

Sex Ratios of Fleas (Siphonaptera: Ceratophyllidae) in Nests of Tree Swallows (Passeriformes: Hirundinidae) Exposed to Different Chemicals

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ABSTRACT Endocrine disruptors have well-known effects on vertebrate population sex ratios, but a variety of chemicals can also affect sex ratios of invertebrates. We tested whether sex ratios of fleas (*Ceratophyllus idius* Jordan and Rothschild) living in the nests of tree swallows (*Tachycineta bicolor* Vieillot) were affected by exposure to environments containing different chemicals. Fleas were collected from nests adjacent to sewage lagoons that received unknown chemical inputs, from nests fumigated with insecticidal vegetation (yarrow, *Achillea millefolium* L.), from nests treated with both commercial flea powder and diatomaceous earth, and from control nests. The proportion of males was lower in nests treated with both commercial flea powder and diatomaceous earth than it was in nests around the sewage lagoon or in nests to which yarrow had been added. These results suggest potentially novel effects of chemicals on invertebrate populations that would not be revealed by, for example, LD₅₀s.

KEY WORDS *Ceratophyllus idius*, fleas, sex ratio, *Tachycineta bicolor*, tree swallow

THERE ARE NUMEROUS ADAPTIVE mechanisms of sex ratio variation in invertebrates, among the simplest being haplodiploidy and parthenogenesis (Hamilton 1967, Bell 1982). But sex ratio variation can also arise as a consequence of environmental, sex-specific effects on development or survival. For example, endocrine disruptors, chemicals that mimic steroid hormones, can cause population sex ratios to deviate in a variety of species of vertebrates (Reeder et al. 1998, Lange et al. 2001, Palanza et al. 2001). Although their physiologies differ radically from those of vertebrates, recent work has shown that invertebrate population sex ratios also can be affected by some endocrine disruptors and other chemicals (e.g., Dodson et al. 1999, Peterson et al. 2001, Watts et al. 2002). One measure of the effects of a chemical is an LD₅₀, which is the dose that kills 50% of a population. However, this parameter may not always be appropriate for estimating long-term population effects; if males suffer higher mortality, re-population may occur more rapidly than if females suffer higher mortality because only a few males may be necessary to fertilize large numbers of females. In this paper, we test for sex ratio variation in fleas (*Ceratophyllus idius* Jordan and Rothschild) in nests of tree swallows (*Tachycineta bicolor* Vieillot) exposed to different chemical environments.

Birds that use cavities, such as old woodpecker nests, often host fleas, the adults of which feed especially on nestlings, but also adult birds. The ecology of *C. idius* has not been well described, but a better-known congener *C. gallinae* (Shrank) afflicts a variety of bird species, such as great tits (*Parus major* L.) (Harper et al. 1992, Heeb et al. 1996). As is the case for tree swallows, many hosts of *C. gallinae* annually reuse tree cavities or nestboxes for breeding. Founding populations of adult fleas are transported to new nest sites by adult birds. After dropping from the birds, fleas begin laying eggs in nesting material. The resulting larvae feed in nesting material. Approximately 23 d elapse between egg and adulthood in *C. gallinae* (Harper et al. 1992). *Ceratophyllus idius* overwinter as adults in abandoned nests (Benton 1982); a few adults may leave with young that are fledging (e.g., Heeb et al. 1996). At fledging, there may be as many as 2000 fleas in a single tree swallow nest (Thomas and Shutler 2001).

Tree swallows are ≈20-g passerines that readily use nest boxes for breeding. In boreal and temperate North America, tree swallows return from their southern wintering grounds in mid- to late April, but do not begin laying until early to late May. It takes ≈36 d from clutch completion to fledging (Robertson et al. 1992). Tree swallows feed primarily on flying insects, particularly those emerging from wetlands (Hussell and Quinney 1986). Because wetlands are repositories for a variety of pesticides and other chemicals, tree swallows have been the focus of a substantial body of toxicology research (Shaw 1983, Kraus 1989, St. Louis

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et al. 1993, Froese et al. 1998, Bishop et al. 1999). However, to our knowledge, there have been no studies on the consequences of these chemicals to the fauna inhabiting tree swallow nests. In this study, we tested for variation in sex ratios of fleas among sites exposed to various kinds and quantities of chemicals. There are too few data from invertebrates to make clear predictions about the direction of sex ratio skew because both male-biased and female-biased sex ratios in aquatic invertebrates have been found in the presence of a variety of chemicals (Dodson et al. 1999, Peterson et al. 2001, Watts et al. 2002).

