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Article

Smaller distance between nest contents and cavity entrance increases risk of ectoparasitism in cavity-nesting birds

Gustavo Tomás, Cristina Ruiz-Castellano, Magdalena Ruiz-Rodríguez and Juan J. Soler

G. Tomás (<https://orcid.org/0000-0001-6701-2055>) ✉ (gtomas@eeza.csic.es), C. Ruiz-Castellano, M. Ruiz-Rodríguez (<https://orcid.org/0000-0002-4202-5180>) and J. J. Soler (<https://orcid.org/0000-0003-2990-1489>), Dept of Functional and Evolutionary Ecology, Estación Experimental de Zonas Áridas (EEZA – CSIC), Almería, Spain. MRR also at: Sorbonne Univ. UPMC Univ. Paris 06, Observatoire Océanologique, Avenue du Fontaulé, Banyuls-Sur-Mer, France.

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Determining costs associated with nest-building behaviour and nest characteristics is of prime importance to understand the evolution of such extended phenotypic traits. We explored a cost of nest building experimentally to determine whether large nests attracted more ectoparasites. This cost may be especially important to cavity-nesting species where larger nests likely reduce the distance between nest contents and the cavity entrance, making the host nest more vulnerable to ectoparasite detection. We manipulated the amount of nest material and the distance between nest and cavity entrance and estimated the abundance of ectoparasites (*Carnus hemapterus* flies) before and after manipulation in nests of spotless starlings *Sturnus unicolor* breeding in nest boxes. Nest size was enlarged and elevated in a first group of nests by adding nest material to the base of the nest (enlarged nests); a second group of nests was elevated with a piece of plastic but without adding nest material (elevated nests); a third group of nests was not manipulated (control nests). Number of flies per nest increased significantly in enlarged and elevated nests compared to control nests. Moreover, distance to cavity entrance after manipulation was negatively associated with the increase in the number of flies per nest (i.e. nests closer to the entrance had more parasites). Thus, nest size, through its effect on distance between nest and cavity entrance, affected fly abundance. Cues produced by nest contents located closer to the cavity entrance may be detected more readily by ectoparasitic flies searching for hosts. These results suggest a novel constraint for the evolution of nest size in cavity-nesting birds.

Keywords: blood-sucking flies, cavity depth, distance to cavity entrance, ectoparasitism risk, host location, nest building, nest depth, nest size

Introduction

Avian nest building behaviour and nest characteristics have attracted a great deal of attention by zoologists, ecologists and evolutionary biologists. Nests act as extended phenotypes evolving through both natural and sexual selection, and provide a diverse array of benefits to their builders (Hansell 2000). Nest materials may, for instance, provide thermal insulation (Hoi et al. 1994, Hansell 2000), act as a physical barrier to predators



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or brood parasites (Soler et al. 2014), be a source of chemical compounds with insecticidal (Clark 1991, Tomás et al. 2012) and/or antimicrobial properties (Mennerat et al. 2009, Peralta-Sánchez et al. 2010, Ruiz-Castellano et al. 2016), or even act as ornaments to attract potential mates (Brouwer and Komdeur 2004, Veiga et al. 2006) and/or to elicit differential reproductive investment by mates in a post-mating sexual selection scenario (Soler et al. 1998, 2001, De Neve and Soler 2002, Moreno 2012, Tomás et al. 2013). Yet there is considerable intraspecific variability in avian nest features, including nest size, which is not fully understood (Soler et al. 1998, Tomás et al. 2006, Cantarero et al. 2013, Maziarz et al. 2015).

Evolution of avian nests is constrained by costs associated with nest-building activity and exaggeration of nest characteristics (Soler et al. 1998). Research on these costs is therefore essential to understand the evolution of nest-building behaviours and nest features. Most studies exploring the costs associated with nest building have focused on birds, and most of them detected energetic costs (Mainwaring and Hartley 2013). Building a large nest might also imply costs in terms of predation (Collias and Collias 1984, Biancucci and Martin 2010) and brood parasitism (Soler et al. 1999, 2014), simply because associated nest building activity and large nests are more easily detected.

