# Are avian ectoparasites more numerous in nest boxes with old nest material?

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**Abstract**: Researchers may reduce the numbers of haematophagous ectoparasites in nest boxes of cavity-nesting birds by removing old nests from boxes and, as a result, eliminate an important selective pressure that could influence the results from nest-box studies of birds. We recorded the numbers of parasites in tree swallow (*Tachycineta bicolor*) boxes in which we manipulated the presence, amount, and quality of old nests. Bird fleas (*Ceratophyllus idius*) were more numerous in boxes with old nests, and there was a positive correlation between nest volume and flea numbers. In one year, there was a positive association between fowl mite (*Ornithonyssus sylviarum*) numbers and nest volume; otherwise, fowl mites and blow flies (*Protocalliphora sialia*) were equally numerous in all nest types. We conclude that ectoparasites whose over-winter survival depends on old nests are more numerous in boxes with old nests, whereas parasites whose over-winter survival is independent of old nests infect nest sites randomly. Also, reinfection and nest microclimate likely contributed to variance in parasite numbers between nest types and years, respectively. We recommend caution when speculating about the possible effects of cleaning boxes on parasites that occur in nests because different species of parasites are not influenced similarly by old nests.

**Résumé** : Les chercheurs peuvent diminuer le nombre d'ectoparasites hématophages dans les boîtes à nids de leurs oiseaux en captivité en enlevant les vieux nids des boîtes, éliminant ainsi une importante pression de sélection susceptible d'influencer les résultats de leurs études ultérieures. Nous avons dénombré les parasites chez les Hirondelles bicolores (*Tachycineta bicolor*) nichant dans des boîtes où la présence, la quantité et la qualité des vieux nids avait été manipulée. Les puces *Ceratophyllus idius* se sont avérées plus nombreuses dans les boîtes contenant des vieux nids et il y avait une corrélation positive entre le volume des nids et le nombre de puces. Au cours d'une année, nous avons observé une association positive entre le nombre d'acariens *Ornithonyssus sylviarum* et le volume des nids, mais, ce cas mis à part, les acariens et les calliphores *Protocalliphora sialia* étaient également nombreux dans tous les types de nids. Les ectoparasites dont la survie en hiver est reliée aux vieux nids sont plus nombreux dans les boîtes contenant des vieux nids, alors que les parasites dont la survie en hiver est indépendante de la présence de vieux nids sont répartis aux hasard à tous les sites de nidation. De plus, les cas de réinfection dans les nids ont probablement influencé la variance dans le nombre de parasites d'un type de nid à l'autre et le microclimat, la variance du nombre de parasites d'une année à l'autre. Les effets possibles du nettoyage des boîtes à nid sur les parasites peuvent donc varier puisque différentes espèces de parasites réagissent différemment à la présence de vieux nids.
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## Introduction

Nest boxes are commonly used during studies of the ecology of cavity-nesting birds because they are accessible, manipulable, and readily accepted by many species. Much of our understanding of avian life history theory is based on long-term studies of birds breeding in boxes, some of which have been conducted for many decades (e.g., Gustafsson and Sutherland 1988; Pettifor et al. 1988).

However, Møller (1989) stated that nest-box studies may produce unrealistic results because researchers remove old nests from boxes at the end of each season, thereby reducing the numbers of ectoparasites in boxes. On occasion, high parasite loads reduce the reproductive success of cavity-nesting birds (e.g., Moss and Camin 1970; Capreol 1983; Clark and Mason 1988; Richner et al. 1993; Winkler 1993; Eeva et al. 1994). Møller therefore suggested that results from nest-box studies should be regarded with caution. This is an important criticism that has generated considerable research into the relationships between parasites and their cavity-nesting hosts (see references in Johnson 1996; Rendell and Verbeek 1996*a*, 1996*b*) because researchers may need to reinterpret the qualitative and quantitative results of previous long-term studies of cavity-nesting birds.

