Green nesting material has a function in mate attraction in the European starling

LYANNE BROUWER & JAN KOMDEUR

Animal Ecology Group, University of Groningen

The function of fresh green nest material has long been debated. It has been suggested that it reduces the number of ectoparasites in nests and on nestlings (nest protection hypothesis), or is used by males to signal condition and paternal quality (male quality hypothesis) or is used as a sexually selected ornament to attract females (courtship hypothesis). We simultaneously tested these three hypotheses in the European starling, *Sturnus vulgaris*, in the field. Green material was carried by male starlings only, and mainly during nest building. It was not used to reduce ectoparasites. Males nesting in nestboxes that were experimentally contaminated with ectoparasites did not carry more green nest material than males nesting in control boxes, and experimental removal and addition of green material had no effect on the number of ectoparasites on the nestlings or on their body mass. Furthermore, the amount of green material carried into a nestbox was not associated with male body mass, paternal incubation attendance or nestling food provisioning. There is two-fold experimental evidence that males use green plant material to attract mates. First, removal of greenery resulted in a significantly lower percentage of nestboxes containing a clutch than the control or addition treatment. Second, unpaired male starlings sang more and carried more greenery into a nestbox when a caged female was positioned adjacent to the nestbox than when a caged male or an empty cage was present. Paired males, when subjected to the same experimental design, did not respond.

The addition of fresh green plant material to dry nest material is widespread among birds (Newton 1979; Wimberger 1984; Clark & Mason 1985). This green material is often replenished daily during the incubation and nestling stage (Brown & Amadon 1968; Beebe 1976; Newton 1979; Wimberger 1984). Several hypotheses regarding its function, although not mutually exclusive, have been proposed.

The 'nest protection' hypothesis suggests that volatile compounds in green nest material have biocidal effects on parasites and pathogens (Widmann 1922; Johnston & Hardy 1962; Wimberger 1984; Clark 1991). In many species parasites and pathogens lead to nest desertion, egg spoilage, and reduced growth and survival of nestlings (Feare 1976; Hitchner 1980; Duffy 1983; Hesse 1985; Møller 1990; Loye & Zuk 1991; Richner et al. 1993; Oppliger et al. 1994; Merino & Potti 1995). A finding consistent with this hypothesis is that species that frequently reuse their nests over successive years and that may suffer more from wintering nest ectoparasites use

fresh green nest material more often than species that build a new nest each year (Wimberger 1984; Clark & Mason 1985).

Green nest material could also be used by males to attract females ('courtship' hypothesis: Kessel 1957; Ali & Ripley 1974; Feare 1984; Fauth et al. 1991). In some species males decorate their nests to attract females. Decorations can consist of brightly coloured flowers (black-throated weaver, *Ploceus benghalensis*, and striated weaver, *Ploceus manyar*: Ali & Ripley 1974), bones and pieces of glass (spotted bowerbird, *Chlamydera maculata*: Borgia 1995), or stones (black wheatear, *Oenanthe leucura*: Moreno et al. 1994). Furthermore, males could use green nest material to signal their condition or paternal quality ('male quality' hypothesis: Gwinner 1997; see also Moreno et al. 1994). Females of species with biparental care could improve their reproductive success by choosing a mate that provides a lot of paternal care (Houston & Davies 1985; Westneat et al. 1990; Smith 1995; Soler et al. 1998; Smith & Härdling 2000). For example, the total number of stones carried by male black wheatears is an indication of paternal quality (Moreno et al. 1994). In contrast to these visual cues, fresh green nest material in dark nest cavities may act as an olfactory cue to attract

Correspondence: J. Komdeur, Animal Ecology Group, Centre for Eco-logical and Evolutionary Studies, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands (email: j.komdeur@biol.rug.nl).

females (Fauth et al. 1991). In this study on the European starling, *Sturnus vulgaris*, we tested experimentally the function of green nest material.

The facultatively polygynous European starling is a semicolonial hole-nesting passerine. Breeding pairs often build nests on the remains of old nests from previous years. Males contribute substantially to both incubation and feeding the young (Møller 1989; Pinxten et al. 1993; Smith et al. 1995; Komdeur et al. 2002) and both measures of paternal care are correlated with reproductive success (Pinxten & Eens 1994; Smith 1995; Komdeur et al. 2002). Only male starlings carry green plant material into their nests (Kessel 1957; Feare 1984; Clark & Mason 1985; Gwinner 1997), some of which is rich in volatile compounds (Clark & Mason 1985). Some studies found that these plants impair the development of blood-sucking mites and bacteria in nests (Clark & Mason 1985, 1988; Clark 1991), but others found no effect (Fauth et al. 1991; Gwinner et al. 2000). Furthermore, a reduction in blood-sucking parasites did not improve the growth or survival of nestlings (Powlesland 1977; Clark & Mason 1985, 1988; Walter & Hudde 1987; Fauth et al. 1991) nor did this result in increased blood haemoglobin levels (Clark & Mason 1988). Given the ambiguity of these results, there is no clear support for the nest protection hypothesis in the starling. Instead it has been suggested that starlings use green material for mate attraction or pair bonding (Eens et al. 1990, 1993; Fauth et al. 1991; Gwinner 1997; Komdeur et al. 2002). Until now, there has been no experimental support from the field for the courtship or the male quality hypotheses (Pinxten & Eens 1990; Fauth et al. 1991; Eens et al. 1993; Pinxten et al. 1995; Gwinner 1997; Komdeur et al. 2002).

In this study we investigated the nest protection and mate attraction hypotheses by manipulating the amount of green material in nests during the nest-building stage within natural limits. In addition, we monitored the frequency of males carrying green material and the singing activity of paired and unpaired males towards an experimentally introduced female and male. The male quality hypothesis was investigated nonexperimentally by relating the amount of green nest material carried in by males to male condition and the level of subsequent paternal care (incubation and food provisioning).

METHODS

Study Population and Data Collection

Starlings were studied at a colony of 112 nestboxes which consisted of three subcolonies (52, 34 and 26 nestboxes) at Vosbergen, near Groningen, The Netherlands, from 17 February to 1 June 2000. Although the subcolonies were separated by 500–1000 m, they can be considered as one colony for various reasons. (1) The distance between the first and the last nestbox in each subcolony also varies up to 500 m. (2) There is exchange of birds between subcolonies within and between years and polygynous males sometimes attend nestboxes and

hold mates simultaneously in different subcolonies (L. Brouwer, J. Komdeur & T. Overveld, personal observation). (3) The average day of laying the first egg and breeding success were similar for the subcolonies (subcolonies I, II and III: laying first egg (in April days): $\overline{X} \pm SE = 18.4 \pm 0.4$, $N = 11$, 19.0 ± 0.4, $N = 11$, and 19.1 ± 0.4, $N = 10$, respectively; number of nestlings per nestbox: $\overline{X} \pm SE = 5.0 \pm 0.33$, $N = 8$, 5.0 ± 0.54, $N = 11$, and 5.8 ± 0.29, $N = 10$, respectively). (4) The colony consisted of uniform nestboxes, situated about 6 m apart at a height of 2.5 m. The population was single-brooded and nested highly synchronously (all 33 females in the study population started laying within 5 days). To create equal conditions before the manipulations, we removed the old nesting material from all nestboxes on 17 February 2000, except for 13 nestboxes in subcolony III (see Nest protection hypothesis below).