Flying ectoparasites may locate active nests by following cues derived from nest-building activities or nest size. Several correlative studies have addressed a potential relationship between nest size and ectoparasite abundance, based on the hypothesis that larger nests can sustain larger ectoparasite populations due to density-dependent processes (Eeva et al. 1994, Heeb et al. 2000, Kleindorfer and Dudaniec 2009), or because ectoparasites have more substrate in which to hide and escape nest sanitation behaviours performed by birds (Soler et al. 2007). Another rarely explored possibility is that larger nests attract more ectoparasites due to easier detectability (Tomás et al. 2008a), as suggested in the case of predators (Collias and Collias 1984, Biancucci and Martin 2010) and brood parasites (Soler et al. 1999, 2014).

Ectoparasites inflict severe costs to breeding adults and nestlings, either directly through blood consumption (Merino and Potti 1995), or indirectly acting as vectors of harmful internal parasites (Merino et al. 2000). Blood-sucking flying ectoparasites, or biting flies, are assumed to locate hosts mainly by means of visual and olfactory cues (Allan et al. 1987, Bowen 1991, Gibson and Torr 1999, Lehane 2005). Recently, a potential role for acoustic cues has also been suggested in host detection by ectoparasites (Tomás and Soler 2016). A large nest can be more easily detected than a small one, either visually or because of the increased number of volatiles released by nest materials (Tomás et al. 2008a). Because nest materials may also trap odours released by birds, larger nests may also retain increased amounts of odorous cues. Relying on chemical or acoustic cues for host detection could be especially important for parasites of hole-nesting birds, where visual cues from nests are constrained.

In hole-nesting birds, nest size should be related to olfactory cues not only because of the association between the amount of nest material and odour intensity, but also because of a relationship between the amount of nest material and distance from nest contents to cavity entrance. Olfactory cues emitted by nests and nestlings would be easier to locate when distance between nest and cavity entrance is reduced because of air flux patterns inside the cavity (Conover 2007). Similarly, acoustic cues emitted by nestlings would be easier to detect when distance between nest and cavity entrance is reduced. Again, natural nests show a remarkable variability regarding the distance between the nest and the cavity entrance (Tomás et al. 2006, Maziarz et al. 2015). Therefore, because distance from the nest to cavity entrance is strongly determined by the amount of nest material (Tomás et al. 2006), nest size of hole-nesting birds may be positively related to risk of parasitism.

Carnus hemapterus (hereafter ‘*Carnus*’) has important negative effects on nestlings (Hoi et al. 2018) and is the most abundant ectoparasite in the spotless starling *Sturnus unicolor* population reported here (Avilés et al. 2009, Azcárate-García et al. 2019). *Carnus* is a 2-mm fly in which the adult is the blood-sucking stage, and is found in nests of an extremely wide diversity of birds (Brake 2011, Calero-Torralbo 2011). After emergence from overwintering pupae inside or around nests, winged adults may disperse, losing their wings once a suitable host is found (Grimaldi 1997, Calero-Torralbo 2011). *Carnus* feeds mainly on nestlings, where it can be found quickly searching for cover in suitable body areas such as the axilla, inguinal region, around the neck or inside ears. It feeds also on incubating adults (López-Rull et al. 2007), yet flies are not found on birds outside the nest (Grimaldi 1997). This suggests that, although there is still a poor understanding of its colonization patterns (Veiga et al. 2020), the main dispersion mode of *Carnus hemapterus* is by active flight, which can be over relatively long distances.

