Home, dirty home: effect of old nest material on nest-site selection and breeding performance in a cavity-nesting raptor

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Abstract

The quality of a breeding site may have major fitness consequences. A fundamental step to understanding the process of nest-site selection is the identification of the information individuals use to choose high-quality nest sites. For secondary cavity-nesting bird species that do not add nest lining material, organic remains (faeces, pellets) accumulated inside nest cavities during previous breeding events may be a cue for high-quality nest-sites, as they contain information about past successful breeding and may improve thermal insulation of eggs during incubation. However, cavities in which breeding was successful might also contain more nest-dwelling ectoparasites than unoccupied cavities, offering an incentive for prospective parents to avoid them. We exposed breeding cavity-nesting lesser kestrels (Falco naumanni) to nestbox dyads consisting of a dirty (with a thick layer of organic substrate) and a clean nestbox (without organic material). Dirty nestboxes were strongly preferred, being occupied earlier and more frequently than clean ones. Hatching success in dirty nestboxes was significantly higher than in clean ones, suggesting a positive effect of organic nest material on incubation efficiency, while nestbox dirtiness did not significantly affect clutch and brood size. Nestlings from dirty nestboxes had significantly higher ectoparasite loads than those from clean nestboxes soon after egg hatching, but this difference was not evident a few days later. Nest substrate did not significantly affect nestling growth. We concluded that nest substrate is a key driver of nest-site choice in lesser kestrels, although the adaptive value of such a strong preference appears elusive and may be context-dependent.

Keywords Carnus hemapterus, ectoparasites, nestbox, nest substrate, nest-site selection.
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Templeton 2002), perceived predation risk (Eggers et al. 2006), presence of parasites (Rosenheim 1988), or a combination of those factors. Nest-site choice may also be context-dependent, with individuals choosing low-quality nest-sites if no better options are available in the surroundings (Stanback and Rockwell 2003).

Cues used by prospecting individuals for choosing their breeding site may be based on direct observations of conspecific presence, which may generate territorial aggregations (‘conspecific attraction’; Stamps 1988), or conspecific behaviour, such as offspring feeding effort by parents, which is expected to provide reliable information about breeding patch quality (Doligez et al. 2002; Pärt and Doligez 2003; Ward 2005). Moreover, they may directly assess conspecifics’ breeding success (quantity/condition of offspring) in a given season and use this information to decide where to settle and breed subsequently (Boulouinier and Danchin 1997).

Prospecting individuals may also exploit indirect cues of conspecific reproduction, such as tracks or signs of reproductive activity occurring in the past. In birds, these may include the density of old nests (e.g. Erckmann et al. 1990; Gergely et al. 2009; Ringhofer and Hasegawa 2014), or, in cavity-nesting species, the presence of old nest material within suitable nest cavities (review in Mazgajski 2007; see also Brown and Shine 2005 for a study of reptiles). The presence of old nest material in nest cavities (nest lining material, faeces, pellets, prey remains, feathers, etc.) does in fact contain information about previous breeding activity: cavities containing such material may be preferred as they may be perceived as being more suitable than similar cavities where no sign of previous reproduction is evident (Brown and Shine 2005; Sumasgutner et al. 2014). At the same time, in species that do not add any material to line their nest, the presence of organic material from previous breeding events may be a further cue to nest-site quality because it may contribute to increase thermal insulation and reduce egg heat loss (Hilton et al. 2004; Mazgajski 2007; Mainwaring et al. 2014), potentially improving incubation efficiency.

In line with the above, experimental removal of old nest material decreased nestbox occupancy in the subsequent breeding season in burrowing owls *Athene cunicularia*, with birds returning from migration avoiding cleaned nestboxes (Riding and Belthoff 2015). Similarly, female Eurasian kestrels *Falco tinnunculus* laid eggs later in experimentally cleaned nestboxes compared to uncleaned ones, indicating a preference for old nest material (Sumasgutner et al. 2014). A preference for nestboxes with old nest material was observed also in some passerine species, such as the pied flycatcher *Ficedula hypoleuca* (Orell et al. 1993; Mappes et al. 1994; Olsson and Allander 1995), the house wren *Troglodytes aedon* (Thompson and Neill 1991) and the eastern bluebird *Sialia sialis* (Davies et al. 1994).

In spite of the potential benefits of choosing cavities with old nest material, some species/populations avoid breeding in previously used cavities (e.g. Merino and Potti 1995; Mazgajski 2003; review in Mazgajski 2007). Breeding in previously used cavities may indeed entail non-trivial costs. Nests containing old nest material may be subjected to increased predation risk due to predators memorizing nest positions (e.g. Sonerud 1985; Nilsson et al. 1991). Importantly, organic nest material is a highly favourable ground for the development of nest-dwelling ectoparasites and pathogens (Rendell and Verbeek 1996). Nest-dwelling parasites infest adults and especially nestlings, eventually impairing individual growth, condition and fitness (Møller et al. 1990; Martinez et al. 2011). Nest parasites can impair fitness either directly (e.g. in the case of blood sucking by haematophagous species; e.g. Heylen and Matthysen 2008; Tomás et al. 2008) or indirectly, transmitting bacterial or viral pathogens and spreading disease (Møller et al. 1990).