From the arrival of starlings in the colony on 20 March 2000 onwards, we checked the nestboxes every other day for the presence of green nesting material. If present, the green material was identified to species level, weighed to the nearest 0.01 g with a 5 g pesola balance and marked with a permanent marker to distinguish new material from material already present. Because eggs are usually laid before 1100 hours (Meijer 1992; Pinxten & Eens 1998) we checked nests daily between 1100 and 1230 hours, for the presence of eggs and start of incubation (determined by feeling the egg temperature). Freshly laid eggs were numbered with indelible ink. Because it was not possible to determine the exact moment of pair formation, we defined courtship time as the period from the first day with greenery in the nest to the laying of the first egg (Gwinner 1997). If a bird was seen carrying green nesting material into the nestbox, we noted the bird's sex, and whether it was accompanied (present within 5 m) by a male or female. All nests with eggs were observed on day 5 or 6 of incubation (incubation day 0: the first day the eggs were assessed as warm) for 90 min between 1000 and 1300 hours. Telescopes, 30–50 m away from the focal box, were used to identify individuals as they arrived at and departed from their nest. When birds were not colour marked, the sexes were distinguished by bill coloration and plumage characteristics of breast and abdomen (Feare 1984). For each sex, we measured incubation attendance (proportion of time spent in the nestbox). From day 11 of incubation we checked each clutch three times daily (between 0800 and 1800 hours) to determine the hatching order of eggs. Nestlings were individually marked by clipping the nails of specific toes immediately after hatching, and were individually colour ringed between 8 and 11 days of age. All nests with broods were monitored for 90 min between 1000 and 1300 hours on day 12 or 13 after the first young in that nest hatched, following the same protocol as above. For each sex, we measured the frequency of food delivery to the young. During the early nestling stage adult male and female starlings were caught with nestbox traps, individually colour banded and weighed (± 0.1 g) with a 100 g pesola balance. Tarsus and wing lengths were measured (± 0.1 mm) with vernier callipers and a ruler, respectively.

Ectoparasite Monitoring

We chose to monitor numbers of the red fowl mite, *Dermanyssus gallinae*, because this was the most common ectoparasite present on all nestlings in our starling colony. This mite can complete three or four generations during the 3-week nestling period of their hosts (Richner & Heeb 1995). Eggs are laid in nest material and eggs, nymphs and adults can be dormant until the next breeding season. All life history stages of *D. gallinae* remain in the nest and feed on the blood of birds (Sikes & Chamberlain 1954). Feeding forms of this mite ascend to the host, feed and leave red spots ('scabs') on the abdomen of nestlings, after which they return to the nest substrate (Feare 1984). As a measure of parasite infestation we scored the abdominal scabs, because this procedure was faster and easier than counting mite numbers. Furthermore, collecting mites from the nesting material would influence their numbers. The percentage of a nestling's abdomen covered with scabs is a reliable measure of mite numbers present (Spearman rank correlation between total number of mites on nestlings and average scab score of all nestlings per nestbox: $r_S = 0.850$, $N = 8$, $P = 0.007$; see also Gwinner et al. 2000). The scabs on the abdomen of each nestling were scored five times, at 3, 6, 9, 12 and 15 days of age, using a 0−2 scale (0: no scabs; 1: less than 50%; 2: more than 50% of the abdomen covered with scabs (as defined by Gwinner et al. 2000; see also Fauth et al. 1991)). Age of the nestlings is based upon known hatching date.

Hypotheses Testing

Nest protection hypothesis

If ectoparasites are harmful to nestlings, we would expect starlings to prefer nestboxes with few ectoparasites. To create nestboxes with high and low numbers of red fowl mites we used the following procedure (see also Nest protection hypothesis in the Results). We collected old nest material of starlings on 17 February 2000 from all 26 nestboxes in subcolony III, and weighed and thoroughly mixed it. The average weight of old nest material per nestbox (175 g) was returned to every other nestbox (infested nestboxes, $N = 13$). The remaining nestboxes were left empty (clean nestboxes, $N = 13$).

Three predictions follow from the nest protection hypothesis. (1) Starlings nesting in infested nestboxes should use more nest greenery than starlings nesting in clean nestboxes. (2) Nestlings reared in nests from which the greenery is removed should carry more parasites than nestlings reared in nests where greenery is added. (3) The types of greenery preferred by starlings should be high in volatile secondary compounds. We did not test the last prediction, because Gwinner (1997) has already shown that the greenery in starling nests is high in volatile compounds. The first two predictions were tested in the following experiment (see also Fig. 1). In subcolony III, five sets of four nestboxes (two infested nestboxes and two clean nestboxes) were selected, such that the date of first appearance of green material in the four nestboxes of each set was the same. Within each set, one infested and one
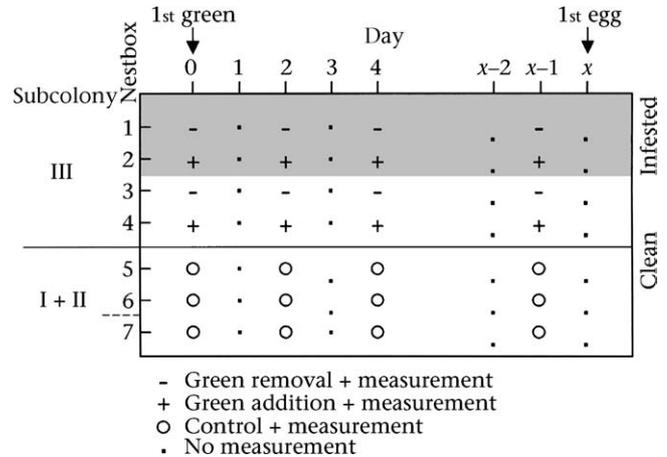
Figure 1. Schematic design to test the nest protection function of green nesting material. In subcolony III sets of four nestboxes (two infested and two clean) were selected, such that the first green material appeared in all four nestboxes on the same day (day = 0). Each nestbox was subjected to either a green removal or a green addition treatment every other day until one of the nestboxes contained an egg (day = x). As a control treatment, five sets comprising two or three clean nestboxes from subcolonies I and II were selected such that the green nest material was not manipulated and that the date of first appearance of green material in the control set was similar to that of one experimental set. The dashed line indicates that some sets comprised two and others three control nestboxes.