In this study, we tested experimentally the association between nest size, distance to cavity entrance, and intensity of parasitism in a population of starlings breeding in nest boxes of identical dimensions and characteristics. We manipulated nest size and distance between nest and cavity entrance shortly after hatching and estimated abundance of *Carnus* ectoparasitic flies in nests before and after manipulation. Nest size was enlarged in a first group of nests by adding nest material under the base of the nest (enlarged nests). A second group of nests was elevated without adding nest materials (elevated nests). In both groups, distance between nest and cavity entrance was reduced. A third group of nests were maintained as controls. We predicted that distance from the nest to cavity entrance was a major determinant of biting fly abundance. Consequently, ectoparasite abundance should be higher in enlarged and elevated nests as compared with control nests. On the contrary, if nest size rather than distance from nest to cavity entrance determines abundance of ectoparasites, enlarged nests should harbour more parasites than elevated or control nests because the amounts of

volatiles released would be positively related to the amount of nest materials. Finally, if distance from nest to cavity entrance predicts probability of nest location by ectoparasites, this distance should be related to abundance of ectoparasites.

Material and methods

Study area and species

Fieldwork was conducted during the 2012 breeding season (April–June) in Guadix, southeastern Spain (37°18'N, 3°11'W). Spotless starlings (hereafter starlings) bred in artificial nest boxes that were installed in 2005 in agro-pastoral habitat. Nest boxes made of cork (internal dimensions: 350 mm [height] × 180 mm [width] × 210 mm [depth]; distance from bottom to hole: 240 mm) were attached to walls of abandoned buildings or tree trunks at 3–4 m above the ground. Typical clutch sizes are four to five eggs and incubation usually starts with the third or fourth egg and lasts 12–13 days (Soler et al. 2008). Nests are built by both sexes, but females carry most nest material (Veiga and Polo 2011) which, in our study area, is mainly dry straw. Other nest material found in starling nests, such as green plants, fresh flowers and feathers, has been interpreted as ornamentation from sexual displays of males (plants) and females (feathers) (Polo and Veiga 2006, Veiga et al. 2006). These hypothetical ornamental materials may also function in protecting offspring from microorganisms (Mennerat et al. 2009, Peralta-Sánchez et al. 2010, Ruiz-Castellano et al. 2016, 2019, Soler et al. 2017) and/or ectoparasitic arthropods (Clark 1991, Tomás et al. 2012).

Nest manipulations

Old nest material was removed from nest boxes before the breeding season (on the last week of March) and transported to the lab, thus ensuring that most parasites overwintering in nest material were removed. Thus, any flies quantified in this study colonized nest boxes from outside experimental nests. From 4 April, nest boxes were checked on a regular basis to allow determining hatching dates (i.e. every four days until egg laying was detected and daily close to the expected hatching date). Three days after the first egg hatched, nestlings were removed temporarily and placed in a container covered with a white paper napkin. Nests were also removed carefully from nest boxes and alternately assigned to one of three treatments, assigning the first nest randomly: 1) enlarged (n = 18), 2) elevated (n = 17) and 3) control (n = 16). In enlarged nests, approximately 10 cm of nest material was added under the base of the nest; dry straw, a common nest material in our starling population, were used for this purpose. This material was collected from the ground in a nearby area but far from any nest, and was thus free of ectoparasites because *Carnus* pupae overwinters in nests (Roulin 1998). In elevated nests, a piece of plastic of approximately 10-cm height was inserted under the nest; we used an empty 5-l plastic container turned upside down and 'covered' with a 2-cm-thick

polystyrene sheet to provide horizontal support for the nest. In control nests, original nest material was returned to nest boxes without further manipulation. Distance between the nest rim and the cavity entrance was measured with a ruler to the nearest 0.5 cm before and after the manipulation. The manipulation led to final nest sizes and distances to cavity entrance that were within the natural range recorded in the study area (authors pers. obs.). Starlings occupy a great diversity of nesting sites and show a remarkable variability in these nest features.