On the whole, although some studies suggest the preference or avoidance of previously used nest cavities (see above), nest-site choice in secondary cavity-nesters appears rather insensitive to the presence of old nest material, with several studies not reporting any clear preference pattern (e.g. Olsson and Allander 1995; Tomás et al. 2007; review in Mazgajski 2007). Furthermore, the adaptive value of breeding in previously used vs. non-used nest cavities has yet to be elucidated. In the majority of studies conducted so far, no significant impact of the presence of old nest material was found on clutch size, fledging success or nestling condition (review in Mazgajski 2007). Statistically significant fitness
effects (mostly negative) of breeding in cavities with old nest material have been reported only occasionally (e.g. Tomás et al. 2007; González-Braojos et al. 2012; review in Mazgajski 2007).

Lesser kestrels *Falco naumanni* appear to make wide use of public, social and environmental information for dispersal, colony-site settlement decisions, and nest-site selection, with breeding success of conspecifics being an important cue (Negro and Hiraldo 1993; Serrano et al. 2001; 2003; Aparicio et al. 2007). In lesser kestrel colonies, most successful breeding attempts take place in previously occupied cavities, which are also occupied earlier compared to seldom used cavities (Negro and Hiraldo 1993). However, to our knowledge, no study has experimentally addressed whether the presence of old nest material is used as a cue for choosing specific nest-sites within a breeding colony. We performed a nestbox choice experiment whereby breeding pairs had the opportunity to select either a nestbox without organic nest material (clean nestbox) or a paired nestbox with a thick organic layer from previous nesting attempts (dirty nestbox). Based on previous studies carried out in this species (Negro and Hiraldo 1993) and in the closely related Eurasian kestrel (Sumasgutner et al. 2014), we expected a preference for settling in dirty nestboxes. In addition, by exploiting a larger sample of unpaired dirty and clean nestboxes and adopting a correlative approach, we assessed whether breeding in dirty vs. clean nestboxes was associated with variation in breeding performance and nestlings’ mortality, ectoparasite load, and early growth patterns.

**Materials and Methods**

**Study species, study area and general methods**

The lesser kestrel is a small (ca. 120 g), colonial breeding, Afro-Palearctic migrant raptor. European individuals reach breeding areas in February/March, and start egg laying between late April and early May. Females lay 3-5 eggs (single brooded), which are incubated for ca. 30 days. Nestlings fledge when ca. 40 days old. Being a secondary cavity-nester, the lesser kestrel does not build its own cavity: it breeds in holes and cavities in rocks, ruins, roof tiles of buildings in urban areas or isolated abandoned farmhouses in the countryside, and it does not add any nest lining material (Cramp 1998). However, it readily settles in nest cavities containing an organic substrate resulting from previous breeding attempts, similarly to other secondary cavity-nesters (Cramp 1998; Negro and Hiraldo 1993).

The study was carried out during April-July 2016 in the city of Matera (Southern Italy; 40°67’ N, 16°60’ E), hosting a large colony of ca. 1000 lesser kestrel pairs (La Gioia et al. 2017). Several hundreds of nestboxes were deployed in 2008-2010 within the framework of the LIFE Project ‘Rapaci Lucani’ (LIFE05NAT/IT/00009), so that presently an unknown (but likely large) fraction of pairs breeds in nestboxes. We relied on 175 nestboxes that were placed on the roof terraces of two large buildings located ca. 500 m apart in the city centre. Nestboxes were made by a hollow refractory brick (300 × 300 × 370 mm external size) closed by two wooden panels (300 × 300 × 20 mm), the frontal one with an entrance hole of 65 mm diameter. Ventilation of the nest chamber was provided by 9 small holes (ca. 10 mm) on the wood panels. The front panel could be easily opened for nest inspection.

Upon deployment, the floor of all nestboxes was coated with a layer of sand and fine gravel to increase insulation towards the cement brick and reduce the probability of egg breakage during nest inspection or egg turning by the female. In February 2016, before arrival of lesser kestrels at the colony site, nestboxes were organized in ‘dyads’ of clean and dirty nestboxes (N = 40 dyads, see below) and ‘unpaired’ nestboxes [24 old (dirty) nestboxes (all of which had been used for breeding and roosting in previous years) and 71 new (clean) nestboxes (deployed in February 2016 and never previously used by lesser kestrels)]. Both dyads and unpaired (dirty and clean) nestboxes were randomly positioned along the entire perimeter of each terrace, at a minimum distance of ca. 2 m from each other. Old nestboxes had never been cleaned after their original deployment (2008-2010). Hence, most old nestboxes had a thick (ca. 5 cm), hard
coating of organic material deriving from previous breeding events spread over the floor of the nestbox (see also Assessment of nest-site preference). The position of all old nestboxes was randomly shuffled in February 2016 to accommodate deployment of new clean nestboxes and to form dyads, as well as to avoid nest recognition bias (see Assessment of nest-site preference).

All nestboxes were regularly checked throughout the breeding season to record breeding bird performance. Nestboxes were checked until the oldest nestling in the brood was ca. 16 days old (we refrained from checking nestboxes after that age because nestlings started wandering outside the nest and freely moved on the terraces, making monitoring difficult and increasing the risk of inducing premature fledging); over this period, each nestbox was checked five times (i.e. five monitoring sessions), with monitoring sessions occurring at an average of 0.8 (range 0–3), 3.0 (2–5), 5.3 (4–9), 7.9 (7–11), and 16.0 (14–18) days from hatching of the first egg in a nestbox, respectively.