clean nestbox were randomly assigned to a 'green removal' treatment, and one infested and one clean nestbox to a 'green addition' treatment. To ensure that the green addition treatment was in the natural range, we added the average weight and average species composition of green material found per nestbox in the colony that day (range 0.15−3.26 g). These manipulations were continued every other day until one of the nestboxes in a set contained an egg. Finally, within a set there were four treatments: two infested nestboxes with either green material added or removed and two clean nestboxes with either green material added or removed (Fig. 1). As a control treatment, five sets comprising two or three occupied clean nestboxes were randomly selected from subcolonies I and II (three sets comprising two nestboxes, two sets comprising three nestboxes), such that the green material was not manipulated (see Courtship hypothesis below) and that the date of first appearance of green material in the nestboxes of one control set was within 2 days of that of the four nestboxes of one experimental set (Fig. 1). To investigate the effect of the treatments on the burden of ectoparasites on the nestlings, we measured the following for each nestling at 3, 6, 9, 12 and 15 days of age: (1) the red scabs on the abdomen on a 0−2 scale; (2) body mass (± 0.1 g) with a 50 g pesola balance.

Male quality hypothesis

To investigate whether the amount of greenery is an indicator of male quality, we related the average daily amount of greenery carried and the total amount of greenery worked into nestboxes by monogamous males to

their body size characteristics, incubation attendance, food-provisioning frequency and reproductive success.

Courtship hypothesis

The indirect courtship hypothesis predicts that a female's choice of nestbox in which to lay her clutch is based on the amount of greenery found when inspecting a nest, which could be an indicator of nest protection or male quality. To test this prediction, we manipulated the amount of greenery in the nestboxes of subcolonies I and II. A set of three nestboxes was selected, such that the first green material appeared in the three nestboxes on the same date. Within each set we randomly assigned the nest to a treatment: no manipulation (control), removal or addition of green material, following the same protocol as described above (Nest protection hypothesis). The experimental addition or removal of green material should have a significant effect on whether a female lays a clutch.

The direct courtship hypothesis predicts that males carry green material into the nestboxes in the presence of a female. To test this prediction, we examined the responses of 15 males, of which seven were unpaired and eight were monogamously paired, towards a cage (40×20 cm and 20 cm high) attached 1 m under the focal nestbox with either no bird, an adult male or an adult female starling. Although starlings can breed polygynously, we expected unpaired males to respond more than monogamously paired males to a caged female. For the unpaired males, the experiments were performed within 1 h of the male being observed singing with 'wing-waving' displays (Feare 1984; Eens et al. 1990) within 2 m of an empty nestbox. For the paired males, all experiments were performed on day 6 of incubation, when males are still capable of attracting a secondary mate (e.g. Eens et al. 1990, 1991; Mountjoy & Lemon 1991; Pinxten & Eens 1998; Komdeur et al. 2002). To test the reaction of paired males, we placed the cage under an empty nestbox ± 6 m from the one inhabited by the paired male, a distance at which male starlings may attract additional females to a nestbox (Sandell & Smith 1996, 1997). The test consisted of sequential presentations of the three cage types. The order of presentation was randomly assigned for the first test and altered for each subsequent test, thereby excluding habituation effects. The subsequent tests were performed with a 10-min interval. The caged birds (one female and one male) were older than 2 years of age. Observations of the response of free-living males started after the first appearance of the male within 5 m of the cage and lasted 30 min. Data were collected using a fixed interval sampling method (Martin & Bateson 1986) and included the carrying of green material into the nestbox and song with 'wing-waving', because this song type plays a role in mate attraction (Feare 1984; Mountjoy & Lemon 1991; Eens et al. 1993). Experiments were conducted from 0900 to 1215 hours. The sequential test used in this study (comparing cage type-specific, provoked behaviour by the same males) was an explicit matched-pairs design. We repeated the experiment with the paired males to test the effect of cage position on male response; for this we attached the cage 1 m under the occupied nestbox of the paired male.

Data Analyses

A total of 33 nestboxes were occupied (contained a clutch), of which 32 were occupied by monogamous breeding pairs. Intraspecific brood parasitism (the presence in a nest of two or more new eggs in 1 day; Yom-Tov 1980) occurred only once and we excluded this nest from further analyses because two females were attending the nest simultaneously. We calculated the total mass (g) of green nesting material present per nestbox as the sum of masses measured from the first day with greenery to the day of laying the first egg in that nestbox. The average daily amount of green nest material present per nestbox was calculated as the quotient of total mass and number of days between the day of first measurement and the day of laying the first egg in that nestbox. As an estimate of body condition for adults and nestlings we used body mass and not the regression residuals derived from the equation of tarsus versus mass, as this regression was not always significant (see discussion in Green 2001). For data that were not normally distributed, even after transformation, we used nonparametric tests. Because not all nestboxes were occupied, samples of the different greenery treatments are small and skewed. Therefore these data were tested by a randomization test. Data were randomly permuted with respect to a treatment and from this the F ratio was calculated. This was repeated 2000 times. We calculated the P value as follows: (number of F ratios that were larger or equal to the original F ratio + 1)/(number of randomizations + 1) (Peres-Neto & Olden 2001). We followed a similar procedure to calculate the t statistic. Multilevel modelling was used to analyse data on nestling scab scores and body mass. In this way we corrected for the effects of the hierarchical structure and nonindependence of the data. The brood, individual nestling and the repeated measurements were included as a level in the model. Models were derived by backward elimination. We determined the significance of explanatory variables by calculating the change in model deviance, which approximates the χ^2 distribution. Interaction terms were tested by including the main effects in the model regardless of their significance. All significance tests are two tailed. For statistical analyses we used SPSS (version 10.0) and MLwiN (version 1.10; Rasbash et al. 2000). Means are expressed $\pm$ SE. Although we tried to control the experimental set-up, sample sizes are often small, resulting in low power.

