection hypothesis', was that these plants contribute in some way to preventing parasites from developing in the nest and attacking nestlings. This hypothesis seemed logical because our Corsican study populations of blue tits suffer from exceptionally high loads of blood-sucking blow fly larvae, the highest loads so far recorded in Europe (Hurtrez-Boussès et al. 1999). Testing this hypothesis in 2005 through manipulation of plants in nests did not provide any evidence of their direct effects

either on nest-dwelling ectoparasite loads or on body size and haematocrit value of nestlings (Mennerat et al. 2008). These results are consistent with most field experiments in other bird species, which did not find any significant effect of fresh plant materials on nest ectoparasite infestation (Rodgers et al. 1988; Fauth et al. 1991; Gwinner et al. 2000; Dawson 2004; Gwinner & Berger 2005; but see Shutler & Campbell 2007). So far, the 'nest protection hypothesis' has not received much empirical support.

However, the year when that experiment (Mennerat et al. 2008) was conducted was exceptionally favourable in terms of food supply during breeding, which may explain why we found neither positive effects of plants nor negative effects of parasites on breeding performance. A similar absence of effects of parasites when breeding conditions are highly favourable was found by Simon et al. (2004), who pointed out the huge yearly variation in breeding conditions in this population.

In this study testing the 'drug hypothesis', nestling feather development, body size and haematocrit were better in 2007 than in 2006. One explanation for such differences is that ambient temperatures were lower and rainfall higher in 2006 (unpublished data, available on request), which certainly made food provisioning

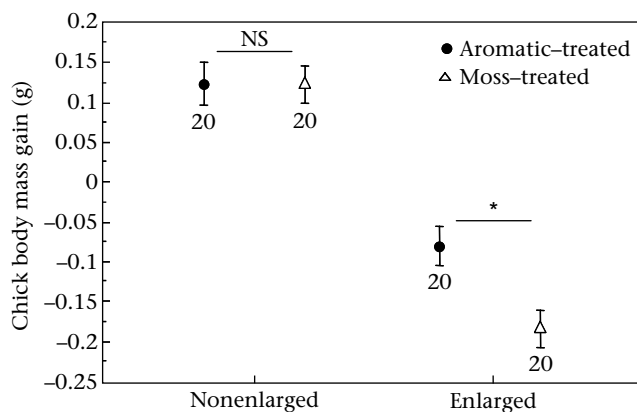


Figure 1. Effects of experimental addition of aromatic plants on chick body mass gain during growth (from 5 to 15 days posthatching), in enlarged and control broods. Chick body mass was corrected using the residuals of its regression against chick age and time of day. In nests containing experimentally added aromatic plants, chicks were approximately 0.1 g heavier than in control (moss-treated) nests. Sample sizes (number of nests) are indicated. Means are given \pm SE. *P* values are those resulting from mixed-effects models with nest and individual (nested within nest) as random factors (see Methods). **P* < 0.05.

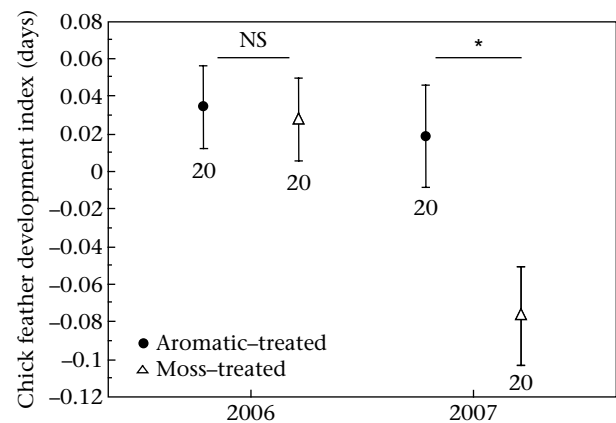


Figure 2. Effects of experimental addition of aromatic plants on chick feather development (from 5 to 15 days posthatching) in 2006 and 2007. Chick feather development index was corrected using the residuals of its regression against chick age and time of day. In nests containing experimentally added aromatic plants, chick feather development was on average 0.1 days faster than in control (moss-treated) nests. Sample sizes (number of nests) are indicated. Means are given \pm SE. *P* values are those resulting from mixed-effects models with nest and individual (nested within nest) as random factors (see Methods). **P* < 0.05.