Study Area and Methods. Research occurred in two different areas in Canada. Four study sites surrounded wetlands within 40 km of Sackville, New Brunswick. The first, River Hebert, is an experimental marsh designed to provide tertiary treatment of domestic wastes before being discharged (Hanson 2002). Secondly treated sewage from ≈ 950 dwellings is pumped into the wetland at a controlled rate. The wetland ecosystem cleans the effluent through uptake by plants and settling. Precise chemical inputs to River Hebert are unknown, and are only monitored indirectly by sampling the biota (Hanson 2002). The other three sites are near Sackville but in Nova Scotia. Eddy, Jolicure, and McIver marshes are wetlands managed for waterfowl that are free of sewage effluent. In addition, as part of a larger study within the four Sackville-area sites, half of the boxes that attracted tree swallows by mid-May had 5 g fresh insecticidal vegetation (yarrow, *Achillea millefolium* L.; see Clark 1991) added to them every second day from when the first nesting material appeared in a box (as much as 2 wk before egg-laying) until laying was complete. Campbell and Shutler (2003) found that yarrow caused roughly a 50% reduction in flea populations within nest boxes.

In British Columbia, nest boxes were erected on two adjacent study sites, both consisting of grazed pasture land interspersed with small wetlands. As part of a second, larger study to test for effects of fleas on tree swallow reproductive ecology, midway through incubation, eggs were temporarily removed from boxes. In half of these boxes, nesting material was dusted with a commercial insecticide containing 38.12% diatomaceous earth (silicon dioxide), 0.2% pyrethrins, and 1.0% piperonyl butoxide, before eggs were returned. These experimental nests were treated again when nestlings were 6-d-old, but in this case only diatomaceous earth (80%) was used to control fleas. Diatomaceous earth is not toxic, but can cause desiccation and death of invertebrates by disrupting the integrity of the exoskeleton.

Swallow boxes were cleaned of the previous year's nests before tree swallows returned in spring. Cleaning does not remove all fleas (R.D.D. and D. S., personal observation), but reduces their numbers so that variation in quality among nest sites is reduced (Møller 1989) to facilitate research on, for example, reproductive ecology. After tree swallows had fledged, nests were collected and frozen (beginning at the end of June and ending in mid-July). Nests were later thawed

Table 1. Median number of fleas per tree swallow nest and proportion of male fleas by site. Sites sharing a letter (rightmost column) were not significantly different in sex ratio

Site	N nests	Median fleas per nest	Mean proportion male	SD
British Columbia	17	335	0.38	0.10 ab
Eddy Road	7	602	0.49	0.15 a
Jolicure	8	584	0.41	0.16 ab
McIver	8	900	0.32	0.13 b
River Hebert	8	606	0.47	0.14 a

and oven-dried before being teased apart to count fleas. We could not tell whether fleas were alive when nests were collected. From a total of 31 New Brunswick nests and 17 British Columbia nests, a mean subsample of 35 fleas (range: 8–84 from the whole population of fleas within a nest) was randomly retrieved (Table 1). The range in subsample size arises because few fleas were available from insecticide-treated British Columbia nests, and because it was easy to obtain large numbers of fleas from some nests. Fleas were cleared in lactic acid at 60°C for 24–48 h, and then serially dehydrated with 70% and then 100% EtOH before being transferred to xylene and mounted on slides. Sexing was done without knowledge of the fleas' sources. Females were distinguished based on their simple spermathecae; males have more elaborate reproductive organs (Rothschild and Clay 1952).

Data were analyzed with SAS software (SAS Institute 1990). Means are reported \pm SD. For univariate tests, nonparametric analyses were used when data were not normally distributed (assessed with Shapiro-Wilk tests). Sex ratios were arcsine-square root transformed for parametric analyses, but for clarity of interpretation, we report original proportion data in the results. Because of small sample sizes, and because we wanted to be alert to any possible perturbations of sex ratios arising from pesticides, we set significance at $P < 0.10$.

Results

A mean of 5.0 ± 1.4 (range: 1–8) fledglings was produced per nest. The median number of fleas per nest was 568.5 (range: 10–1610; medians by site are presented in Table 1, and by treatment in Table 2). Among controls, the median number of fleas per nest (for British Columbia = 570.5, $N = 10$; for New Brunswick = 800.0, $N = 15$; Kruskal-Wallis $\chi^2 = 1.0$, $P = 0.32$) and mean proportion of male fleas (for British Columbia = 0.42 ± 0.09 , $N = 10$; for New Brunswick = 0.36 ± 0.16 , $N = 15$; $F_{1,23} = 1.1$, $P = 0.31$) did not differ by geographic location, so we pooled controls from both provinces. Males made up 41% of the 1681 total fleas we subsampled from all sites. In the remaining tests, nests were the units of analysis, and the proportion of males in any nest ranged from 0.10 to 0.73 (Fig. 1).