RESULTS

Timing of Carrying Greenery

Only male starlings ($N = 15$ males) were observed to carry green material into nestboxes. On most occasions (80%), the male carrying and adding the green material was accompanied by a female, but never by another male. All green material was collected from herbs, bushes or trees within 6 m of the nestbox (60 observations of seven males). Incorporation of green plants into nests started on average 8.7 ± 1.0 days before laying of the first egg ($N = 31$). However, in one nestbox with a clutch no green material was observed. The variation in the pattern of first
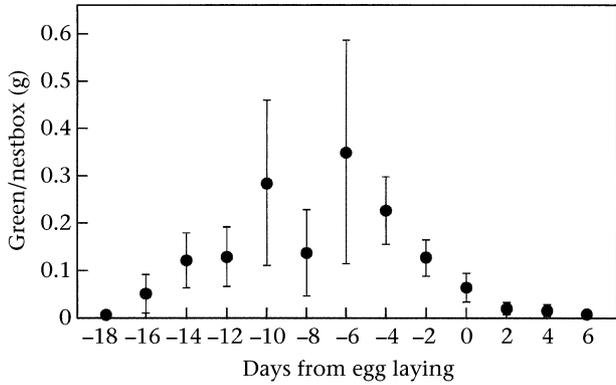
Figure 2. Average $\pm$ SE daily mass of greenery carried into nestboxes occupied by breeding pairs of starlings in relation to the onset of laying (day 0 is laying of the first egg; mass $= -0.002X^2 + 0.047X - 0.12$, $r^2 = 0.65$, $F_{2,11} = 9.10$, $P = 0.011$; $N = 31$ nestboxes observed).

appearance of green material in nests varied considerably between males (range 18–0 days before laying of the first egg). The average daily mass of green material carried into a nestbox increased towards a peak 6 days before egg laying and decreased to almost zero during laying (Fig. 2). Green material was carried into the nestbox in small amounts (average daily mass carried per male: 0.18 ± 0.05 g; average total mass carried per male: 1.28 ± 0.22 g; 359 observation days of 31 males).

Hypotheses Testing

The manipulations of green nesting material during the nest-building stage had a significant effect on whether a nestbox contained a clutch. The green removal treatment resulted in a significantly lower percentage of nestboxes containing a clutch than the control and green addition treatments (Table 1). However, the removal and addition of green nesting material had no detectable effect on the total amount of green nesting material carried into the nestbox by starlings, clutch size, or the number of nestlings and fledglings (Table 1).

Nest protection hypothesis

Our data do not support the nest protection hypothesis for three reasons.

(1) The total amount of green nest material carried into unmanipulated nestboxes (green nest material not experimentally removed or added) was not correlated with the average scab scores of all nestlings in a nestbox (Spearman correlation: $r_S = 0.30$, $N = 10$, $P = 0.402$). Furthermore, the removal and addition of green nesting material had no effect on the scab scores on the abdomen of nestlings (Table 2) or on nestling body mass (Table 3). Because the manipulation of greenery during nest building had no effect on the total amount of green nesting material carried into the nestbox, we pooled the nestboxes with greenery manipulations over infested and clean nestboxes to analyse the effect of nestbox type on breeding performance.

(2) In subcolony III (with an equal number of infested and clean nestboxes) starlings showed no preference for clean nestboxes for depositing green nest material (76.9% of clean nestboxes with green nest material ($N = 13$) versus 84.6% of infested nestboxes with green nest material ($N = 13$); $\chi_1^2 = 0.25$, $P = 0.618$) and for egg laying (23.1% clean nestboxes with a clutch ($N = 13$) versus 53.9% infested nestboxes with a clutch ($N = 13$); $\chi_1^2 = 2.60$, $P = 0.107$). When we compared the occupied infested nestboxes with the occupied clean nestboxes in the entire colony, green material was not carried more into infested nestboxes than into clean nestboxes (Table 4). Furthermore, the average clutch size, number of nestlings and fledglings produced and nestling body mass were similar between nestbox types (Tables 3 and 4).

(3) Nestlings in infested nests had significantly higher scab scores than those in clean nests, but the manipulation of green material had no significant effect on the scab scores on the abdomen of nestlings (Fig. 3, Table 2). The scab scores we found were within the range of the average scab scores on starling nestlings in herb (green) and dried grass (control) nests found by Gwinner et al. (2000) (Fig. 3). Nestling body mass was not significantly associated with the number of scabs on the abdomen of a nestling, although there was a slight decrease in body mass with increasing scab score (Table 3).

Courtship hypothesis

The removal and addition of green nest material during the nest-building stage had no effect on average time spent on courtship (Table 1). The total amount of green nest material carried into a nestbox was significantly

Table 1. The influence of removal and addition of green nest material during the nest-building stage on courtship time (interval between first appearance of greenery to first egg), total mass of greenery carried into the nestbox by starlings, and breeding performance of starling pairs

	Removal	N	Control	N	Addition	N	Statistics	P
Percentage with clutch	40.0	10	91.7	12	100.0	10	$\chi_{2,30} = 12.59$	0.002
Mass greenery (g)	0.93 ± 0.46	4	0.85 ± 0.27	11	1.69 ± 0.47	10	$F = 1.42$	0.266
Clutch size	5.50 ± 0.29	4	5.60 ± 0.15	11	5.00 ± 0.37	10	$F = 1.61$	0.229
Number of nestlings	5.25 ± 0.25	4	5.60 ± 0.22	10	4.50 ± 0.76	8	$F = 1.39$	0.275
Number of fledglings	4.25 ± 0.48	4	2.70 ± 0.82	10	2.63 ± 0.84	8	$F = 0.76$	0.461
Courtship time (days)	11.00 ± 3.72	4	8.36 ± 1.36	11	11.10 ± 2.14	10	$F = 0.64$	0.546

P value derived from a randomization test, $N =$ number of nests. Means are shown $\pm$ SE.

Table 2. Model summaries examining the effect of nestbox type (clean or infested), greenery manipulation and the total mass of greenery in the nestbox on the scab scores of nestlings ($N=528$)

Explanatory term	Coefficient $\pm$ SE	χ^2_1	P
Nestling hatching order	**−0.07 ± 0.02**	**12.5**	**<0.001**
Nestbox type	**0.43 ± 0.13**	**7.95**	**0.005**
Brood size	−0.09 ± 0.06	2.16	0.142
Greenery manipulation (g)	0.21 ± 0.14	2.00	0.157
Mass of greenery (g)	0.02 ± 0.06	0.18	0.671
Nestbox type × greenery manipulation	−0.18 ± 0.25	0.25	0.488
Greenery manipulation × mass of greenery	0.07 ± 0.11	0.43	0.511

Brood size defined as brood size at day 3. Summaries derived from the normal response mixed-modelling procedure in MLwiN. Explanatory terms included in the final model are shown in bold.

correlated with courtship time (Spearman correlation: $r_S=0.37$, $N=31$, $P=0.041$), whereas the average daily amount of green material carried into a nestbox was not correlated with courtship time ($r_S=0.30$, $N=31$, $P=0.107$). The duration of time spent singing and the frequency of carrying greenery were significantly associated with male mating status and the sex of the bird present in the cage (Fig. 4). Unpaired males sang and carried greenery into the nestbox significantly more often in the presence of a caged female than in the presence of a caged male. They also carried more green material in the presence of a caged male than in the presence of an empty cage (song duration: GLM repeated measures: female > male: $F_{1,6}=7.87$, $P=0.031$; male > empty: $F_{1,6}=11.10$, $P=0.016$; green carrying: Wilcoxon signed-ranks test: female > male: $T=-2.37$, $N=7$, $P=0.018$; male > empty: $T=-2.23$, $N=7$, $P=0.026$). The frequency of carrying greenery was positively correlated with the time spent singing (Spearman correlation: $r_S=0.641$, $N=21$, $P=0.002$). The order in which the different cages were presented had no effect on the results. In contrast to unpaired males, paired males did not show any response, either in song or carrying greenery, towards any of the cage types. The position of the cage, either attached under the paired male's occupied nestbox or under an unoccupied nestbox ±6 m from the focal one, had no effect on the male's response ($N=8$ paired males).