At the heart of Møller's criticism was an assumption: parasites are more numerous in boxes where old nest material is present than in boxes from which old nests were removed. Until recently, this basic assumption had not been tested. Little is actually known about how numbers of parasites are affected by nest reuse by cavity-nesting birds. Many factors other than the presence of old material may affect the numbers of parasites in nests, including (*i*) other arthropods that are predators or parasites of parasites (e.g., the predatory mite

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*Cheletomorpha lepidopterorum*; Burtt et al. 1991; the parasitoid wasp *Nasonia vitripennis*; Gold and Dahlsten 1989); (*ii*) density-dependent factors such as the volume of nest material (Whitworth 1976) or the availability of hosts (Burtt et al. 1991); (*iii*) the infection process; and finally, (*iv*) microclimate variability in nest sites (e.g., Erbelding-Denk and Trillmich 1990), which can influence the development of parasites (Holland 1985).

We hypothesized that the life cycle of each species of parasite is another, and perhaps the most significant, factor affecting the numbers of parasites in the nests of cavity-nesting birds. Parasites that depend on nest material for shelter or sustenance outside of the host's breeding season (e.g., fleas, Holland 1985; fowl mites, Sikes and Chamberlain 1954) should be more numerous in cavities with old material than in cavities from which old material has been removed. Species that are not dependent on old material outside of the host's breeding season (e.g., blow flies; Sabrosky et al. 1989), and that disperse away from the nest, should be equally numerous in cavities with and without old nest material.

We tested this hypothesis by examining the numbers of fleas (*Ceratophyllus idius* Jordan and Rothschild), fowl mites (*Ornithonyssus sylviarum* Canestrini and Franzago), and blow flies (*Protocalliphora sialia* Shannon and Dobroscky) collected from nest boxes of breeding tree swallows (*Tachycineta bicolor*) during experiments conducted on a nest-box population in British Columbia. Some boxes contained old nest material, some did not. Further, we describe the results of an experiment performed on a nest-box population of tree swallows in Ontario that addressed qualitatively how old nest material affects the over-winter survival of fleas (*Ceratophyllus idius*).

Similar experiments were conducted concurrently with, or since, ours. Contrary to our predictions, Mappes et al. (1994) counted more fleas (*Ceratophyllus gallinae*) in clean boxes of pied flycatchers (*Ficedula hypoleuca*) than in boxes with old nests, but Johnson (1996) showed that the blow fly *Protocalliphora parorum* infested nests of house wrens (*Troglodytes aedon*) randomly with respect to the presence or absence of old nest material, as we would have predicted. Our study differs from these because we examine how other characteristics of a nest, such as the amount of nest material, affect the numbers of parasites found there. We studied three species of ectoparasites from the same nests, instead of only one, so we looked for associations between the numbers of each parasite type and another in nests. Finally, because some of our nests were used consecutively for 3 years, we examine how repeated use of nest material affects the numbers of parasites found in boxes.

## Species studied

### Tree swallows

Tree swallows (Hirundinidae) have been studied extensively in nest-box and natural populations (Robertson et al. 1992). They return to nesting areas at the end of March to acquire and defend territories immediately around cavities (Muldal et al. 1985). Some pairs defend two or more cavities, possibly for the entire breeding season (Rendell and Robertson 1994). Pairs are socially monogamous and single-brooded. Females build the nest using dead grass or pine needles and line the nest cup with feathers. Females build alone, although males also bring feathers to the nest. Nest building begins in mid-April and egg laying in early to mid-May. Clutches of two to eight eggs are laid and most females in the population lay synchronously (Stutchbury and Robertson 1988). Incubation lasts 12–14 days and the nestling period is 16–21 days, during which both parents feed the young. After fledging, young birds remain with their parents for an indefinite period before they migrate south in September or October. Several types of haematophagous parasites feed on tree swallows, but the three types known from our study areas are discussed below.

### Blow flies

Blow flies (Diptera: Calliphoridae) overwinter as adults in crevices in cavities, behind tree bark, and, from our observations, rarely in cavity nest material (Sabrosky et al. 1989). Adults disperse in spring and enter nest cavities when the hosts have nestlings. Females lay eggs singly or in batches in nest material, typically within a week after the host's nestlings hatch. Blow fly eggs hatch in 24 h, whereupon the larvae immediately begin feeding on the nestlings. Each of the first two instars lasts 1–2 days, during which the small larvae take one or two blood meals. The third and final instar lasts about 7 days, during which two or three blood meals are taken per day. The pupal stage lasts 2–3 weeks. Adults emerge in June and July and may or may not seek mates and hosts in the same season. The blow fly *P. sialia* is found in tree swallow nests across North America.