Sex ratio of flea populations was not significantly related to flea densities within nests (Fig. 1, $r_s = 0.22$,

Table 2. Median number of fleas per tree swallow nest and mean proportion of male fleas by treatment.

Chemical exposure	N nests	Median fleas per nest	Mean proportion male	SD
Control	25	768	0.38	0.14 ab
Commercial insecticide/ diatomaceous earth	7	55	0.33	0.09 b
Sewage	8	606	0.47	0.14 a
Yarrow	8	546	0.48	0.13 a

Commercial insecticide and diatomaceous earth were used only in the nests from British Columbia. By chance, all nests from River Hebert for which fleas were retrieved had not had yarrow added to them, so that "River Hebert" (Table 1) and "sewage" have the same statistics. Sites sharing a letter (rightmost column) were not significantly different in sex ratio.

$P = 0.14$), or to the number of swallows fledging from nests (same statistics as for flea density). Sex bias varied among sites (Table 1, General Linear Model $F_{4, 43} = 2.1, P = 0.10$; note that sample sizes were too small to test separately for effects of treatment and site). Pairwise comparisons indicated that McIver Marsh had a lower proportion of males than did Eddy Road Marsh or River Hebert (post hoc least squares means test [option PDIF in SAS 1990], $P_s < 0.04$, Table 1). Sex proportion also varied among treatments (Table 2, General Linear Model $F_{3, 44} = 2.5, P = 0.08$). Nests treated with commercial insecticide/diatomaceous earth had a lower proportion of male fleas than did nests near tertiary sewage treatment, and nests to which yarrow was added (post hoc least squares means test, $P < 0.05$; Table 2).

Discussion

At conception, sex in fleas is determined via Mendelian segregation of sex chromosomes. Thus, males and females are initially produced in equal numbers (Marshall 1981a, b). However, female fleas are generally larger and live longer than males (Rothschild and Clay 1952). As a consequence, populations are typically female-biased, and the overall 41% males we observed is within the ranges reported for unmanipulated populations of both bird (Rothschild and Clay

1952, Marshall 1981a) and mammal fleas (Morlan 1955, Schwan 1993). Presumably, these sex ratios represent outcomes in natural environments. We found some evidence that sex ratios vary geographically; these differences could be the result of microclimatic differences, diet (e.g., Uçkan and Ergin 2002), genetic variation at a population level, or a combination of these.

Our data also suggest skews in flea sex ratios beyond those of natural environments; these skews may have been a consequence of chemical exposure. Specific mechanisms involved in provoking shifts in sex ratio remain to be elucidated. The chemicals to which our fleas were exposed could cause direct sex-specific mortality. Alternatively, if emergence times of sexes differ, differential exposure may result in one sex suffering higher mortality or impaired development relative to the other (Marshall 1981a, b; Bossard et al. 2000). Similarly, because males are able to fertilize multiple females (Rothschild and Clay 1952, Hsu and Wu 2000), pursuit of mates may make them more active than females, and this may expose them to a broader spectrum of environmental hazards, such as pesticides. However, as we note above, we collected both living and dead fleas, so that differences in activity as adults should not affect sex ratios of the fleas we collected. Nonetheless, measurement of sex proportions should take temporal factors into account for the reasons given above. In our study, all of our fleas were collected after tree swallows had fledged; because >95% of fledging occurs within two weeks (D. S., unpublished data), temporal influences on flea sex ratios should be minimal.

Regardless of the mechanisms, perturbations of invertebrate sex ratios have potentially important implications, especially if our results for fleas have generality for other arthropods. At an applied level, pesticides that primarily affect males may have limited effects on long-term population trends. This may be more pronounced in species where the sexes have different niches (e.g., mosquitoes where females are sanguivorous while males are nectarivorous). From a conservation perspective, if females are differentially and negatively affected, this could affect the rate at which populations recover, imperiling species that are already at low numbers (e.g., Sutherland 2002). These are but a few scenarios associated with chemical sex ratio disruptors, and we urge further attention to their potential impact.

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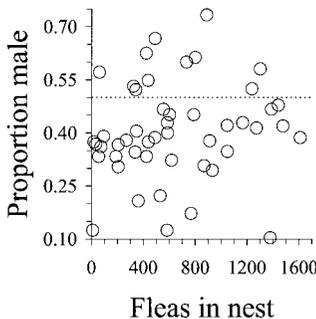


Fig. 1. Relationship between the proportion of male fleas and the number of fleas in a tree swallow nest. Dotted line indicates an even sex ratio.

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