Male quality hypothesis

Paternal care of monogamous males showed a consistent pattern during the breeding cycle. When the effects of clutch and brood size were controlled, male incubation

attendance was positively correlated with male food provisioning to nestlings (Spearman correlation: $r_S=0.80$, $N=12$, $P=0.002$). However, there was no relation between either the total or the average daily amount of green nest material carried into the nestbox and either measure of male paternal care (total amount of green: incubation attendance: $r_S=0.24$, $N=14$, $P=0.401$; food provisioning: $r_S=-0.16$, $N=11$, $P=0.631$; daily amount of green: incubation attendance: $r_S=-0.10$, $N=14$, $P=0.742$; food provisioning: $r_S=-0.10$, $N=11$, $P=0.769$). The total and average daily amount of green nest material carried into the nestbox were not correlated with male body size characteristics (body mass, wing and tarsus length: total amount of green: $r_S \geq -0.52$, $N=8$, $P \geq 0.188$; daily amount of green: $r_S \geq -0.51$, $N=8$, $P \geq 0.201$). Nor was the total amount of greenery correlated with female body size characteristics (body mass, wing and tarsus length: $r_S \leq 0.17$, $N=26$, $P \geq 0.386$), clutch size ($r_S=-0.23$, $N=31$, $P=0.217$) or number of fledglings ($r_S = -0.22$, $N = 29$, $P = 0.261$).

DISCUSSION

Green Nest Material and Nest Protection

There is ample evidence that nest ectoparasites have a negative effect on the survival and fecundity of breeding adult birds (Richner et al. 1993; reviewed in Møller et al. 1990). High ectoparasite numbers may result in depressed growth and survival of the young because of blood loss (Møller 1990; Clayton & Tompkins 1995; Potti & Merino 1996) or physiological stress (Hofstad et al. 1984), and in

Table 3. Model summaries examining the effect of nestbox type (clean or infested), greenery manipulation and total mass of greenery in the nestbox on nestling body mass ($N=499$)

Explanatory term	Coefficient $\pm$ SE	χ^2_1	P
Nestling hatching order	**−1.80 ± 0.55**	**10.4**	**0.003**
Scab score	−2.21 ± 1.28	2.90	0.089
Brood size	2.63 ± 2.30	1.30	0.254
Mass of greenery (g)	2.42 ± 2.17	1.23	0.268
Nestbox type	4.13 ± 4.51	0.83	0.362
Greenery manipulation (g)	−2.08 ± 4.48	0.22	0.642
Greenery manipulation × mass of greenery	−2.82 ± 3.08	0.74	0.390
Nestbox type × greenery manipulation	−3.38 ± 9.78	0.11	0.740

Brood size defined as brood size at day 3. Summaries derived from the normal response mixed-modelling procedure in MLwiN. Explanatory terms included in the final model are shown in bold.

Table 4. Breeding performance of starlings occupying infested and clean nestboxes

| Nestbox type	Infested	N	Clean	N	t	df	P
Mass of greenery (g)	1.56 ± 0.56	7	1.81 ± 0.65	25	0.65	30	0.532
Clutch size	5.86 ± 0.40	7	5.36 ± 0.16	25	1.32	30	0.178
Number of nestlings	5.71 ± 0.36	7	5.32 ± 0.19	22	1.00	27	0.342
Number of fledglings	3.71 ± 0.87	7	3.05 ± 0.47	22	0.76	27	0.457

P value derived from a randomization test, N = number of nests. Means are shown ± SE.

decreased postfledging survival (Moss & Camin 1971; Arendt 1985a, b; Brown & Brown 1986). One option to minimize ectoparasite load is the use of clean nest sites (Clark & Mason 1988). However, evidence for this is conflicting, even within species: some studies show that hole-nesting species prefer parasite-free nestboxes (great tit, *Parus major*: Oppliger et al. 1994), whereas others do not discriminate between nestboxes with or without parasites (great tit, blue tit, *Parus caeruleus*, marsh tit, *Parus palustris*: Olsson & Allander 1995), or even prefer nestboxes containing parasites (house wren, *Troglodytes aedon*: Thompson & Neill 1991; pied flycatcher, *Ficedula hypoleuca*: Orell et al. 1993; Olsson & Allander 1995). We found that starlings did not avoid infested nestboxes, even though these contained more ectoparasites than clean nestboxes. Starlings could have changed the effectiveness of our experimental manipulation of ectoparasite content by cleaning nestboxes themselves (Feare 1984). This is not the case in our population because infested nest material remained in the nestboxes throughout the different nesting stages until fledging and the manipulations of ectoparasite content before nest building had a significant effect on ectoparasite numbers on nestlings. Given the high proportion of clean nestboxes without a nest (75%, N=99), the absence of a preference for clean nestboxes was not due to a forced choice.

Another possibility for controlling nest ectoparasites is the incorporation of green plant material into the nest substrate to repel or kill ectoparasites. However, published evidence for the nest protection hypothesis is weak. For example, there is no evidence that the use of green plant material by North American and European Falconiformes serves to lower the incidence of ectoparasites, although its use is correlated with the use of old nests (Wimberger 1984). Our study does not support the nest protection hypothesis for three reasons.