Upon hatching, nestlings were individually marked with different combinations of small black dots on the down of the nape using a non-toxic black permanent marker, then ringed with metal rings when ca. 10 days old. Nestling body mass (accuracy of 0.1 g using an electronic scale) and ectoparasite load (see below) were recorded from the first to the fourth monitoring session, while tarsus (accuracy 0.1 mm with dial calliper) and forearm length we report in this study (accuracy 1 mm with a ruler) were recorded at the fourth monitoring session only. At the fourth monitoring session, a small (ca. 200 µl) blood sample was collected in capillary tubes by the puncturing brachial vein with sterile needles in order to determine nestling sex. This was achieved by means of polymerase chain reaction amplification of the sex-specific avian CHD-1 gene, following standard protocols (Griffiths et al. 1998).

Each nestling in a given nestbox was ranked according to hatch order. When two or more nestlings were first found hatched on the same monitoring session, rank was assigned based on body mass (larger nestlings had higher rank). The first hatched nestling was assigned the highest rank (i.e. rank 1), while subsequent nestlings were assigned lower ranks (i.e. 2 to 5; no more than 5 nestlings were found in each nestbox). As there were no statistically significant sex differences in body mass at hatching (body mass recorded within 1 day of hatching, mixed model with nestbox identity as a random intercept effect, effect of sex: \( F_{1,167} = 0.01, P = 0.98 \)), sex did not confound nestling rank assignment.

As proxies of breeding performance, we used clutch size (number of eggs laid), hatching success (proportion of eggs hatched in a clutch), and brood size (number of nestlings in the nest), the latter being recorded at each monitoring session.

As a part of a parallel study, unrelated to the present one, in a sample of 44 nestboxes (20 belonging to dyads and 24 unpaired) out of the 98 where the clutch size was completed and incubation started, we performed a food supplementation by which we provided laying pairs with laboratory mice after the laying of the first egg and during the early nestling period. Pairs breeding in non-supplemented nestboxes served as controls. This concomitant experiment, whose results will be reported elsewhere (S. Podofillini et al., manuscript in preparation), could not alter nestbox occupation patterns because supplementation started after a given nestbox had been chosen by the kestrels (i.e. after the first egg had been laid).

Assessment of nest-site preference

Nest-site preference was experimentally investigated based on 40 nestbox dyads, A dyad consisted of two paired nestboxes placed side-by-side (the sides were touching each other), one of which was ‘dirty’ while the other was ‘clean’, with the two front panels with the entrance holes pointing towards the same direction (Figure 1). In this way, we aimed at forcing the choice between the dirty and the clean nestbox while eliminating any confounding effect due to nest
orientation, position (e.g. shaded vs. unshaded, disturbance level), nestbox wear (see below), predation risk and surrounding habitat quality.

When assembling dyads, one old nestbox, in which clear signs of previous breeding attempts were obvious, was paired with an identical, brand-new nestbox. Old nestboxes, besides containing compressed organic material (mostly consisting of prey remains, regurgitated pellets, faeces, feathers, etc.), had a rather worn external appearance (i.e. faded colouration), including front panels. To remove any confounding effect of external nestbox wear on nest-site preference, we shuffled front panels and nest material between old and new nestboxes according to all eight possible combinations (Figure 1), each of which was applied five times (there were five dyads for each combination). The old nest material was carefully removed from any old nestbox included in a dyad, vigorously minced, shaken and placed back either into the old or the new nestbox according to the predetermined combinations. To avoid any side bias, the old nestbox was placed alternately on the left or the right side. Hence, dirty nestboxes within a dyad were characterized by the presence of old, organic nest material (a cue of previous breeding attempts) while clean nestboxes did not have any organic nest material but only a thin layer of gravel and sand on the bottom of the nestbox (no cue of previous breeding attempts). Dyads were randomly interspersed among unpaired nestboxes along the perimeter of terraces, and were positioned at a minimum distance of 2 m from nearby dyads or unpaired nestboxes (see also Study species, study area and general methods).

Since lesser kestrels show a high natal and breeding philopatry (57% of first-time breeders recruit to the natal colony, and ca. 72% of adults return to the colony where they bred in the previous year; Negro et al. 1997; Serrano et al. 2001), nest-site preference could be affected by previous experience and recognition of previous year’s nest-sites. To avoid this bias, in February 2016 all old nestboxes (either included in dyads or not) were randomly shuffled along the perimeter of terraces.

Nest-site preference was determined by assessing the settlement of a breeding pair in each nestbox of the dyad (laying of eggs). Laying date of the first egg was used to establish which of the two nestboxes of a dyad was occupied first (in case both nestboxes of a dyad were occupied). Lesser kestrel females may occasionally start laying one egg in a nest and then lay the other eggs in nearby nests, especially when several identical nestboxes are placed nearby (authors’ pers. obs). This was not the case in our dyads, where occupancy mostly occurred in only one of the two nestboxes, and when both nestboxes of a dyad were occupied, we found different females in the nests. In one dyad, however, a single egg was laid in a clean nestbox and then abandoned. This dyad was considered in the analyses of nest site preferences, but excluding it did not alter our conclusions (see Results).