### Fowl mites

Fowl mites (Parasitiformes: Dermanyssidae) reach nests as adults by transport on a bird or hatch there (Sikes and Chamberlain 1954). Adults may overwinter in nest material but most spend their entire lives on adult or nestling hosts. They have short life cycles of 5–7 days and each female may lay two to five eggs, potentially resulting in rapid, exponential increases in population size. The eggs hatch in 1 or 2 days depending on temperature and humidity, larvae molt into protonymphs in less than a day, protonymphs feed twice and molt into deutonymphs in 1–3 days, and nonfeeding deutonymphs become adults in less than a day. Adults reproduce after two blood meals. The northern fowl mite, *O. sylviarum*, parasitizes several families of birds throughout the Holarctic region.

### Fleas

Fleas (Siphonaptera: Ceratophyllidae) are holometabolous insects with a four-stage life cycle (Holland 1985). Eggs are laid a few at a time in nest material or on hosts. Development of eggs is asynchronous and embryogenesis lasts from 2 to 12 or more days. The larvae are free-living and feed on organic material in the nest structure during three instars, but they do not take blood meals from the host. The pupal stage is spent in a silken cocoon. When they emerge, the adults are perfectly formed and ready to feed. Adults feed exclusively on blood. The bird flea *C. idius* parasitizes all Nearctic members of the family Hirundinidae.

## Materials and methods

### British Columbia experiments

#### *Study site and nest boxes*

This research was conducted at the Creston Valley Wildlife Management Area, Creston, southeastern British Columbia (49°05′N, 116°35′W), during 1991 and 1992. Creston is open, wetland habitat divided into shallow ponds by dikes. Dikes are bounded by water (0.5–1.5 m deep) on at least one side. Tree swallows have bred in about 160 nest boxes at Creston for several years. The boxes are made of cedar or plywood and mounted about 1 m off the ground on wooden posts with metal predator guards.

Our experiments included four box types: (*i*) clean (C) boxes with no old nest material, (*ii*) sham (S, 1991 only) boxes which contained old nest material that had been microwaved; (*iii*) clean boxes with inserts that reduced the internal volume of the box (CI, 1992 only), and (*iv*) old (O) boxes that contained old nest material. Old nest material was available at Creston because boxes were not cleaned out after the 1990 breeding season. Old nests were removed from C boxes and the inside was swept with a wire brush to loosen duff and bird droppings. Care was taken to clean in the cracks of boxes, where possible, to kill or flush out hidden parasites. S boxes received the same treatment as C boxes, except that after cleaning, previously microwaved nest material was inserted. We collected 50 old nests from boxes at Creston and microwaved each nest separately in a Look cooking bag for 5 min at high power in a Toshiba (625 W) oven. The nests were then replaced in clean Ziploc bags. We know that microwaving sterilized the nests, because we sifted 3 of the 50 nests after the procedure and found that all arthropods inside were dead. Old nest material was left in place in O boxes, and they were not cleaned. Nest material used at both S and O boxes showed evidence that they had been occupied the previous year, such as dead nestlings and bird droppings. Therefore, any parasites in these boxes presumably would have had access to hosts previously, and could have multiplied. CI boxes were used for an experiment on nest-building behavior (Rendell and Verbeek 1996*a*). CI boxes were created by randomly choosing 15 C boxes and inserting a Styrofoam and plywood floor that filled the lower 8 cm of each box, therefore they were clean boxes that mimicked the smaller cavity typical of boxes with old nests.

In 1991, C, S, and O boxes were used. Boxes were distributed in pairs as part of an experiment on box preference (Rendell and Verbeek 1996*a*). Boxes within a pair were 3 m apart and pairs of boxes were 40 m apart. We refer to a pair of boxes as a territory. Seventy-nine territories were established in the marsh, alternating in the following manner: C × S, C × O, O × S, S × C, O × C, S × O, etc. In 1992 the boxes were redistributed and C, CI, and O boxes were used for experiments. One hundred and twenty-five boxes were arranged singly, 30–40 m apart. C (including CI) and O boxes were arranged along dikes throughout the marsh as follows: C1, O1, O2, C2, C3, O3, etc. In both years, by distributing boxes and territories in a regular order throughout the study area, we subjected all box types to similar environmental conditions in an attempt to standardize for any subtle, yet unknown, effects of microhabitat variability on local parasite populations. Tree swallows occupied all 79 territories in 1991 and 112 of 125 (90%) boxes in 1992.