Cavity temperature and humidity

The microclimate of the cavity may cause differences in abundance and survival of certain ectoparasites (Bennett and Whitworth 1991, Chilton et al. 2000, Heeb et al. 2000, Castaño-Vázquez et al. 2018). Therefore, temperature and humidity were recorded in a subsample of nests (n = 26; nine enlarged, nine elevated and eight control nests) to test whether nest manipulations affected microclimate of the cavity, which could subsequently affect ectoparasite abundance. For this purpose, a datalogger (Thermocron iButton, Dallas Semiconductor, Dallas, TX), was attached to the inner lateral wall of the nest box above the nest rim (Ardia and Clotfelter 2007). The datalogger recorded temperature ($\pm 0.5^\circ\text{C}$) and relative humidity ($\pm 0.01\%$) at 12-min intervals over 48 h, beginning at 8 pm on day 3 of the nestling stage, thus providing 240 measurements per nest. Mean, minimum and maximum temperature and humidity for each nest were obtained.

Ectoparasite abundance

Although patterns of *Carnus* colonization vary among nests, in starling nests, *Carnus* abundance often peaks shortly after hatching, reaching highest abundance when day 5 of the nestling stage (authors unpubl.; Dawson and Bortolotti 1997, Roulin 1998, Liker et al. 2001). As nestlings age, *Carnus* abundance decreases but estimation is less reliable with increasing feather development in nestlings. Therefore, the abundance of *Carnus* flies was estimated twice: when nestlings were 3 days (immediately before nest manipulation) and 5 days old to look for experimental differences. The window for nest manipulations and ectoparasite quantification was narrow out of necessity, as manipulating nests before day 3 is not recommended due to risk of abandonment, and estimating *Carnus* abundance after day 5, when feathers start growing, becomes less reliable (authors pers. obs.). Nestling mass was recorded at day 3 with an electronic balance (Pocket-240; Gram Precision, Barcelona, Spain) to the nearest 0.1 g, and within-nest means were used in analyses to avoid pseudoreplication.

Nestlings were removed simultaneously to avoid any *Carnus* falling off nestlings, and placed in a container covered with a clean white paper napkin. No fly was observed in nests after removal of nestlings. One nestling at a time was carefully examined, forcing all *Carnus* to fall off into another container until no flies were present on any nestling.

Finally, all *Carnus* were counted in the container and returned to the nestlings that were then placed back in the nest. This method employed for estimation of *Carnus* abundance has been previously shown to be reliable (Roulin 1998) and highly repeatable (Veiga et al. 2020). Five of the 51 nests were not visited on nestling day 5 because of cold and/or rainy weather (which may increase probability of nest abandonment), and five additional nests were depredated or abandoned. Nests that were not measured on day 5 were not biased relative to their assigned experimental treatment (four enlarged, two elevated and four control nests) and thus, 41 nests were used to explore the effect of the experimental treatments.

At nestling day 8, a sticky trap was installed on nest boxes to sample biting flies other than *Carnus*. The trap consisted of a plastic Petri dish placed inside the nest box close to the roof facing downwards, supported on a wire mesh and covered with oil (Tomás et al. 2008b). This sampling method has been designed to estimate abundances of biting flies such as Simuliid blackflies and Ceratopogonid biting midges that rest on the roof inside nest boxes (Tomás et al. 2008b). The trap was in use until nestling day 14, when Petri dishes were collected and screened for biting flies. Latex gloves were always worn when handling nests and nestlings to avoid releasing odorous cues that might interfere with the experiment.

Statistical analyses

Mean, maximum and minimum temperature and humidity inside cavities were compared among treatments with ANOVAs including hatching date (1 = 1 April) as a continuous variable. Comparisons of ectoparasite abundance at days 3 and 5 after hatching were performed using repeated measures ANOVAs, with number of flies per nest at the two sampling ages as the repeated measures (dependent variable), and hatching date, brood size and mean nestling body mass as between-factor variables. The effect of the experimental treatments on ectoparasite abundance (i.e. response variable) was therefore estimated by the interaction between experimental treatment and the repeated measure of number of flies per nest (i.e. difference in total number of flies counted in all nestlings of a nest at nestling days 3 and 5). The interactions between repeated measures and between factors therefore estimated the effects of hatching date, brood size and nestling body mass on the change detected in ectoparasite abundance estimated at the two different nestling ages.