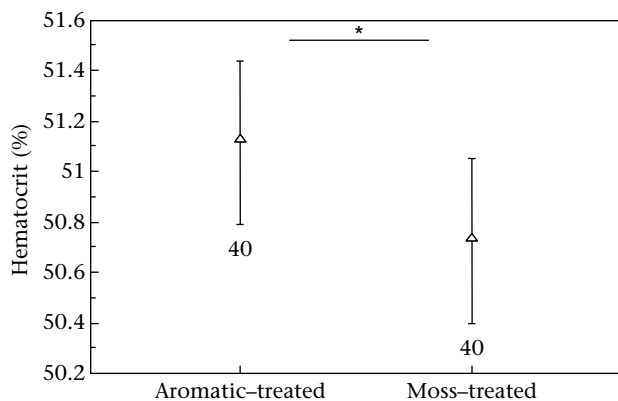


Figure 3. Effects of experimental addition of aromatic plants on nestling haematocrit at day 15 posthatching. Sample sizes (number of nests) are indicated. Means are given \pm SE. The *P* value is that resulting from a mixed-effects model with nest as random factor (see Methods). **P* < 0.05.

by the adults much more difficult in 2006 than in 2007, although food abundance was higher, as measured by the amount of caterpillar frass falling from the trees (Zandt 1994; peak values collected in the study site: 2006: 412 g/m² per day; 2007: 314 g/m² per day). Such variation in environmental constraints complicated the investigation of the effects of aromatic plants.

To disentangle the effects of plants from those of the environment, we used the 2 × 2 experimental protocol described above. We found that plants had a positive effect on chick mass growth, but only in enlarged broods, which confirms that plants have beneficial effects under constraining breeding conditions. This is consistent with the finding that in the European starling nest greenery increases chick body mass especially under unfavourable conditions (Gwinner et al. 2000; Gwinner & Berger 2005). Surprisingly, aromatic plants positively affected chick feather development in the best year (2007), when chicks were larger and had higher haematocrit levels, which partly contradicted our predictions. Whatever the proximate mechanisms involved, this means that aromatic plants can affect some aspects of chick growth even when breeding conditions are relatively good. To explain this unexpected result, we propose that the absence of an effect of plants on feather development in 2006 may have resulted from higher plasma concentrations of corticosterone in nestlings under higher environmental stress. In blue tit nestlings, a negative correlation was recently found between the mean ambient temperature during growth and the levels of corticosterone metabolites (Lobato et al. 2008). Food deprivation during growth is also known to lead to increased corticosterone levels in nestlings of the barn swallow, *Hirundo rustica* (Saino et al. 2003; but see Gil et al. 2008). Since corticosterone inhibits feather growth (e.g. Romero et al. 2006), higher levels in nestlings may have masked the beneficial effects of aromatic plants on feather growth in 2006.

The positive effects of aromatic plants on chick mass gain and feather development did not translate into a significant effect on chick body size at fledging. This is consistent with results from a previous experiment carried out in 2005 in the same study population, where addition of aromatic plants also had no effect on chick body size at fledging (Mennerat et al. 2008). We do not know yet the precise proximate mechanisms whereby plants affect the birds, but here we offer a potential explanation. A faster growth does not necessarily mean a larger final size, but can also be related to other components of chick condition such as their immune response (Soler et al. 2003; Brommer 2004). As found in a recent field experimental study, aromatic plants strongly reduce both the richness and density of bacteria on the ventral skin of nestlings (A. Mennerat, P. Mirleau, J. Blondel, P. Perret, M. M. Lambrechts &

P. Heeb, unpublished data). Since immune function is traded off against growth in altricial birds, any mechanism reducing immune challenge during growth would, as a side-effect, improve body growth rate (Sheldon & Verhulst 1996; Soler et al. 2003; Brommer 2004). From this perspective, our results support the hypothesis proposed by Gwinner et al. (2000) that fresh plants added to nests relate in some way to the immune system of birds.