#### *Nest switches*

We tried to control for covariation between host phenotype, breeding success, and the box type used by tree swallows (Rendell and Verbeek 1996*b*). After females settled in boxes and built nests, we moved old nest material from one nest, cleaned the box, and put the new material back. This was done within pairs of boxes on randomly chosen territories in 1991. In 1992, nest switches were made between some O boxes and their nearest C neighbor, but CI boxes were not disturbed. To give an example from 1992: if a female settled at box C5, we inserted the O5 nest material underneath the new C5 nest material. The C5 nest was now considered an O nest and box O5 now became a C box, because we had removed the old material from under the new nest, cleaned the box, and put the new nest back. Nest switches were made late in the nest-building stage, just before egg laying, in late April and early May. Females often built only small nests in S and O boxes in 1991 and in O boxes in 1992 (Rendell and Verbeek 1996*a*), so nest switches were performed only at those boxes where we were sure that nest handling and manipulation would not destroy a new nest structure. In total, nests were switched at 44 of 79 (56%) territories in 1991 and at 14 of 64 (22%) neighboring C–O pairs in 1992.

#### *Successive use of nest material*

Because nest material was used and reused by tree swallows from 1990 to 1992, we recognized three types of boxes in 1992, based on the number of times the oldest nest material had been used. The material brought to C and CI boxes was being used for the first time in 1992, the material in some O boxes in 1992 had been used only in 1991 and so was being used for the second time, and the material in some O boxes had been in use since 1990 and so was being used for the third time. The ability to distinguish these box types according to the age of the nest material allowed us to analyze how the numbers of the three types of ectoparasites changed with nest reuse.

#### *Ectoparasite counts*

''Hand counts'' of adult fowl mites were performed at nests within 24 h after the last nestling fledged. One of us (W.B.R.) placed a hand in the nest for 10 s and then estimated the number of mites on the hand and arm in 10's, 100's, and 1000's (Møller 1990). Fowl mites were not counted from Berlese funnel collections (see below), so the only estimates of their numbers were from hand counts.

We collected 30 nests in 1991 ($n_C = 11$, $n_S = 10$, $n_O = 9$) and 103 nests in 1992 ($n_C = 36$, $n_{CI} = 13$, $n_O = 54$), immediately after the mite hand count, and stored each in a sealed and marked Ziploc bag. In both years, W.B.R. sifted through each nest by hand to count the number of adult fleas and blow fly adults, puparia, and third-instar larvae. Each nest was placed on a white sheet and the grass, feathers, and nest duff were separated. Adult fleas, alive or dead and regardless of the age of the material, were totalled for each nest. We distinguished between fleas from the collection year and those that were obviously from a previous year, which were brittle and desiccated, and subtracted the latter from the totals. When counting the blow flies in nests with old material, we distinguished between puparia from the study year and those from a previous year by adding only whole puparia with an intact pupa to the numbers of third-instar larvae and newly emerged adults. For 1991 nests, 11 nests were sifted within 1 month of collection, while the remaining 19 were frozen and stored until January 1992. All of the 1992 nests were sifted within a month of collection.

In 1992, before sifting, nests were dried in modified Berlese funnels (Murphy 1962) to flush out live parasites. Each nest was put in a separate plastic funnel with the stem wrapped in cotton and inserted into a vial of 75% alcohol. Lamps with 60- to 100-W bulbs were centered about 10 cm above a nest for 24–72 h. When nest material was too bulky to dry all at once, it was halved and dried, one half after the other, using the same funnel and vial. Once a nest was dry, it was returned to its original bag and its vial was labelled. The fleas and blow flies from the Berlese funnels were added to those collected by sifting.

#### *Host breeding*

Regular checks of tree swallow boxes enabled us to record the host's reproductive output and nesting phenology in both years (Rendell and Verbeek 1996*a*, 1996*b*).