First, both the total amount of green plant material carried into unmanipulated nestboxes and the experimental addition or removal of greenery had no effect on nestlings' scab scores, body mass and survival to fledging. Previous studies also found that the use of green plants in starling nests had no effect on ectoparasite numbers (Gwinner et al. 2000) and nestling condition (Clark & Mason 1988; Fauth et al. 1991), even though the green plant species that were used contained high levels of volatile compounds (Clark & Mason 1985; Gwinner 1997; Lambert 1997). However, some of these studies found that nestlings from nests where greenery was added during the incubation stage had a higher immune response (measured as haematocrit level; Clark & Mason 1988; Gwinner et al. 2000) and a higher first-year survival (Gwinner et al.
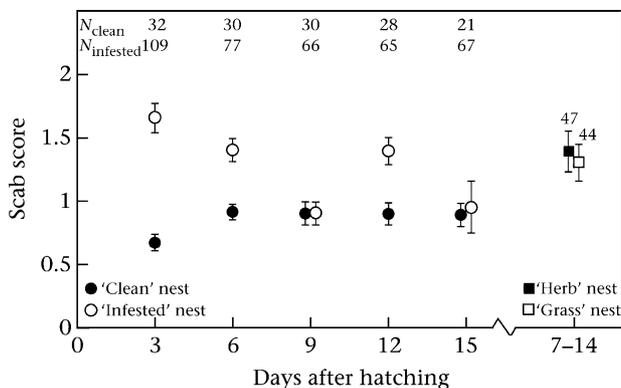
Figure 3. Influence of nestbox type and nestling's age on the abdominal scab scores in starlings (see also Table 2; N = number of nestlings). As a comparison, the scab scores on starling nestlings in herb (green) and dried grass (control) nests as found by Gwinner et al. (2000) are given.
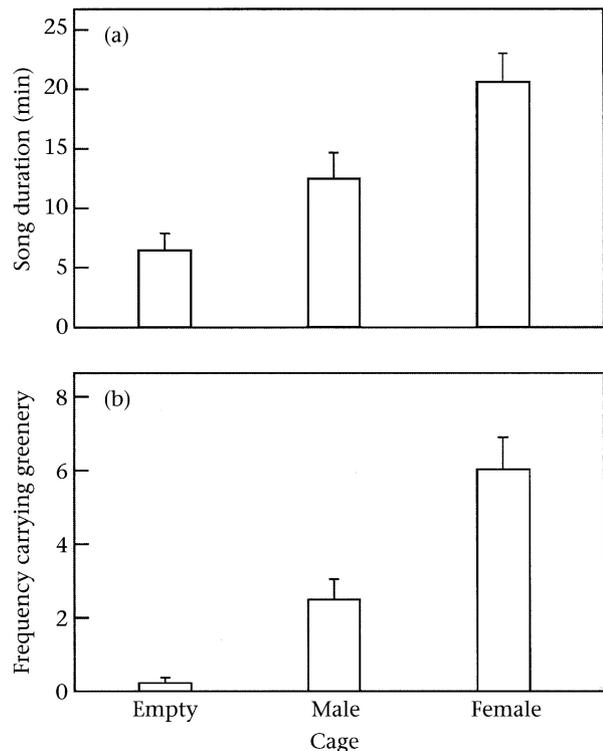
Figure 4. (a) Average song duration and (b) frequency of carrying greenery per 30 min of unpaired starling males in the presence of an empty cage, a caged adult male or a caged adult female starling.

2000) than nestlings from nests where no greenery was added. These results are possibly attributable to an improvement of the immune system that helps the nestlings to cope better with the harmful activities of ectoparasites (Gwinner et al. 2000). However, these experiments, where green plant material was manipulated after the clutch was completed, do not represent the natural conditions under which green nesting material is used, because starlings rarely add greenery to the nest during the incubation stage (Gwinner 1997; this study). Also, the greenery was manipulated outside the natural range, as the total mass of greenery added (40 g; Gwinner et al. 2000) was far more than the average total mass added by male starlings in that population (1.4 g; Gwinner 1997).

Second, regardless of the presence of higher numbers of ectoparasites in infested nests, no more green plant material was carried into infested than into clean nestboxes. Another study on starlings even found that experimentally infested nestboxes contained significantly less greenery than clean nestboxes (Gwinner 1997), the opposite to that predicted. Perhaps starlings cannot quickly assess future parasite abundance in potential nest sites. Alternatively, given the absence of an effect of ectoparasites on reproductive success among starlings there may be little pressure on starlings to avoid nestboxes with a high ectoparasite load. In this study the scab scores of nestlings had no effect on nestling body mass. Other studies did not find an effect of parasite infestation on nestling mass or survival either (Clark & Mason 1988; Fauth et al. 1991; Gwinner et al. 2000). It could be that the infestation was not high enough to be really harmful for the chicks.

Third, a prediction of the nest protection hypothesis is that green nest material should be carried into the nestbox not only by males but also by females. Furthermore, it should be added during the entire breeding cycle. However, it has been argued that green plants need to dry out to release their volatile compounds efficiently (H. Gwinner, personal communication). In that case, green plants added early would be more effective through the nestling period than those added later. Also, the decrease in carrying of green nest material once egg laying has started might be a result of females spending 60% (or more) of their time in their nestbox from the moment that egg laying starts (Pinxten & Eens 1997) which makes it more difficult for males to enter the nestbox. Furthermore, males intensively mate-guard their female from this period onwards (and they probably cannot mate-guard and collect green nest material effectively at the same time). To reject the nest protection hypothesis confidently, other populations with a higher ectoparasite abundance should be studied, where it might be easier to demonstrate an effect of green nest material.

Courtship Display and Male Quality

In agreement with other studies (Kessel 1957; Feare 1984; Clark & Mason 1985; Gwinner 1997), our study confirmed that green nest material was carried into the nestbox by male starlings only. Furthermore, the presence of green nest material in a nestbox during the nest-building stage affected a female's choice to lay a clutch in that nestbox. The green removal treatment resulted in a significantly lower percentage of nestboxes containing a clutch than the control and green addition treatments. However, female choice was independent of the amount of greenery in the nestbox, because the addition of green nest material did not increase the chance of a nestbox containing a clutch. If the amount of greenery was important, male starlings should have carried more green nest material into the nestbox after we had removed it, which was not the case. These findings support the idea that the amount of green material does not serve as a male signal. In contrast to the stone-carrying behaviour of the black wheatear (Moreno et al. 1994), the amount of green plant material carried into the nestbox by male starlings was not correlated with male paternal care or body size characteristics. This came as no surprise, because the carrying of green material is unlikely to be an energetically expensive behaviour. In our study the average daily amount and the total amount of green material carried into the nestbox was small and observations showed that the green material was collected within 6 m of the nestbox.

We have empirical and experimental data that support the mate attraction function of green material. In agreement with previous studies (Eens et al. 1993; Gwinner 1997), this study showed that males collect greenery particularly when females are in close proximity to the nestbox, and often in an eye-catching manner. The carrying of green material reached a peak around 6 days before laying and stopped during laying, which corresponds with the period of pair formation (between day 7 and day 4 preceding laying; Eens et al. 1994). The average daily mass and the total mass of green nest material carried per male into the nestbox were similar to those observed in a German population (average daily mass: 0.18 ± 0.05 g ($N=31$) versus 0.4 ± 0.2 ($N=13$, Gwinner 1997); $t_{43}=1.49$, $P=0.144$; average total mass: 1.28 ± 0.22 g ($N=31$) versus 1.4 ± 0.6 ($N=13$, Gwinner 1997); $t_{43}=0.23$, $P=0.819$). Only unpaired males started collecting and carrying green nest material into their nestbox in the presence of a caged female. The frequency of carrying green material was positively correlated with singing time. Singing is already known to have a function in territoriality, but is thought to be even more important in mate attraction (Feare 1984; Mountjoy & Lemon 1991; Eens et al. 1993).