Change in parasitism intensity experienced by enlarged and elevated nests was similar (Results) and, thus, these two groups were considered together when analysing predictions derived from the hypothesis that distance from the nest to cavity entrance, rather than amount of nest material, affects detectability by parasites. The effect of the experiment on ectoparasite abundance was also explored using initial and final distance to entrance rather than experimental treatments. In this General Linear Model, change in number of flies per nest between days 3 and 5 of nestling age was used as the dependent variable, distance to entrance before and after the manipulation as continuous independent variables, and hatching date, brood size and nestling body mass as covariables.

All statistical tests were performed in Statistica 12.0 (StatSoft Inc. 2007). Residuals of the models were tested for normality (Kolmogorov–Smirnov tests: all $p > 0.10$).

Results

Before the manipulation, distance to entrance did not differ between nests subsequently assigned to enlarged (mean \pm SE: 17.4 ± 0.5 cm), elevated (16.5 ± 0.5 cm) and control nests (16.9 ± 0.5 cm; Anova: $F_{2,47} = 0.88$, $p = 0.42$). After the manipulation, distance to entrance differed significantly among treatments: (enlarged nests: 7.1 ± 0.5 cm; elevated nests: 3.6 ± 0.5 cm; control nests: 16.7 ± 0.6 cm; Anova: $F_{2,47} = 143.22$, $p < 0.001$). Distance to entrance of elevated nests was slightly shorter than distance to entrance of enlarged nests (post-hoc Unequal N Honestly Significant Difference test: $p < 0.001$), while distance to entrance of the two experimental treatments was approximately half the distance to entrance of control nests (enlarged versus control: $p < 0.001$; elevated versus control: $p < 0.001$).

Experimental treatments did not create significant differences in microclimate of the cavities. Mean, maximum and minimum temperature and relative humidity did not differ among treatments (Table 1). Hatching date was positively related to mean and maximum temperature, and negatively related to mean, maximum and minimum relative humidity (Table 1).

Abundance of ectoparasitic flies other than *Carnus* was very low in starling nests: Simuliid biting flies were only found in sticky traps in one control nest, and thus we will not refer to them further. No other parasites (e.g. Calliphorid

Table 1. Comparison of microclimate of the cavities (temperature and relative humidity) after experimental manipulation of spotless starling nests. Partial correlation coefficients (β) for the relationships of microclimate variables with hatching date are shown.

		Treatment					Hatching date			
		Enlarged	Elevated	Control	$F_{2,22}$	p	$F_{1,22}$	p	$\beta \pm$ SE	
Temperature (°C)	Mean	22.9 ± 0.4	22.6 ± 0.4	22.3 ± 0.4	0.00	1.00	13.66	0.001	0.63 ± 0.17	
	Maximum	33.3 ± 1.0	32.3 ± 1.0	32.8 ± 1.0	0.47	0.63	14.28	0.001	0.65 ± 0.17	
	Minimum	13.6 ± 0.7	12.8 ± 0.7	13.2 ± 0.7	0.33	0.72	0.34	0.57	0.13 ± 0.22	
Relative humidity (%)	Mean	35.6 ± 1.2	36.1 ± 1.2	36.6 ± 1.2	0.00	1.00	4.98	0.04	-0.44 ± 0.20	
	Maximum	63.0 ± 2.6	63.7 ± 2.6	64.3 ± 2.8	0.03	0.97	4.56	0.04	-0.43 ± 0.20	
	Minimum	17.3 ± 0.8	17.4 ± 0.8	17.9 ± 0.9	0.43	0.65	26.11	<0.001	-0.76 ± 0.15	

Table 2. Results from repeated measures Anova with number of flies per nest estimated in starling nests three and five days after hatching as repeated measures (i.e. age effect), in relation to body mass, brood size, hatching date and experimental treatments. Interactions between age and between factor variables estimate the effects of between factor variables on change in number of flies per nest associated with age. Effect size (partial eta-squared) and observed power (alpha=0.05) are also shown.

