Diversity and Abundance of Ectoparasitic Blow Flies *Protocalliphora* (Diptera: Calliphoridae) and Their *Nasonia* (Hymenoptera: Pteromalidae) Parasitoids in Tree Swallow Nests Within Agricultural Lands of Southern Québec, Canada

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Ann. Entomol. Soc. Am. 105(3): 471–478 (2012); DOI: http://dx.doi.org/10.1603/AN11155

ABSTRACT We described the community composition of the ectoparasitic flies *Protocalliphora* Hough (Diptera: Calliphoridae) and their *Nasonia* (Ashmead) (Hymenoptera: Pteromalidae) parasitoids collected from tree swallow, *Tachycineta bicolor* (Vieillot), nests in southern Québec, Canada, in 2008 and 2009. The prevalence of nest infestation by *Protocalliphora* was 70.8% in 2008 and 34.6% in 2009. The average parasitic burden of *Protocalliphora* spp. was estimated at 5.53 (±5.61 SD) pupae per nestling for 2008 and 4.66 (±9.31 SD) pupae per nestling for 2009. The percentage of nests containing *Protocalliphora* pupae parasitized by *Nasonia* spp. was of 85.3% in 2008 and 67.2% in 2009. Three species of *Protocalliphora* were collected—*Protocalliphora sialia* Shannon & Dobroscky, *Protocalliphora bennetti* Whitworth, and *Protocalliphora metallica* (Townsend)—and two species of *Nasonia*, *Nasonia vitripennis* (Walker) and *Nasonia giraulti* Darling. This is the first record of *P. bennetti* and *N. giraulti* in the province of Québec. Our findings provide further evidence for observation made previously that altricial bird nests are more frequently and more heavily infested by blow flies in western than in eastern North America. Our data also suggest that more than a quarter of the tree swallow within the study area are exposed to high levels of ectoparasitism that could negatively affect their health.

KEY WORDS *Protocalliphora*, *Nasonia*, agricultural landscape, parasitism

Larvae of Holarctic bird blow f1ies in the genera *Protocalliphora* Hough and *Trypocalliphora* Peus (Diptera: Calliphoridae) are obligate blood-feeding parasites of nestling birds, a unique life history among the Calliphoridae. To date, 28 species of *Protocalliphora* and one species of *Trypocalliphora* parasitizing 139 bird species have been recorded from the Nearctic region (Sabrosky et al. 1989, Whitworth 2003). Birds with the highest infestation rates are those that nest repeatedly in a given habitat and build large nests, such as hawks (*Acipiter* and *Buteo* spp.), magpies (*Pica* spp.), crows (*Corvus* spp.), and cavity-nest breeders such as European starlings (*Sturnus vulgaris* L.) and tree swallows (*Tachycineta bicolor* Vieillot) (Bennett and Whitworth 1992).

Adult *Protocalliphora* are rarely collected in the field but immatures (larvae and pupae) are typically found after close examination of nestlings or nest materials. There is a paucity of information on the life history of these bird ectoparasites; most of the literature on *Protocalliphora* focuses on taxonomy, host records, and host fitness costs after parasitism (Dawson et al. 2005, Simon et al. 2005, Hannam 2006). Generally, both male and female *Protocalliphora* overwinter as adults, and females lay their eggs as soon as nestlings hatch (T.L.W., personal observation). Development from egg to adult takes on average 23–38 d, and although most *Protocalliphora* species are univoltine, some are capable having more than one breeding cycle per season by parasitizing the second brood of the bird species within their habitat (Bennett and Whitworth 1991).

Blow fly larvae are typically anchored to the most accessible part of the nestling (feet, legs and belly); some species also can be found in the auditory and nasal cavities (Whitworth and Bennett 1992). *Protocalliphora* larvae must take two to three bloodmeals to complete their development (Sabrosky et al. 1989). Amounts of blood ingested vary according to species, reaching on average 0.08 g in the large *Protocalliphora rognesi* Thompson & Pont (Bennett and Whitworth 1991). In the most extreme scenario, five or six of the largest *P. rognesi* larvae, capable of each ingesting 0.2 g of blood, could exsanguinate an 18-d-old bank swallow [*Riparia riparia* (L.)] in a single day (Whitworth and

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Bennett 1992). However, most instances of nestling mortality have been associated with organ damage because of parasitism by obligate subcutaneous larvae of *Trypocalliphora braueri* Hendel (Sabrosky et al. 1989, Howe 1992, Matsuoka et al. 1997).