**Nestling ectoparasite load**

We assessed ectoparasite load of nestlings by estimating infestation by a common, small (ca. 2 mm) haematophagous ectoparasitic fly (*Carnus hemapterus*, Diptera: Carnidae). Adult flies infest nestlings of several cavity-nesting bird species (Capelle and Whitworth 1973). Females lay eggs in the organic nest material and the saprophagous larvae thrive in the nest substrate, where they feed on detritus. The life-cycle of this ectoparasitic fly is synchronized with that of its hosts: the peak of emergence of adult parasites from the nest material coincides with the hatching of hosts’ eggs (Roulin 1998). Pupae are able to overwinter inside nest organic material, waiting for potential hosts to settle (Roulin 1998; Valera et al. 2006).

Nestlings were inspected to estimate the number of adult flies on the *furcula* (interclavicular depression) and on the right and left *axillae* (underwings) from the first to the fourth monitoring session. We could not accurately count all flies as they were fast-moving and hid rapidly within the nestling down upon handling. Hence, nestling ectoparasite
load was rapidly scored upon handling each nestling by estimating visible flies for each body district on a 0-3 scale (0: no ectoparasites, 1: 1–3 flies, 2: 4–6 flies and 3: > 6 flies) and then averaged between all body districts before statistical analyses.

Statistical analyses

Nest-site preferences were assessed based on the sample of 40 dyads. The number of dyads with occupied dirty vs. clean nestboxes were compared by means of a binomial tests for deviation from equality.

The effects of nestbox dirtiness on laying date, breeding performance, nestling mortality, ectoparasite load and growth patterns were assessed based on pooling data collected both from dyads and unpaired nestboxes, This was necessary because of the very low sample size of occupied clean nestboxes belonging to dyads (see Results). The effect of nestbox dirtiness on proxies of breeding performance [clutch size, hatching success, brood size at 8 and 16 days from hatching of the first egg] was evaluated by generalized linear models (GLMs) with nestbox dirtiness (clean vs. dirty) and laying date (day of laying of the first egg) as predictors (to control for seasonal variation in breeding performance). Hatching success was expressed as the proportion of eggs hatched on clutch size, and tested in a binomial GLM using the events/trials syntax. In models of clutch and brood size (count variables) we assumed a Poisson error distribution. To reduce noise in estimates of egg hatching success and nestling survival, we excluded from the analysis all 16 nests where clutch size was completed but no eggs hatched (likely deserted by parents; 16% of the 98 nestboxes where clutch size was completed; see Results). This did not affect our conclusions concerning the effect of dirtiness on other breeding parameters because the proportion of nests abandoned before hatching (nestboxes where no egg hatched) did not significantly differ between clean (0.22) and dirty (0.12) nestboxes [binomial GLM: effect of dirtiness, estimate (SE): -0.39 (0.59), Z = -0.66, P = 0.51; effect of laying date, estimate (SE): 0.07 (0.04), Z = 1.68, P = 0.09], though there was a trend for clean nestboxes to be abandoned more frequently than dirty ones.

The effect of nestbox dirtiness on nestling mortality was investigated using a binomial mixed model whereby mortality of each nestling (0 = alive, 1 = found dead or disappeared) at the fifth monitoring session was the dependent variable, while nestbox dirtiness, nestling rank, brood size (maximum brood size across all monitoring sessions), laying date and ectoparasite load (maximum ectoparasite load across all monitoring sessions) were included as covariates. Nestbox identity was included as a random intercept effect.

To assess the effect of nestbox dirtiness on ectoparasite load, we ran a linear mixed model with nestbox dirtiness, nestling rank, brood size and laying date as predictors. We also included monitoring session as a four-level fixed factor to control for variation in ectoparasite infestation throughout the course of the nestling period. Two-way interactions between dirtiness and all other predictors were also included in the initial model. Nestling and nestbox identity were included as random intercept effects.

We evaluated the effects of nestbox dirtiness on body mass using a linear mixed model including nestbox dirtiness, nestling age, nestling rank, brood size (number of nestling in the nestbox at each check), laying date, ectoparasite load and two-way interactions between dirtiness and nestling rank, brood size, or ectoparasite load, as well as the two-way interaction between nestling rank and nestling age (to account for differential growth of nestlings differing in rank) as fixed predictors; nestling and nestbox identity were included as random intercept effects. The models of tarsus and forearm length had a fixed effect structure identical to the model of body mass, but as we had a single measurement per nestling, we included only nest identity as a random intercept effect. Moreover, in these models brood size and ectoparasite load referred to the maximum values recorded for that nestbox/nestling during the four monitoring sessions. Age effects on growth were controlled for by including the linear term of age only. Despite generally growth curves are
sigmoidal-shaped (Starck and Ricklefs 1998), nestling growth of lesser kestrels up to 11 days (out of a nestling period of ca. 30 days) did not significantly deviate from linearity (details not shown for brevity).

In all models, two-way interaction terms were removed in a single step if non-significant ($P > 0.05$). Full models (including all non-significant interactions) are reported in Supplementary material.

Since the lesser kestrel is sexually size dimorphic, females being heavier and larger than males (Cramp 1998), we performed exploratory analyses on the subsample of 209 nestlings (out of 244 hatched) that were alive at the fourth monitoring session (when blood sampling was performed) to investigate possible effects of nestling sex ($0 =$ female, $1 =$ male) on the response variables. Mixed models (with the same random intercept effects as detailed above) did not reveal any statistically significant difference in response variables according to sex [parasite load: estimate (SE): -0.07 (0.04), $F_{1,169} = 3.71$, $P = 0.06$; body mass: -1.55 (1.65), $F_{1,200} = 0.52$; tarsus length: -0.20 (0.48), $F_{1,185} = 0.18$, $P = 0.67$; forearm length: estimate (SE): -0.38 (0.84), $F_{1,187} = 0.20$, $P = 0.65$]. Hence, for simplicity and to avoid sacrificing sample size for some of the analyses, we did not consider sex effects any further in the analyses. These results indicate that nestling parasite load is not significantly different between sexes and that sexual size dimorphism is not yet evident during the early nestling stage.