### Ontario experiment

The objective of this simple experiment, using the same host and bird flea species, was to corroborate the results of our British Columbia experiments concerning the importance of old nest material on over-winter survival of bird flea populations in nest boxes. This experiment was conducted near the Queen's University Biological Station

**Table 1.** Numbers of three types of ectoparasites per tree swallow nest at Creston, B.C., 1991–1992, from all box types combined.

| Ectoparasite | Median | Range | Percentiles | Observed | $n$ |
|---|---|---|---|---|---|
| **1991** | | | | | |
| Fowl mites* | 1 | 0–300 | 0, 3 | 37 | 68 |
| Bird fleas** | 20 | 4–386 | 9.25, 62 | 30 | 30 |
| Blow flies | 29 | 0–107 | 3, 57 | 28 | 30 |
| **1992** | | | | | |
| Fowl mites | 0 | 0–1 | 0, 0 | 1 | 103 |
| Bird fleas | 48.5 | 2–836 | 15, 118.5 | 102 | 102 |
| Blow flies | 28 | 0–128 | 14, 45 | 96 | 103 |

**Note:** Values are given as medians, ranges, and 25th and 75th percentiles, with the number of nests where parasites were found and the number of nests sampled. Significant differences between years, Mann–Whitney tests, two-tailed: *, $P < 0.0001$; **, $P < 0.05$.

(Queen's), Chaffey's Lock, Ontario (44°34′N, 76°20′W), from 1994 to 1995. Tree swallows have nested in standardized plywood boxes near Queen's for 20 years. Boxes were mounted on aluminum poles with predator guards and were distributed in rows and columns (i.e., grids) in hayfields (70 boxes were occupied in 1994) and at one site over water (50 boxes were occupied in 1994). At the aquatic site, boxes were mounted on aluminum poles or wooden posts in 1.5 m of water. Along rows and columns of all grids, boxes were 40 m from each other but 28 m from the next nearest box along a diagonal.

In July and August 1994, after the breeding season, boxes were cleaned out in the terrestrial grids only, while boxes at the aquatic site were left alone. All pairs of tree swallows in the study had reached an advanced stage of incubation or the nestling stage, so any fleas in nests could have fed on either adult or nestling hosts.

In the last week of March 1995, W.B.R. visited all boxes on the terrestrial and aquatic grids. Boxes were opened, examined, and in the case of the aquatic grid, cleaned out. We recorded how many nests and boxes contained fleas in all grids. Old nests at the aquatic site were sifted briefly to look for living adult and larval fleas. At this time of the season, few tree swallows had returned from migration; in fact, the first migrants were seen the same week that the boxes were examined for fleas. According to our hypothesis, and the results from our British Columbia experiments, we expected that adult and larval fleas would be abundant in boxes at the aquatic site but absent from boxes on the terrestrial grids.

### Statistical analysis

To minimize any possible effects of season on our statistical analyses of reproductive data for tree swallows (Stutchbury and Robertson 1988; Rendell and Verbeek 1996*b*), we used only those nests in which the first egg was laid before 1 June. Any variation in sample sizes between tests is due to missing values. We used nonparametric statistics (SAS Institute Inc. 1985; Siegel and Castellan 1988), performing Spearman's rank-order correlations, and Mann–Whitney and Kruskal–Wallis tests of medians, using the significance level $\alpha = 0.05$. Our analyses were performed on the absolute numbers of each type of parasite for each nest. The data from 1991 and 1992 were not combined because of different experimental methods used between years, significant differences in host reproductive success and nestling sizes between years (Rendell and Verbeek 1996*b*), and significant differences in the numbers of fowl mites and fleas at nests between years.

## Results

### British Columbia experiments

Fowl mites were found in approximately half of the nest boxes sampled in 1991 but in only one box in 1992, a statistically significant difference (Table 1). Infestations exceeding 100 mites occurred only twice, so fowl mites were relatively uncommon. In contrast, bird fleas were observed in all nests collected in both years of the study, with the median number of fleas per nest higher in 1992 (Table 1). Blow flies were found in >90% of nests sampled each year. The median numbers of blow flies per nest did not differ between years (Table 1).