At the upper trophic level, puparia of *Protocalliphora* are often found parasitized by small, gregarious "jewel wasps" of the genus *Nasonia* (Ashmead) (Hymenoptera: Pteromalidae). All four known species of *Nasonia* are found in the Nearctic region, and only *Nasonia vitripennis* (Walker), a generalist that develops in the pupae of a variety of calyptrate Diptera species (Whiting 1967), also is found in the Palaearctic region. The three other *Nasonia* species only parasitize *Protocalliphora* pupae and are restricted to eastern (*Nasonia giraulti* Darling, *Nasonia oneida* Raychoudhury & Desjardins) and western North America (*Nasonia longicornis* Darling) (Darling and Werren 1990, Raychoudhury et al. 2010). For the past 40 yr, *Nasonia* parasitoids, especially *N. vitripennis*, have been extensively used as model organisms for studies investigating mating behavior, genomics, speciation and especially sex allocation and sex ratio distorters (Werren and Loehlin 2009, Godfray 2010). However, very little is actually known of their ecology and behavior in their natural habitat (Grillenberger et al. 2008).

Although the literature has been divided with regard to the impact of *Protocalliphora* larvae on their bird hosts, it now seems clear that they can negatively influence health and survivorship of nestlings and fledglings by exacerbating effects of unfavorable environmental conditions (e.g., low food abundance). Such negative impacts are more likely to occur in species that can be frequently afflicted by high loads of *Protocalliphora* larvae, such as the tree swallow (Bennett and Whitworth 1992, Hannam 2006, Thomas et al. 2007). Indeed, tree swallows renest in the same habitat each year; conditions conducive to maintenance of large *Protocalliphora* populations (Bent 1963, Bennett and Whitworth 1992). To date, 11 species of *Protocalliphora* have been recorded parasitizing tree swallow nestlings, six of which occur in the Eastern Nearctic (Sabrosky et al. 1989; Whitworth 2002, 2003). Moreover, the population of this bird species, like that of most aerial insectivores found in North America and Europe, has been decreasing over most of its range during the past 30 yr (Benton et al. 2002, Nebel et al. 2010). This generalized population decline of aerial insectivores is hypothesized to be linked to a decrease in flying insect abundance caused by large-scale ecosystem modifications, notably agricultural intensification (Benton et al. 2002, Ghilain and Bélisle 2008, Nebel et al. 2010). In the event that *Protocalliphora* abound in agricultural landscapes, these blow flies have the potential to exacerbate the negative impact that agricultural intensification has on aerial insectivores through reduced prey abundance. Nevertheless, the impact of *Protocalliphora* on farmland bird species also will depend on the capacity of *Nasonia* parasitoids to control these blow flies in agricultural landscapes.

The faunistics and ecology of the *Protocalliphora* and *Nasonia* associated with tree swallows are poorly known in eastern Canada where tree swallows are experiencing important population decline (Nebel et al. 2010). The objectives of this study were to 1) describe the assemblages of *Protocalliphora* and *Nasonia* species found in tree swallow nests in 40 farms in southern Québec, Canada; 2) estimate the parasitic load of *Protocalliphora* on their bird hosts; and 3) establish levels of parasitism by *Nasonia.*

Materials and Methods

Study Area and Nest Box Network. The 10 200-km^2 study areas included a network of 400 bird nest boxes distributed among 40 farms within the Montérégie and Estrie regions in southern Québec (Fig. 1). The area is characterized by an east–west gradient of agricultural intensification where dairy farming and small-scale, familial farms are replaced by large-scale, continuous row cropping with full mechanization and high input of pesticides as well as of organic and chemical fertilizers (Bélanger and Grenier 2002, Jobin et al. 2003; Fig. 1). Nest boxes were built according to North American Bluebird Society's specifications (i.e., eastern/western bluebird model) and were erected in winter 2004. Ten nest boxes per farm were placed 50 m apart along drainage ditches or fence lines that bordered agricultural fields or pastures. All boxes were mounted on a metal post 1.5 m above the ground and with the opening facing southeast. See Ghilain and Bélisle (2008) for the detailed farm selection protocol.

Specimen Collection and Identification. Before their fall migration, tree swallows typically visit nests that were used during the breeding season, probably to ascertain the quality of potential future breeding sites (Bent 1963, Doligez et al. 2004, Ghilain and Bélisle 2008, Winkler et al. 2011). To avoid interference with the bird's habitat quality assessment, insect specimens were collected from the field using two protocols. First, two of the 10 available tree swallow nests in each of the 40 farms were examined for *Protocalliphora* pupae immediately upon fledging, i.e., between 25 June and 16 July in both 2008 and 2009. The nest was carefully removed from the box, and all pupae in nest material and in the nest box were collected. The nest was put back into the box upon completion of sampling. Pupae were individually placed into meshed capped plastic bottles (4 cm in length by 7 cm in height) for rearing. They were maintained in a mixture of sawdust and 1% boric acid at room temperature (≈22°C) until the emergence of the adult flies or their parasitoids (5–12 d). Adults were kept alive for 48 h for maturation and hardening of the integuments and killed by freezing (−30°C) for 7 d. Adult *Protocalliphora* were pinned and air-dried, and their puparia were stored individually in 90% ethanol. Adult *Nasonia* were stored in 90% ethanol at −20°C.