To check for the possible confounding effects of the food supplementation experiment on breeding performance traits, nestling ectoparasite load, body mass and skeletal growth, all relevant models were re-run while including food supplementation (supplemented vs. control) as a fixed effect. The effect of food supplementation was never statistically significant ($P$-values always $> 0.14$; additional details not shown for brevity). Hence, for simplicity we did not consider this variable further.

Mixed models were fitted using the \textit{lmer} or \textit{glmer} function of the ‘lme4’ library (Bates et al. 2014) for R 3.3.1 (R Core Team 2014). Degrees of freedom for linear mixed models were estimated using the Kenward-Rogers approximation (‘pbkrtest’ library; Halekoh & Højsgaard 2014). Non-Gaussian GLMs and mixed models were not overdispersed (see Results; overdispersion for non-Gaussian mixed models was checked using the ‘blmeco’ library; Korner-Nievergelt 2015).

Results

Nestbox occupancy, nest-site preference, and laying date

Among unpaired nestboxes, old nestboxes were occupied significantly more often than new ones [old nestboxes: 20/24 (83.3%), new nestboxes: 34/71 (47.9%); $\chi^2 = 9.19$, $df = 1$, $P = 0.002$]. In the nest-site selection experiment, 38 out of 40 dyads had at least one nestbox occupied (i.e. 95% of dyads had at least one nestbox occupied). Among the 38 dyads with at least one nestbox occupied, in 31 cases only the dirty nestbox was occupied, in 1 case only the clean nestbox was occupied (binomial test, $P < 0.001$), and in 6 cases both nestboxes were occupied. Among the latter 6 dyads, the dirty nestbox was occupied earlier in 5 out of 6 cases, the mean laying date in the dirty nestbox of the dyad being 12.0 (4.1 \textit{SE}) days earlier than in the clean one (Wilcoxon matched-pairs test: $Z = 2.02$, $P = 0.043$). Considering both unpaired nestboxes and dyads, mean laying date in dirty nestboxes was May 13 (1.0 \textit{SE}, $N = 57$), while it was May 18 (1.3 \textit{SE}, $N = 41$) in clean ones ($t_{56} = 2.89$, $P = 0.005$).

Nestbox dirtiness, breeding performance and nestling mortality
The effects of nestbox dirtiness (clean vs. dirty) on clutch size, hatching success and brood size was analysed in the sample of 82 nestboxes where at least one egg hatched.

Clutch size did not significantly differ between clean and dirty nestboxes (Table 1), while hatching success of eggs laid in dirty nestboxes (percentage hatched = 86%) was slightly but significantly higher than that of eggs laid in clean nestboxes (76%) (Table 1). In spite of a significantly higher hatching success in dirty nestboxes, brood size did not significantly differ between clean and dirty nestboxes (Table 1). Breeding performance of lesser kestrels did not significantly vary across the breeding season, as shown by the lack of significant effects of laying date (Table 1).

The probability that a nestling had died by the last monitoring session was not significantly affected by nestbox dirtiness (Table 2), while it was significantly higher among low-ranking nestlings (Table 2).

Nestling ectoparasite load, body mass and size in relation to nestbox dirtiness

Nestling ectoparasite load was recorded in 70 nestboxes (28 clean, 42 dirty). The model of ectoparasite load revealed a statistically significant nestbox dirtiness × monitoring session interaction (Table 3, Figure 2): post-hoc tests indicated that mean ectoparasite load was significantly higher in dirty nestboxes soon after the first eggs had hatched (i.e. in the first monitoring session) ($P = 0.003$), whereas the effect of dirtiness on ectoparasite load became non-significant in all subsequent monitoring sessions (all $P$-values > 0.40). Moreover, ectoparasite load strongly decreased with nestling rank, high-ranking nestlings being more infested than low-ranking (smaller and late hatched) ones (Table 3). Finally, ectoparasite load markedly decreased in the course of the breeding season, late clutches being significantly less infested than early ones (Table 3). Two-way interactions between nestbox dirtiness and other predictors were not significant and were thus removed from the model (all $P > 0.33$; see Table S1 in Supplementary material for details).

Nestling body mass was not significantly affected by nestbox dirtiness (Table 3), while it significantly decreased in more parasitized nestlings, in low-ranking ones, and among nestlings reared in larger broods (Table 3). Moreover, early nestling growth was significantly lower in low-ranking nestlings, as shown by the negative sign of the significant age × nestling rank interaction (Table 3). Other two-way interactions with nestbox dirtiness were not significant and were removed from the model (all $P > 0.60$; see Table S1 in Supplementary material for details).

Tarsus and forearm length recorded at the last monitoring session were not significantly affected by nestbox dirtiness, while they were both lower in low-ranking nestling (Table 3). Tarsus (but not forearm) length was significantly larger in nestlings reared in larger broods (Table 3). Two-way interactions between dirtiness and other predictors were not significant and were removed from the models (tarsus length, all $P > 0.30$; forearm length, all $P > 0.20$; see Table S1 in Supplementary material).