There was no effect of season on the numbers of parasites recovered from nests when all nests were combined. The numbers of each species of parasite per nest and dates of first egg, hatching, and fledging of tree swallows were not correlated (Spearman's rank-order correlations ($\rho$), all $P \geq 0.13$, 1991: for mites, $n_{\mathrm{egg}} = 68$, $n_{\mathrm{hatch}} = 64$, $n_{\mathrm{fledge}} = 62$; for fleas and blow flies, $n_{\mathrm{egg,\ hatch}} = 30$, $n_{\mathrm{fledge}} = 29$; 1992: for mites and blow flies, $n_{\mathrm{egg,\ hatch}} = 103$, $n_{\mathrm{fledge}} = 87$; for fleas, $n_{\mathrm{egg,\ hatch}} = 102$, $n_{\mathrm{fledge}} = 87$).

The number of a particular species of parasite in a nest was not correlated with the numbers of either of the other species in a nest when all nests were combined (all $P \geq 0.12$, $n_{91} = 30$, $n_{92} = 102$).

The median number of fleas, but not blow flies or fowl mites, per nest was significantly different in C and in S and O box types in 1991 and among C, CI, and O box types in 1992 (Table 2). In 1991, the number of fleas per nest was greater in O and S boxes than in C boxes. In 1992, fleas were more numerous in O than in C or CI boxes and more numerous in C than in CI boxes.

The median number of fleas, but not blow flies or fowl mites, per nest was significantly lower in box types with new nest material than in those with old material being used for the second or third time (Table 3). Fleas were significantly more numerous in nests with old nest material that was being used for the third time than in second-use nests.

The number of fleas per nest was significantly positively correlated with the total volume of nest material (old + new material) in boxes in 1991 and 1992 for all box types combined (1991: $\rho = 0.57$, $n = 29$, $P = 0.0012$; 1992: $\rho = 0.42$, $n = 103$, $P < 0.0001$). The number of fleas per nest was significantly and negatively correlated with the proportion of the total volume of a nest that was composed of new material in both years, when all box types were combined (1991: $\rho = -0.45$, $n = 30$, $P = 0.013$; 1992: $\rho = -0.39$, $n = 103$, $P < 0.0001$). Therefore, with an increase in the proportion of the total volume of nest material that was new, the number of fleas per nest decreased. Within box types, the number of fleas per nest was positively correlated with the total volume of nest material in O boxes in 1992 ($\rho = 0.31$, $n = 54$, $P = 0.023$) but not in 1991 ($P = 0.27$, $n = 8$). The number of fleas per nest was not correlated with the total volume of nest material within other box types (all $P \geq 0.18$, 1991: $n_{\mathrm{C}} = 11$, $n_{\mathrm{S}} = 10$; 1992: $n_{\mathrm{C}} = 36$, $n_{\mathrm{CI}} = 13$).

In 1991, the number of fowl mites per nest was correlated with the total volume of nest material when all box types were combined ($\rho = 0.25$, $n = 64$, $P = 0.046$). In 1991, the number of fowl mites per nest was positively correlated with the total volume of nest material in O boxes ($\rho = 0.46$, $n = 20$; $P = 0.04$) but not in C or S boxes (both $P \geq 0.24$, $n_{\mathrm{C}} = 25$, $n_{\mathrm{S}} = 19$). These analyses were not performed on data from fowl mites in 1992.

**Table 2.** Numbers of three types of ectoparasites per experimental tree swallow nest at Creston, B.C., 1991–1992.

| | 1991 | | | | | | | | | 1992 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | | | S | | | O | | | C | | | CI | | | O | | |
| Ectoparasites | Median | Range | $n$ | Median | Range | $n$ | Median | Range | $n$ | Median | Range | $n$ | Median | Range | $n$ | Median | Range | $n$ |
| Fowl mites | 1 | 0–75 | 25 | 1 | 0–300 | 21 | 2.5 | 0–300 | 22 | 0 | 0–1 | 36 | 0 | 0 | 13 | 0 | 0 | 54 |
| Bird fleas* | 10*a* | 4–23 | 11 | 26*b* | 4–386 | 10 | 36*b* | 7–284 | 9 | 33*a* | 2–836 | 36 | 13*b* | 2–70 | 12 | 77.5*c* | 5–396 | 54 |
| Blow flies | 54 | 0–107 | 11 | 6 | 1–83 | 10 | 30 | 0–92 | 9 | 30.5 | 0–123 | 36 | 37 | 4–116 | 13 | 26 | 0–128 | 54 |

*Kruskal–Wallis test, df = 2, two-tailed, 1991: $P < 0.003$; 1992: $P < 0.0001$. Median values followed by a different letter differ significantly from each other (multiple comparison test; Siegel and Castellan 1988).