	SS	df	MS	F	p	Effect size	Power
Between effects							
Treatment	238.0	2,35	119.0	0.72	0.50	0.04	0.16
Body mass	536.3	1,35	536.3	3.23	0.08	0.08	0.42
Brood size	841.7	1,35	841.7	5.08	0.03	0.13	0.59
Hatching date	401.2	1,35	401.2	2.42	0.13	0.06	0.33
Within effects							
Age	56.4	1,35	56.4	1.01	0.32	0.03	0.16
Age×Treatment	313.6	2,35	156.8	2.81	0.07	0.14	0.52
Age×Body mass	255.8	1,35	255.8	4.59	0.04	0.12	0.55
Age×Brood size	37.7	1,35	37.7	0.68	0.42	0.02	0.13
Age×Hatching date	0.25	1,35	0.25	0.00	0.95	0.00	0.05

blowflies) were apparently present in starling nests. On the contrary, *Carnus* was highly prevalent in nests, both at nestling day 3 (94.1%, n = 51) and 5 (95.1%, n = 41) of nestling age. Before the experiment, three days after nestlings hatched, abundance of *Carnus* parasites in nests under different experimental treatments did not differ significantly (enlarged nests: 12.4 ± 3.0 ; elevated nests: 8.8 ± 3.1 ; control nests: 16.0 ± 3.2 ; Anova: $F_{2,48} = 1.34$, $p = 0.27$). Similarly, there were no significant differences among experimental treatments in hatching date (enlarged nests: 39.6 ± 1.0 ; elevated nests: 38.0 ± 1.0 ; control nests: 39.1 ± 1.0 ; Anova: $F_{2,48} = 0.65$, $p = 0.53$), brood size (enlarged nests: 3.2 ± 0.2 ; elevated nests: 3.5 ± 0.2 ; control nests: 3.4 ± 0.2 ; Anova: $F_{2,48} = 0.82$, $p = 0.45$) and mean nestling mass (enlarged nests: 19.6 ± 1.0 g; elevated nests: 19.7 ± 1.1 g; control nests: 19.8 ± 1.1 ; Anova: $F_{2,48} = 0.01$, $p = 0.99$) at three days of age.

Number of flies per nest at day 3 was higher in nests with larger brood sizes (β [SE] = 0.32 [0.15]) and tended to be higher in nests with heavier nestlings (β [SE] = 0.35 [0.15]) (between effects in Table 2). Number of flies per nest tended to decrease with age (day 3: 12.00 ± 1.99 , n = 51; day 5: 10.68 ± 1.39 , n = 41), mostly in control nests as compared to enlarged and elevated nests (Table 2, Fig. 1). Change in number of flies per nest in enlarged (-1.0 ± 2.9 flies) and elevated nests (2.5 ± 2.8 flies) was similar (repeated measures Anova, interaction with treatment, $F_{1,27} = 1.10$, $p = 0.30$). Thus, these two groups were considered together when analyzing the prediction that distance from the nest to cavity entrance, rather than amount of nest material, affects detectability by parasites. In this case, treatment explained change in number of flies per nest between days 3 and 5 after hatching (repeated measures Anova, interaction between age and treatment, $F_{1,36} = 4.35$, $p = 0.04$). Number of flies per nest was increased in enlarged and elevated nests compared to control nests (Fig. 1) and was higher in nests with larger brood sizes ($F_{1,36} = 5.22$, $p = 0.03$).

Finally, and in accordance with the hypothesis that distance to cavity entrance affects risk of ectoparasitism, we found a statistically significant negative association between difference in number of flies per nest and distance to entrance

after manipulation (β [SE] = -0.45 [0.16]; $F_{1,34} = 8.33$, $p = 0.007$; Fig. 2), once the negative effect of nestling body mass was controlled for, i.e. number of flies per nest decreased more in nests with heavier nestlings (β [SE] = -0.41 [0.16]; $F_{1,34} = 6.84$, $p = 0.01$).