Discussion

Studies addressing the preference for dirty vs. clean nestboxes in secondary cavity-nesters have provided conflicting evidence, highlighting broad interpopulation and interspecific differences in preference patterns (see Introduction and review by Mazgajski 2007). Part of this variability may be due to different experimental designs that were not specifically aimed at testing the effect of cues of previous breeding attempts on nest-site choice (Mazgajski 2007). In our carefully designed nestbox choice experiment, lesser kestrels showed a strong preference for nestboxes previously used by conspecifics, breeding pairs settling earlier and more frequently in nestboxes with a dirty substrate. The
preference for dirty nestboxes is consistent with two possible explanations. First, it is consistent with the idea that the breeders exploit cues about previous breeding attempts by conspecifics to choose their nest cavity or colony site (Negro and Hiraldo 1993; Serrano et al. 2001; 2003; Aparicio et al. 2007). Secondly, it may reflect preference for a more comfortable nest substrate by females. The organic material contained in old nests, being ca. 5 cm thick, may improve thermal insulation of the egg laying substrate, reducing heat loss, increasing incubation efficiency, and ultimately lowering the energetic costs of incubation (Mainwaring et al. 2014). Energy demands during incubation largely depend on the rate at which eggs lose heat (Deeming 2002). Incubating birds, especially those (as the lesser kestrel) that lay eggs directly on the substrate without lining their nest cavity, are therefore expected to preferentially lay eggs on those substrates that minimize the energetic costs of incubation (Deeming 2002; Mainwaring et al. 2014). Females may have been roosting in both nestboxes of a dyad before egg laying, and this might have promoted the choice for the likely more suitable organic nest substrate. Finally, earlier egg laying in dirty vs. clean nestboxes is in accordance with the hypothesis that the sequence of cavity occupation in lesser kestrels follows a despotic distribution (Negro and Hiraldo 1993; see also Sumasgutner et al. 2014), with early-settling individuals (likely older and experienced breeders; Catry et al. 2017) preferentially settling in dirty nestboxes compared to clean ones.

With regards to the fitness consequences of settling in a dirty nestbox, we envisage three possible explanations for the ca. 10% greater hatching success in dirty vs. clean nestboxes. First, the organic material could allow establishing a favourable nest microclimate through improved thermal insulation and humidity stabilization (Hooge et al. 1999, Ardia et al. 2006), possibly increasing egg viability (Cook et al. 2003). Indeed, previous studies have shown that nest position and content are important factors in affecting thermal insulation and in buffering the potential negative effects of harsh environmental conditions on embryo development (Hilton et al. 2004; Mainwaring et al. 2014). Secondly, eggs laid on soft, organic rather than mineral substrate may suffer a lower risk of breakage and/or be more efficiently incubated, resulting in lower egg failure rates. Alternatively, a higher hatching success in dirty nestboxes may be due to a better incubation performance/higher phenotypic quality of early settling (older/more experienced; Catry et al. 2017) pairs occupying these nestboxes.

The higher C. hemapterus load of nestlings hatched in dirty vs. clean nestboxes is likely due to the higher parasite load of dirty vs. clean nestboxes. C. hemapterus flies undergo a prolonged diapause when hosts are absent from the nest cavity, and adult emergence is synchronized with nestling hatching (Roulin 1998). However, ectoparasite load of nestlings raised in clean vs. dirty nestboxes became very similar within a few days after hatching of the first egg, likely because of ectoparasite dispersal between nearby nestboxes to limit competition for access to hosts (e.g. Dawson and Bortolotti 1997). Moreover, ectoparasite load strongly decreased over the course of the breeding season, late broods being significantly less parasitized than early ones. The seasonal decline of C. hemapterus load is in line with previous studies (e.g. Dawson and Bortolotti 1997; Sumasgutner et al. 2014), and may be due to natural variation in abundance through the parasite life-cycle (Roulin 1998).

The lack of significant effects of nestbox dirtiness on nestlings’ early growth patterns suggests that the higher ectoparasite load of dirty nestboxes is of seemingly minor importance for nestling fitness (Sumasgutner et al. 2014), in spite of the higher C. hemapterus parasitism of nestlings hatched in dirty nestboxes that we observed soon after hatching. Together with the observation that breeding success in dirty nestboxes was not lower than in clean ones, this finding suggests that breeding in dirty nestboxes does not entail fitness costs (e.g. Sumasgutner et al. 2014).

On the whole, our results did not provide strong evidence that breeding in dirty nestboxes provides fitness payoffs in terms of improved reproductive output. Studies of nest-site or breeding habitat choice commonly assume that observed preference patterns are adaptive, implying that settlement decisions reflect fitness benefits (in terms of higher breeding success and/or survival; see Orians and Wittmerberger 1991; Martin et al. 1998; Chalfoun and Schmidt 2012), but this
assumption has only seldom been tested (Brambilla and Ficetola 2012). In secondary-cavity nesters, the effects of nest dirtiness on reproductive parameters are unclear; the majority of studies have shown no obvious effects of nest material from previous breeding events on fitness traits, though some studies have documented weak statistically significant (mostly negative) effects (Mazgajski 2007). Our findings are thus in line with such previous evidence. We note however that the detection of significant fitness effects of nest-site preference for previously used nests may be context-dependent. It is known that lesser kestrels use conspecific presence as a major cue when deciding where to nest and when to breed (Serrano et al. 2003), and our study site may in fact act as a single huge colony of ca. 1000 breeding pairs (La Gioia et al. 2017). In this context, selection of different nest-sites may not be so relevant in terms of fitness because the high number of individuals occurring at this colony may indicate favourable breeding conditions (for instance, larger colonies are mostly settled in sites that are less accessible to predators; Serrano et al. 2004). However, in a different context, with small colonies that are sparsely distributed through the landscape (thus more difficult to be detected by prospecting individual kestrels), the presence of organic material derived from previous breeding attempts in a cavity would be an important cue for settlement at a suitable breeding site and could have significant fitness consequences.