**Table 3.** Numbers of three types of ectoparasites per tree swallow nest from boxes in which the nest material was new and from those in which the material was being used for the second or third time, 1992.

| | New nest material | | | Second-use material | | | Third-use material | | |
|---|---|---|---|---|---|---|---|---|---|
| Ectoparasites | Median | Range | $n$ | Median | Range | $n$ | Median | Range | $n$ |
| Fowl mites | 0 | 0–1 | 49 | 0 | 0 | 42 | 0 | 0 | 12 |
| Bird fleas* | 23*a* | 2–836 | 48 | 58*b* | 5–396 | 42 | 121*c* | 41–286 | 12 |
| Blow flies | 32 | 0–123 | 49 | 29.5 | 0–128 | 42 | 22 | 2–92 | 12 |

*Kruskal–Wallis test, df = 2, two-tailed, $P < 0.0001$. Median values followed by a different letter differ significantly from each other (multiple comparison test; Siegel and Castellan 1988).

The number of blow flies per nest was not correlated with the total volume of nest material when all box types were combined (all $P \geq 0.07$, $n_{91} = 29$, $n_{92} = 103$), nor within a box type (1991: all $P \geq 0.16$, $n_{\mathrm{C}} = 11$, $n_{\mathrm{S}} = 10$, $n_{\mathrm{O}} = 8$; 1992: all $P \geq 0.20$, $n_{\mathrm{C}} = 36$, $n_{\mathrm{CI}} = 13$, $n_{\mathrm{O}} = 54$).

### Ontario experiment

During the 20-year period of the tree swallow project at Queen's, fleas have not been observed during the breeding season in boxes of tree swallows or other cavity-nesting species (i.e., eastern bluebirds, *Sialia sialis*, and house wrens, *T. aedon*) using boxes there (R.J. Robertson, personal communication; W.B.R., personal observation). For example, they were not reported during examinations of 335 tree swallow nests for pupae and larvae of the blow fly *P. sialia*, described in Rogers et al. (1991). Fleas likely have not been reported because nests of this population are typically cleaned out of boxes in late summer. In late March 1995, on the four terrestrial grids where boxes had been cleaned out the previous year, 0 of 70 boxes contained fleas of any age. However, in the same week on the aquatic grid, where nest boxes had not been cleaned out, 17 of 50 (34%) boxes and nests contained active adult fleas and larvae.

## Discussion

The results of this study generally support the hypothesis that those ectoparasites whose survival depends on nest material in the absence of the host, such as fleas, are more numerous in nest boxes with old nest material, while other types that do not depend on old nest material outside of the host's breeding season, such as blow flies, apparently infest cavities randomly regardless of the presence of old nest material. Fleas were most numerous in boxes with old nest material compared with boxes with microwaved nests (although not significantly so; Table 2) and boxes from which old nests had been removed. The numbers of fleas in boxes increased with repeated use of nest material, whereas blow flies were equally common in boxes with material of all ages. Further, despite the fact that fleas had never previously been observed in boxes of tree swallows at Queen's (Ontario), simply leaving old nest material in some boxes between years allowed some fleas and larvae to overwinter.

Johnson (1996) found that larvae of the blow fly *P. parorum* were not more numerous in either clean or "dirty" boxes of house wrens in Wyoming, which is similar to our predicted results for *P. sialia*. However, the results of an experiment similar to ours by Mappes et al. (1994) are in stark contrast to ours. They found that fleas (*C. gallinae*) were more numerous in *clean* boxes than in those with old nest material, a result they attributed to possible differences in microclimate and the invertebrate community between clean and old nest boxes. Other factors may also explain the difference between our results and those of Mappes et al. (1994). Both studies show that the numbers of fleas in boxes are highly variable intra-annually. Also, our results for fleas and fowl mites show that interyear variability in numbers of a parasite in nests may also be considerable. Thus, intra- and inter-annual variation in numbers, combined with the relatively small sample sizes for fleas from C and O boxes in the single year of their study, may have contributed to differences between the studies. Further, fleas establish themselves in nests via the infection process, travelling on their hosts. Stochastic variation in host flea loads could lead to relatively clean nests becoming heavily parasitized. One such event apparently occurred in a clean box during our British Columbia experiment in 1992, where we recorded the highest number of fleas (i.e., 836) from any box type in either year of the study. Finally, Eeva et al. (1994) found a negative correlation between nest moisture and the numbers of fleas (*C. gallinae*) present in nest boxes of pied flycatchers and great tits (*Parus major*). Thus, possible differences in the amount of moisture in boxes with old nests between Mappes et al. (1994) and this study may also have affected the results of flea counts.