Discussion

Our main findings are that ectoparasite abundance in experimentally enlarged and elevated nests was larger than in control nests, and that distance from nest to cavity entrance after the experimental manipulation was negatively related to change in ectoparasite abundance. The abundance of *Carnus hemapterus* flies was not different between the two experimental treatments (enlarged and elevated nests), which indicates that the effects detected were not due to adding additional nesting material (i.e. to chemicals originated from nest material; Tomás et al. 2008a), but to olfactory cues derived from

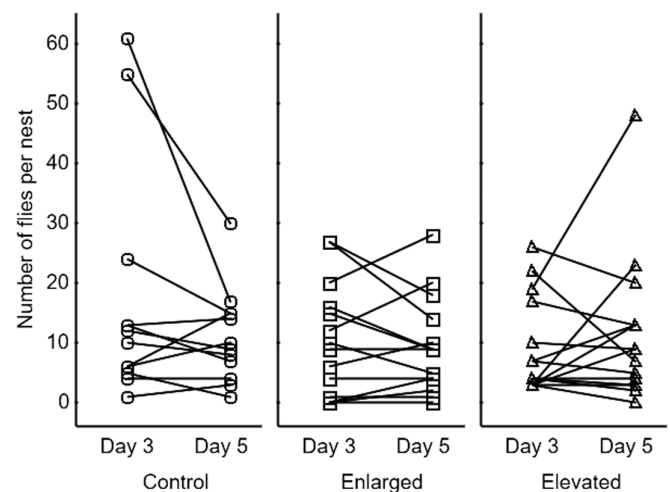


Figure 1. Number of *Carnus hemapterus* flies per nest before (day 3 post hatching) and after (day 5) manipulation of distance to cavity entrance in control, enlarged and elevated spotless starling nests.

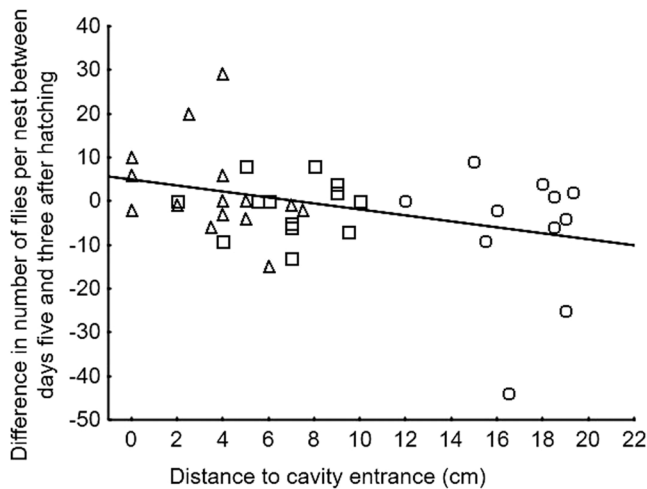


Figure 2. Difference in number of *Carnus hemapterus* flies per nest (number of flies at day 5 after hatching – number of flies at day 3) in relation to distance to cavity entrance after manipulation in spotless starling nests; Δ : elevated nests, \square : enlarged nests, \circ : control nests. Regression line is shown.

nestling physiological activity (Azcárate-García et al. 2019), and/or auditory cues derived from begging behaviour (Tomás and Soler 2016). Nestlings in nests closer to the cavity entrance would be more easily located by ectoparasites. These results cannot be attributed to differences in cavity microclimate derived from the experimental manipulations. Our findings may have broad implications for understanding the ecology and evolution of cavity-nesting birds. Below we discuss alternative explanations, and the importance of considering the selective pressure exerted by parasitism for understanding the evolution of nest-building behaviour in hole-nesting birds, where larger nests would attract more parasites due to a reduction in the distance from nest contents to cavity entrance.