Other findings emerging from this study, unrelated to nestbox dirtiness, are briefly discussed below.

First, parasite load negatively affected body mass growth, suggesting that intense *C. hemapterus* parasitism may entail fitness costs for nestlings (e.g. Hoi et al. 2010). Alternatively, the negative effect of *C. hemapterus* parasitism on nestling body mass may be indirect, resulting from higher parasitism in clutches with low-quality nestlings (i.e. nestlings with a smaller cutaneous immune response; Bize et al. 2008), or from greater exposure to pathogens that may be transmitted through *C. hemapterus* blood meals.

Second, the higher *C. hemapterus* load in high- vs. low-ranking nestlings is consistent with the idea that ectoparasites’ host selection is non-random. *C. hemapterus* seem to aggregate in larger numbers on older/heavier nestlings, suggesting avoidance of smaller and/or poorer condition nestlings within broods (e.g. Dawson and Bortolotti 1997; Valera et al. 2004; Bize et al. 2008; Hoi et al. 2010; but see Roulin et al. 2003). This may occur because: 1) parasites can less easily obtain abundant/high-quality food resources from such hosts, decreasing their own fitness; 2) lesser kestrels show a relatively large hatching asynchrony [days between hatching of the first and the last egg in a clutch: 2 days (range 1-10); our unpubl. data], whereby early hatched hosts are the only target of parasites before hatching of their younger siblings; 3) smaller hosts simply provide less resources for parasites (in terms of total blood amount flow/feeding space available on the nestling skin). The fact that the per gram ectoparasite load (ectoparasite load/body mass) was not significantly predicted by nestling rank is in line with the third explanation (see Table S2 and Figure S1 in Supplementary material), though hatching asynchrony may also contribute to explain nestling rank effects on ectoparasite load.

Third, nestlings from larger broods had a lower body mass, but longer tarsi and forearm. This suggests that brood size may modulate early growth trajectories, perhaps via an effect on sibling competition (see also Gil et al. 2008). A larger skeletal size may provide competitive advantages in obtaining food items, as it may lead to dominance in sib-sib interactions once parents arrive at the nest with prey, and the payoff of a larger skeletal size may be greater in larger broods where sibling competition is higher (Schew and Ricklefs 1998).

In conclusion, we provide strong evidence that the presence of organic material from previous nesting attempts in the nest cavity is a key driver of nest-site choice, in line with lesser kestrels exploiting cues of conspecific presence for deciding where to settle and breed, and with the idea that organic nest material provides females with a comfortable substrate for egg laying and incubation. We emphasize that these findings may have bearings for projects aimed at improving the conservation status of the lesser kestrel, a species of European conservation priority that has suffered
severe population declines and range contractions in the course of the 20th century (BirdLife International 2015). As the availability of suitable nest-sites has been identified as an important factor limiting population growth (Negro and Hiraldo 1993), many conservation projects rely on provisioning large numbers of nestboxes (Iñigo and Barov 2010; La Gioia et al. 2017). We propose that adding old nest material to newly deployed nestboxes may represent a cheap and effective way to enhance their occupation rate, hence increasing the effectiveness of conservation measures aimed at improving the conservation status of lesser kestrel breeding populations.

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Study from the Naumanni76 team (paper#01).

Author contributions

DR, JGC, MV, SPo and MG conceived the study and wrote the paper, with inputs from EDC and NS; SPo, AC, EF, SPi, LS, JGC, EDC, MG and DR conducted fieldwork and collected the data; SP, DR, JGC and NS analyzed the data.

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Figure 1 Schematic illustration of the different combinations adopted to randomize nest material, front panel and cement block in dyads of adjacent clean and dirty nestboxes. The combinations were illustrated using white panels and white cubes for panels and cement blocks installed for first time in 2016; brown panels and grey cubes for old frontal and cement block; white holes: clean nestboxes; white and grey holes: dirty nestboxes. The dirty nestbox was alternately placed on the left or right side, to avoid any side bias. A dyad was interspersed in random order between unpaired nestboxes or other dyads along the perimeters of the terraces of two buildings, and was at a minimum distance of 2 m from any nearby dyad/unpaired nestbox.
Figure 2 Nestling ectoparasite load in each of the four monitoring sessions. Filled dots represent the mean ectoparasite load of nestlings reared in dirty nestboxes while empty dots refer to nestlings reared in clean nestboxes ($N = 70$ nests, 244 nestlings). Error bars represent 1 SE.
Table 1. Effect of nestbox dirtiness on breeding performance. Mean values (1 SE) of breeding parameters are reported (binomial SE for hatching success). Estimates are from Poisson or binomial GLMs (for hatching success). Models were not overdispersed (dispersion parameter always < 1.26).