Our hypothesis is not supported by our data on fowl mites. Fowl mites were not more numerous in nests with old material than in nests with new material at any time. There was a weak positive correlation between the number of fowl mites and the total volume of nest material in O boxes in 1991, and when all box types were combined, but these associations were driven by results from only two nests with infestations. In 1992, fowl mites were rare, so factors other than old material must have affected their numbers. We suggest that cavity microclimate was responsible for the disappearance of fowl mites from our population that year. The major difference between 1991 and 1992 was the average temperature during the breeding season. Temperatures were significantly higher in March, May, and June of 1992 than in the same months in 1991 (average daily temperature in March, April, May, and June: 2.6, 8.3, 12.2, and 14.7°C in 1991; 6.5, 9.3, 13.7, and 19.4°C in 1992; Mann-Whitney tests, two-tailed, $P < 0.05$). In June 1992, the maximum daily temperature exceeded 30°C for 9 consecutive days, resulting in extreme heat stress for tree swallow nestlings and causing all the young to die in several nests (Rendell and Verbeek 1996*b*). Fowl mites may have been influenced by high temperature and humidity in the boxes because their generation time is known to be sensitive to changes in temperature and humidity (Sikes and Chamberlain 1954).

The amount of nest material also influenced the numbers of fleas in different box types. Fleas were most numerous in O boxes with more material, suggesting that density-dependent factors, such as space limitation, can affect their numbers. Larger nests probably have more food for larvae and may ensure a more stable microclimate for fleas in winter. Fowl mites were more numerous in O boxes with more material in 1991, perhaps for similar reasons. Whitworth (1976), Pinkowski (1977), and Gold and Dahlsten (1989) all found that the number of blow flies in a nest increased with nest size, although this was not observed in our study, nor in two others (Rogers et al. 1991; Wittmann and Beason 1992).

The numbers of fleas and fowl mites were unexpectedly large in S nests in 1991 and in C nests in 1992 because these boxes had been cleaned, and in addition, the material in S nests had been microwaved. These results were probably due to two factors: reinfection by adult hosts, and some fleas and fowl mites possibly remaining in boxes after cleaning. Tree swallows visit many nest sites during and after the breeding season (Lombardo 1986), and this might increase the likelihood of reinfection. Fleas and fowl mites may also have reached S and C nests on nest material. The fact that high numbers of parasites may still develop in boxes (see also Mappes et al. 1994) after old nests have been cleaned out shows that the habit of removing old nests from boxes does not eliminate the possible influence of parasites on the breeding ecology of birds. Johnson (1996) states, however, that annual nest removal may drive the numbers of relatively host-specific parasites down to unnaturally low levels. Our results for fleas in boxes in Ontario provide some support for this idea. Obviously, the complete elimination of parasites from boxes can only be achieved by cleaning out nests and fumigating boxes with pesticides.

Our results for fleas show that Møller's (1989) assumption that parasites are more numerous in boxes of cavity-nesting birds that contain old nest material is partly correct. Given that some parasites increase in numbers with nest reuse (this study), and that some species of parasite can reduce the reproductive success of cavity-nesting birds (e.g., Richner et al. 1993), his critique of box studies is justified. But, like others (Koenig et al. 1992; Johnson 1996; Rendell and Verbeek 1996*b*), we caution that it is difficult to generalize about how the numbers of some types of parasites will change in boxes because there are numerous factors that can affect their numbers within and between seasons. We have shown that one such factor is the degree to which a species of parasite depends on nest material for survival during its life cycle, but also that the quantity of nest material in a cavity, reinfection, and cavity microclimate can also affect the numbers of haematophagous parasites in boxes. In the future, researchers studying cavity-nesting birds should consider not only the possible effects of ectoparasites on the bird species under investigation, but how characteristics of the life cycles of parasites and the nest cavity influence the community of parasites in nest boxes.

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