Our results supporting the mate attraction function are in agreement with the findings of three other studies on starlings. An experimental study showed that males with an increased testosterone level, which is known to have a function in male courtship, sang more and carried more green material into the nestbox, but had a lower incubation and feeding rate than control males (De Ridder et al. 2000). Male starlings with damage to a specific part of the hypothalamus, which is known to have a function in male courtship, gathered significantly less green nest material than control males (Riters & Ball 1999). The carrying of green nest material into a nestbox by males (analyses controlled for singing activity) was positively

associated with the probability of mate acquisition (Komdeur et al. 2002).

However, our data also suggest that the carrying of green material may serve as an 'intrasexual signal' for nest occupation (Eens et al. 1993; Gwinner 1997) or territory occupation (Wimberger 1984), because on some occasions males deposited greenery in the absence of females, and sometimes the focal males deposited greenery in the presence of a caged male (although significantly less than in the presence of a caged female). Male golden eagles, *Aquila chrysaetos*, add green material in all nests within their territory (they maintain several on a territory) throughout the year which is thought to have a territorial function (Newton 1979; Wimberger 1984).

The origin of starlings carrying greenery into nestboxes is unknown. It could be that originally the incorporation of green plant material into the nests may have protected the nestlings against ectoparasites at a certain level of infestation, but that because of environmental changes, or just in our population, the parasite load may have become too low to have a negative effect on nestlings. Therefore the carrying of greenery may have gradually evolved from a nest protection cue to a mate attraction cue, controlled by females that still prefer males that carry greenery into the nest.

Acknowledgments

References

Ali, S. & Ripley, S. D. 1974. *Handbook of the Birds of India and Pakistan*. Oxford: Oxford University Press.

Arendt, W. J. 1985a. *Philornis* ectoparasitism of pearly-eyed thrashers. I. Impact on growth and development of nestlings. *Auk*, **102**, 270–280.

Arendt, W. J. 1985b. *Philornis* ectoparasitism of pearly-eyed thrashers. II. Effects on adults and reproduction. *Auk*, **102**, 281–292.

Beebe, F. L. 1976. *Hawks, Falcons and Falconry*. Saanichton, British Columbia: Hancock House.

Borgia, G. 1995. Complex male display and female choice in the spotted bowerbird: specialized functions for different bower decorations. *Animal Behaviour*, **49**, 1291–1301.

Brown, C. R. & Brown, M. B. 1986. Ectoparasitism as a cost of coloniality in cliff swallows (*Hirundo pyrrhonota*). *Ecology*, **67**, 1206–1218.

Brown, L. & Amadon, D. 1968. *Eagles, Hawks and Falcons of the World*. New York: McGraw-Hill.

Clark, L. 1991. The nest protection hypothesis: the adaptive use of plant secondary compounds by European starlings. In: *Bird–Parasite Interaction* (Ed. by J. E. Loye & M. Zuk), pp. 205–221. Oxford: Oxford University Press.

Clark, L. & Mason, J. R. 1985. Use of nest material as insecticidal and antipathogenic agents by the European starling. *Oecologia*, **67**, 169–176.

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

Clayton, D. H. & Tompkins, D. M. 1995. Comparative effects of mites and lice on the reproductive success of rock doves (*Columba livia*). *Parasitology*, **110**, 195–206.

De Ridder, E., Pinxten, R. & Eens, M. 2000. Experimental evidence of a testosterone-induced shift from paternal to mating behaviour in a facultatively polygynous songbird. *Behavioral Ecology and Sociobiology*, **49**, 24–30.

Duffy, D. C. 1983. The ecology of tick parasitism on densely nesting Peruvian seabirds. *Ecology*, **64**, 110–119.

Eens, M., Pinxten, R. & Verheyen, R. F. 1990. On the function of singing and wing-waving in the European starling *Sturnus vulgaris*. *Bird Study*, **37**, 48–52.

Eens, M., Pinxten, R. & Verheyen, R. F. 1991. Male song as a cue for mate choice in the European starling. *Behaviour*, **116**, 210–238.

Eens, M., Pinxten, R. & Verheyen, R. F. 1993. Function of the song and song repertoire in the European starling (*Sturnus vulgaris*): an aviary experiment. *Behaviour*, **125**, 51–66.

Eens, M., Pinxten, R. & Verheyen, R. F. 1994. Variation in singing activity during the breeding cycle of the European starling *Sturnus vulgaris*. *Belgian Journal of Zoology*, **124**, 167–174.

Fauth, P. T., Krementz, D. G. & Hines, J. E. 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.

Feare, C. J. 1984. *The Starling*. Oxford: Oxford University Press.

Green, A. J. 2001. Mass/length residuals: measures of body condition or generators of spurious results? *Ecology*, **82**, 1473–1483.

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. *Animal Behaviour*, **59**, 301–309.

Hesse, G. H. 1985. Brutverlust von Uferschwalben *Riparia riparia* durch massive Parasitierung nestbewohnender Flöhe und Zecken. *Ornithologische Mitteilungen*, **37**, 31–32.

Hitchner, S. B. 1980. *Isolation and Identification of Avian Pathogens*. College Station, Texas: American Association of Avian Pathologists.

Hofstad, M. S., Barnes, H. J., Calnek, B. W., Reid, W. M. & Yoder, H. W. 1984. *Diseases of Poultry*. 8th edn. Ames, Iowa: Iowa Sate University Press.

Houston, A. I. & Davies, N. B. 1985. The evolution of cooperation and life history in the dunnock, *Prunella modularis*. In: *Behavioural Ecology: Ecological Consequences of Adaptive Behaviour* (Ed. by R. Sibly & R. Smith), pp. 471–487. Oxford: Blackwell Scientific.

Johnston, R. F. & Hardy, J. W. 1962. Behavior of the purple martin. *Wilson Bulletin*, **74**, 243–262.

Kessel, B. 1957. A study of the breeding biology of the European starling (*Sturnus vulgaris* L.) in North America. *American Midland Naturalist*, **58**, 257–331.

Komdeur, J., Wiersma, P. & Magrath, M. 2002. Paternal care and male mate-attraction effort in the European starling is adjusted to clutch size. *Proceedings of the Royal Society of London, Series B*, **269**, 1253–1261.

Lambert, K. 1997. *Eintrag von grünem Nistmaterial beim Starling, Sturnus vulgaris.* Diplomarbeit, Universität Würzburg.

Loye, J. L. & Zuk, M. 1991. *Bird–Parasite Interactions. Ecology, Evolution and Behavior.* Oxford: Oxford University Press.