|                | Clean | Dirty | Estimate (SE) | Z    | P    |
|----------------|-------|-------|---------------|------|------|
| **Clutch size (N = 82)** |       |       |               |      |      |
| Dirtiness      | 4.10 (0.14) | 4.34 (0.10) | 0.05 (0.11) | 0.47 | 0.64 |
| Laying date    | -     | -     | -0.01 (0.01)  | -0.25| 0.80 |
| **Hatching success (N = 82)** |       |       |               |      |      |
| Dirtiness      | 0.76 (0.04) | 0.86 (0.03) | 0.65 (0.29) | 2.29 | 0.022|
| Laying date    | -     | -     | 0.01 (0.02)   | 0.04 | 0.97 |
| **Brood size, day 7 (N = 82)** |       |       |               |      |      |
| Dirtiness      | 2.59 (0.24) | 3.16 (0.18) | 0.21 (0.14) | 1.54 | 0.12 |
| Laying date    | -     | -     | 0.01 (0.01)   | 0.55 | 0.58 |
| **Brood size, day 15 (N = 82)** |       |       |               |      |      |
| Dirtiness      | 2.25 (0.21) | 2.70 (0.17) | 0.19 (0.15) | 1.30 | 0.19 |
| Laying date    | -     | -     | 0.01 (0.01)   | 0.38 | 0.70 |

Table 2. Binomial mixed model of the effect of nestbox dirtiness on the probability that a nestling died at 15 days from start of egg hatching. Nestbox identity was included as a random effect. The model was not overdispersed (dispersion parameter = 0.81).

|                | Estimate (SE) | Z    | P    |
|----------------|---------------|------|------|
| Dirtiness      | -0.77 (0.78)  | 0.99 | 0.32 |
| Nestling rank  | 1.30 (0.26)   | 4.95 | < 0.001|
| Brood size     | -0.22 (0.36)  | 0.62 | 0.53 |
| Laying date    | -0.08 (0.05)  | 1.63 | 0.10 |
| Ectoparasite load | -0.57 (0.48) | 1.18 | 0.24 |
Table 3. Mixed models of the effects of nestbox dirtiness on nestling ectoparasite load, body mass, tarsus and forearm length, while accounting for the concomitant effects of different predictors. Models for ectoparasite load and body mass included nestbox and nestling identity as random effects, while models for tarsus and forearm length included only nestbox identity as a random effect.

| Ectoparasite load (N = 70 nests and 244 nestlings) | F   | df  | P     | Estimate (SE) |
|---------------------------------------------------|-----|-----|-------|---------------|
| Dirtiness                                         | 1.95| 1, 67| 0.17  | -             |
| Session                                           | 0.44| 3, 593| 0.73  | -             |
| Nestling rank                                     | 11.29| 1, 189| < 0.001| -0.05 (0.01) |
| Brood size                                        | 0.11| 1, 314| 0.75  | -0.01 (0.02)  |
| Laying date                                       | 32.90| 1, 77 | < 0.001| -0.02 (0.01)  |
| Dirtiness × session                               | 3.41| 3, 581| 0.017 | -             |

| Body mass (N = 70 nests and 244 nestlings; covariates centred on their mean value) | F   | df  | P     | Estimate (SE) |
|---------------------------------------------------------------------------------|-----|-----|-------|---------------|
| Dirtiness                                                                       | 0.01| 1, 51| 0.82  | -             |
| Age                                                                            | 4960.8| 1, 580| < 0.001| 6.96 (0.10)  |
| Nestling rank                                                                  | 120.2| 1, 144| < 0.001| -4.01 (0.37) |
| Brood size                                                                     | 5.2 | 1, 294| 0.023 | -1.01 (0.44) |
| Laying date                                                                    | 3.4 | 1, 68 | 0.07  | -0.15 (0.08) |
| Ectoparasite load                                                              | 4.3 | 1, 697| 0.038 | -1.55 (0.75) |
| Age × nestling rank                                                            | 123.5| 1, 601| < 0.001| -0.97 (0.08) |

| Tarsus length (N = 63 nests and 202 nestlings) | F   | df  | P     | Estimate (SE) |
|------------------------------------------------|-----|-----|-------|---------------|
| Dirtiness                                       | 0.36| 1, 53| 0.55  | -             |
| Age                                             | 212.1| 1, 168| < 0.001| 1.61 (0.11)  |
| Nestling rank                                   | 63.4| 1, 173| < 0.001| -0.97 (0.12) |
| Brood size                                      | 4.73| 1, 71 | 0.033 | 0.43 (0.20)  |
| Laying date                                     | 0.03| 1, 61 | 0.86  | 0.01 (0.03)  |
| Ectoparasite load                               | 0.65| 1, 194| 0.42  | -0.21 (0.23) |

| Forearm length (N = 63 nests and 203 nestlings) | F   | df  | P     | Estimate (SE) |
|------------------------------------------------|-----|-----|-------|---------------|
| Dirtiness                                       | 3.29| 1, 145| 0.08  | -             |
| Age                                             | 222.0| 1, 123| < 0.001| 2.78 (0.19)  |
| Nestling rank                                   | 63.6| 1, 181| < 0.001| -1.82 (0.23) |
| Brood size                                      | 3.89| 1, 67 | 0.053 | 0.58 (0.30)  |
| Laying date                                     | 1.49| 1, 57 | 0.23  | 0.05 (0.04)  |
| Ectoparasite load                               | 0.01| 1, 175| 0.98  | -0.01 (0.46) |