Given that old nest material with any overwintering parasites was removed from experimental nest boxes before nests had been initiated, we are confident that the vast majority of ectoparasites detected during the nestling phase were from nearby areas. Abundance of *Carnus* at a given nestling stage is a balance between increasing abundance by flies that are newly arrived (immigration) and decreasing abundance due to completion of life cycle, death or elimination by host birds. Thus, we did not necessarily expect a differential increase among treatments, but rather differences between post- and pre-experimental abundances, due to this dynamic balance regulating ectoparasite abundance in every nest at a given time (Dawson and Bortolotti 1997, Liker et al. 2001, Veiga et al. 2018). Moreover, it has been previously shown that *Carnus* has detrimental effects on their bird hosts (Wiebe 2009, Hoi et al. 2018), including starlings (Avilés et al. 2009, Tomás et al. 2018). Consequently, our experimental results suggest the existence of costs for starlings building large nests, mediated by blood-sucking parasites.

In an attempt to differentiate between the effects of olfactory cues derived from nest material and those derived from

nestling activity as attractors of flying ectoparasites, we elevated the nests with either plant nest material or with artificial plastic material, while assuming that the former but not the latter may include detectable olfactory cues for parasites. We found non-significant differences among these experimental treatments, suggesting that the distance between the nest and the cavity entrance, rather than amount of volatiles derived from nest materials, was the cause of the patterns detected. However, we cannot rule out the hypothesis that parasites cue on volatile emissions from nest material, vegetable or other. This alternative explanation could be a function of the nest material used for the experimental manipulation of nest size. We chose dry straw, following the rationale that it is the main nest material used by starlings in this and other populations (Cramp 1998, Veiga and Polo 2011). However, this is apparently a nest material with limited odorous properties (Tomás et al. 2012), which may make it difficult to detect. This hypothesis is however non-exclusive and may operate additively with the effect of distance to the cavity entrance, or with other nest materials when considering other species. Another hypothetical scenario predicting a positive relationship between nest size and parasite abundance is that ectoparasites in larger nests have more space to hide and escape from nest sanitation behaviour performed by adult birds (Heeb et al. 2000, Soler et al. 2007). We found that number of flies per nest increased in elevated nests with no additional material, which does not support this hypothesis.

Finally, our results may also be of interest in the debate regarding suitability of using artificial nest boxes for studying factors affecting the risk of parasitism or related life-history traits. Lambrechts et al. (2010) urged researchers to report detailed characteristics of nest boxes because those may affect or confound conclusions derived from nest-box studies. Our results indicate that distance to the cavity entrance should be reported as an important parameter affecting ectoparasite load, therefore supporting this claim. Furthermore, cavity-nesting birds may prefer deeper cavities for breeding to reduce risk of ectoparasitism and associated diseases, in line with what has been suggested regarding predation risk (Wesołowski 2002, Wesołowski and Rowinski 2012, Kaliński et al. 2014, Mainwaring et al. 2015, Fokkema et al. 2018). Other characteristics of the nesting cavity such as orientation of the entrance hole (Goodenough et al. 2008), location of the cavity (e.g. tree or wall) (Lambrechts et al. 2010), or proximity to other cavities occupied in the current or the previous season (Veiga et al. 2020), will surely affect nest colonization success by ectoparasites and deserve further research.

To conclude, our results suggest that building a large nest in a cavity carries an important and overlooked cost by reducing the distance between nest contents (eggs, nestlings or incubating/brooding/roosting adult) and the cavity entrance, facilitating nest detection by ectoparasites. This may be a strong evolutionary force restricting nest size in cavities, and therefore the benefits of building larger nests derived from its functioning in scenarios of natural and/or sexual selection (Soler et al. 1998, Moreno 2012, Mainwaring et al. 2014).

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Author contributions – GT, CRC and JJS designed the study. GT, CRC, MRR and JJS conducted field work. JJS and GT performed the statistical analyses and wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

Conflict of interest – The authors declare that they have no conflict of interest.

Permits – All applicable institutional and/or national guidelines for the care and use of animals were followed. Authorization to conduct the study was granted by the Dirección General de Gestión del Medio Natural, Consejería de Medio Ambiente, Junta de Andalucía.

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Data availability statement

Data used in this paper are available from the CSIC Institutional Repository, with the accession number: <<http://dx.doi.org/10.20350/digitalCSIC/12512>>.

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