Martin, P. & Bateson, P. 1986. *Measuring Behaviour: an Introductory Guide.* Cambridge: Cambridge University Press.

Meijer, T. 1992. Egg-laying patterns in captive starlings. *Ardea*, **80**, 301–310.

Merino, S. & Potti, J. 1995. Mites and blowflies decrease growth and survival in nestling pied flycatchers. *Oikos*, **73**, 95–103.

Møller, A. P. 1989. Parasites, predators and nest boxes: facts and artefacts in nest box studies of birds? *Oikos*, **56**, 421–423.

Møller, A. P. 1990. Effects of parasitism by a hematophagous mite on reproduction in the barn swallow. *Ecology*, **71**, 2345–2357.

Møller, A. P., Allander, K. & Dufva, R. 1990. Fitness effects of parasites on passerine birds: a review. In: *Population Biology of Passerine Birds. An Integrated Approach* (Ed. by J. Blondel, A. Gosler, J.-D. Lebreton & R. McCleery), pp. 269–280. Berlin: Springer-Verlag.

Moreno, J., Soler, M., Møller, A. P. & Linden, M. 1994. The function of stone carrying in the black wheatear, *Oenanthe leucura. Animal Behaviour*, **47**, 1297–1309.

Moss, W. W. & Camin, J. H. 1971. Nest parasitism, productivity and clutch size in purple martins. *Science*, **168**, 1000–1003.

Mountjoy, D. J. & Lemon, R. E. 1991. Song as an attractant for male and female European starlings, and the influence of song complexity on their response. *Behavioral Ecology and Sociobiology*, **28**, 97–100.

Newton, I. 1979. *Population Ecology of Raptors.* Vermillion, South Dakota: Buteo Books.

Olsson, K. & Allander, K. 1995. Do fleas, and/or old nest material, influence nest-site preference in hole-nesting passerines? *Ethology*, **101**, 160–170.

Oppliger, A., Richner, H. & Christe, P. 1994. Effect of an ectoparasite on lay date, nest site choice, desertion and hatching success in the great tit (*Parus major*). *Behavioral Ecology*, **5**, 130–134.

Orell, M., Rytkönen, S. & Ilomäki, K. 1993. Do pied flycatchers prefer nest boxes with old nest material? *Annales Zoologici Fennici*, **30**, 313–316.

Peres-Neto, P. R. & Olden, D. 2001. Assessing the robustness of randomization tests: examples from behavioural studies. *Animal Behaviour*, **61**, 79–86.

Pinxten, R. & Eens, M. 1990. Polygyny in the European starling: effect on female reproductive success. *Animal Behaviour*, **40**, 1035–1047.

Pinxten, R. & Eens, M. 1994. Male feeding of nestlings in the facultatively polygynous European starling: allocation patterns and effect on female reproductive success. *Behaviour*, **129**, 113–140.

Pinxten, R. & Eens, M. 1997. Copulation and mate-guarding patterns in polygynous European starlings. *Animal Behaviour*, **54**, 45–58.

Pinxten, R. & Eens, M. 1998. Male starlings sing most in the late morning, following egg-laying: a strategy to protect their paternity? *Behaviour*, **135**, 1197–1211.

Pinxten, R., Eens, M. & Verheyen, R. F. 1993. Male and female nest attendance during incubation in the facultatively polygynous European starling. *Ardea*, **81**, 125–133.

Pinxten, R., Eens, M. & Verheyen, R. F. 1995. Response of male European starlings to experimental removal of their mate during different stages of the breeding cycle. *Behaviour*, **132**, 301–317.

Potti, J. & Merino, S. 1996. Parasites and the ontogeny of sexual size dimorphism in a passerine bird. *Proceedings of the Royal Society of London, Series B*, **263**, 9–12.

Powlesland, R. G. 1977. Effects of the haematophagous mite *Ornithonyssus bursa* on nestling starlings in New Zealand. *New Zealand Journal of Zoology*, **4**, 85–94.

Rasbash, J., Browne, W., Goldstein, H., Yang, M., Plewis, I., Healy, M., Woodhouse, G., Draper, D., Langford, I. & Lewis, T. 2000. *A User's Guide to MLwiN.* 2nd edn. London: Institute of Education.

Richner, H. & Heeb, P. 1995. Are clutch and brood size patterns in birds shaped by ectoparasites? *Oikos*, **73**, 435–441.

Richner, H., Oppliger, A. & Christe, P. 1993. Effect of an ectoparasite on reproduction in great tits. *Journal of Animal Ecology*, **62**, 703–710.

Riters, L. V. & Ball, G. F. 1999. Lesions to the medial preoptic area affect singing in the male European starling (*Sturnus vulgaris*). *Hormones and Behavior*, **36**, 276–286.

Sandell, M. I. & Smith, H. G. 1996. Already mated females constrain male mating success in the European starling. *Proceedings of the Royal Society of London, Series B*, **263**, 743–747.

Sandell, M. I. & Smith, H. G. 1997. Female aggression in the European starling during the breeding season. *Animal Behaviour*, **53**, 13–23.

Sikes, R. K. & Chamberlain, R. W. 1954. Laboratory observations on three species of bird mites. *Journal of Parasitology*, **40**, 491–497.

Smith, H. G. 1995. Experimental demonstration of a trade-off between mate attraction and paternal care. *Proceedings of the Royal Society of London, Series B*, **260**, 45–51.

Smith, H. G. & Härdling, R. 2000. Clutch size evolution under sexual conflict enhances the stability of mating systems. *Proceedings of the Royal Society of London, Series B*, **267**, 2163–2170.

Smith, H. G., Sandell, M. I. & Bruun, M. 1995. Paternal care in the European starling, *Sturnus vulgaris*: incubation. *Animal Behaviour*, **50**, 323–331.

Soler, J. J., Cuervo, J. J., Møller, A. P. & de Lope, F. 1998. Nest building is a sexually selected behaviour in the barn swallow. *Animal Behaviour*, **56**, 1435–1442.

Thompson, C. F. & Neill, A. J. 1991. House wrens do not prefer clean nest boxes. *Animal Behaviour*, **42**, 1022–1024.

Walter, G. & Hudde, H. 1987. *Carnus hemapterus* (Milichiidae, Diptera), an ectoparasite of nestlings. *Journal of Ornithology*, **128**, 251–255.

Westneat, D. F., Sherman, P. W. & Morton, M. L. 1990. The ecology and evolution of extra-pair copulations in birds. In: *Current Ornithology. Vol. 7* (Ed. by D. M. Power), pp. 331–369. London: Plenum.

Widmann, O. 1922. Extracts from the diary of Otto Widmann. *Transaction of the Academy of Science of St Louis*, **24**, 1–77.

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

Yom-Tov, Y. 1980. Intraspecific nest parasitism in birds. *Biology Reviews*, **55**, 93–108.