Our results also confirm the finding by Gwinner et al. (2000) that chick haematocrit is positively affected by fresh plants added to the nest. Haematocrit is a major determinant of chick aerobic capacity, which is strongly related to postfledging survival in this population (Thomas et al. 2007). Therefore, higher haematocrit of nestlings in nests containing aromatic plants is probably beneficial in terms of fitness. We also found, however, that chick haematocrit is significantly higher in enlarged broods, which at first may seem contradictory. Nevertheless, it is consistent with the positive relationship previously found in this population between haematocrit and brood size (e.g. Mennerat et al. 2008). Since haematocrit is the ratio between the erythrocyte volume and the total blood volume, higher haematocrit values can result either from higher erythrocyte volume (reflecting an increase in erythropoiesis) or from lower total blood volume (reflecting dehydration of the chicks). One could argue that chicks facing higher constraints would, as a response, increase their metabolic capacity by producing more erythrocytes. This is unlikely to occur in our study population, where chick metabolic capacity decreases both with decreasing food availability and with increasing parasite load (Thomas et al. 2007). Therefore, we propose that higher haematocrit in enlarged broods relates to the dehydration status of nestlings (lower plasma volume) under food constraints, as has been suggested for pigeons, *Columba livia* (Kasprzak et al. 2006). Consistent with this hypothesis, haematocrit was negatively related to time of day at measure (Table 1), which may be explained by a better hydration status (lower haematocrit) at the end of the day than in the morning, that is, after the long night fast. A similar relationship was found in nocturnal bats such as the black myotis, *Myotis nigricans*, and the little brown bat, *M. lucifugus*, which had lower haematocrit in the morning, when they returned from feeding, than at the end of the day (Studier & Ewing 1971). The effects of aromatic plants on haematocrit, in contrast, cannot be simply explained in terms of dehydration of the nestlings and we could not find any reason why aromatic compounds in the nest would lead to lower plasma volume of nestlings. As a conclusion, we therefore propose that the effects of aromatic plants on nestling haematocrit reflect some related positive effects on their health (e.g. via a decrease in immune challenge during growth). This interpretation would, of course, need further checking, for example by assessing the dehydration status of nestlings when measuring haematocrit.

Finally, the positive effects of aromatic plants on nestlings might result from another, indirect mechanism related to sexual selection. A symmetrical hypothesis to the 'courtship hypothesis' investigated in male starlings (e.g. Gwinner 1997; Brouwer & Komdeur 2004) would state that, in blue tits, aromatic plants could be a signal of female quality. Males, perceiving aromatic odours emanating from their nests, could adjust their parental investment accordingly, which would result in better growth or condition of their chicks. In this study, we found no significant change in parental feeding behaviour, as recorded in a subsample of nests with video cameras (unpublished data). In addition, in a previous experiment, both male and female feeding blue tits were more reluctant to enter the nest cavity after experimental addition of aromatic plants to their nests (Mennerat 2008). Therefore, there is little support so far for the hypothesis that male blue tits would increase their feeding effort in response to aromatic plants added to their nests.

In conclusion, aromatic plants are likely to have longer-term consequences than the observed subtle, albeit significant, benefits

on chick growth and development. Addressing these questions which concern the health of the birds, a timely theme of research, will be a challenging issue requiring several more years of nest monitoring to assess the ultimate effects of aromatic plants on offspring survival and recruitment in the breeding population. In the shorter term, investigating the immune consequences of aromatic plants in blue tit nests will be the next and exciting step towards a better understanding of the effects of aromatic plants on blue tit nestlings. Undoubtedly, this will provide new insights into the adaptive value of this uncommon behaviour.

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