The second sampling protocol took place during winters 2008–2009 and 2009–2010, several months after the birds had left. All nests (including those sampled in the first place) were collected and brought back to the laboratory for examination. Empty *Procalliphora* puparia were removed from nest material,

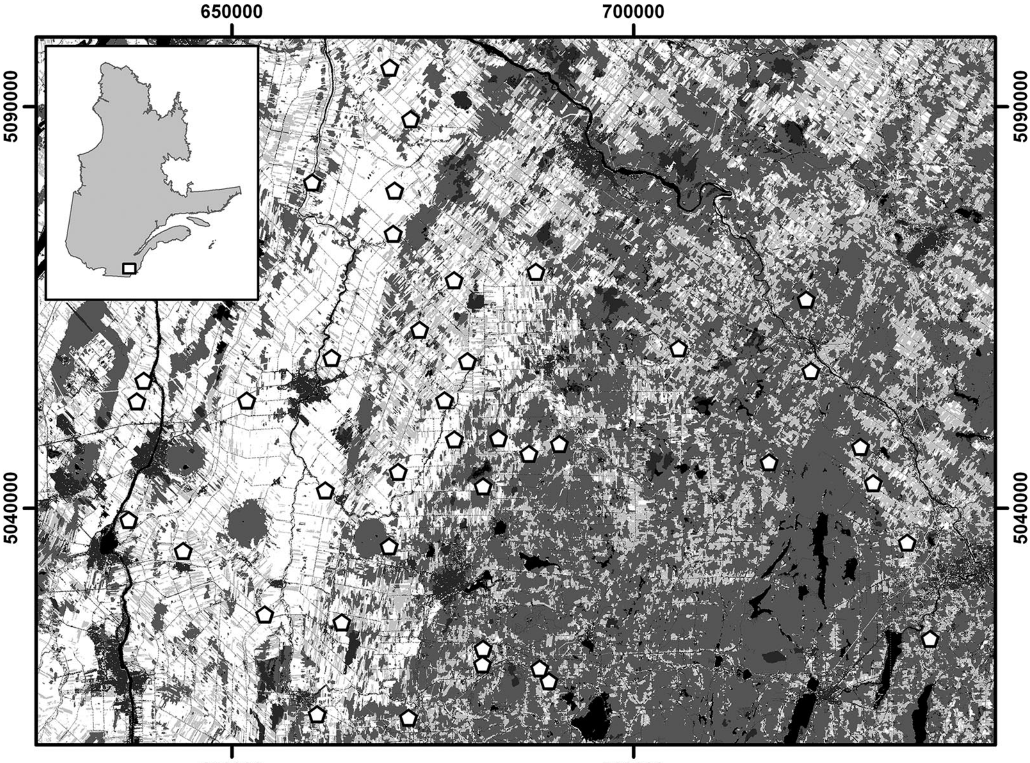

Fig. 1. Distribution of the 40 farms along a gradient of agricultural intensification in southern Québec, Canada, 2008–2009. Land cover types are based on a mosaic of classified LANDSAT-TM satellite images (Canadian Wildlife Service 2004) and include water (black), urban (dark gray), forest (mid-tone gray), extensive cultures (e.g., hayfields and pastures; light gray), and intensive cultures (e.g., maize [*Zea mays* L.]rsqb], cereals, and soybean [*Glycine max* (L.) Merr.]; white). Open pentagons indicate farm locations. Coordinates are UTM (Zone 18; NAD83) and refer to the number of meters from a reference point.

air-dried, and subsequently stored at room temperature in vials. Pupae from which insects failed to emerge were dissected and examined.

Adults *Protocalliphora* were sexed and keyed to species by using Sabrosky et al. (1989) and Whitworth (2003). Because *Protocalliphora* adults are rarely caught and most specimens in collections consist of puparia, much effort has been invested into developing reliable identification keys based on morphological structures of puparia (Whitworth 2006). The puparia of emerged adults as well as those collected during the winter were first examined with a stereomicroscope for markings associated with either adult fly emergence (missing or broken emergence cap) or wasp emergence (exit holes 0.5–1 mm in diameter) and then keyed to species by using Whitworth (2002, 2003). All puparia that could not be identified at low magnification were mounted on slides (Whitworth 2002) for examination with a compound microscope.

Nasonia specimens were examined and photographed for measurements necessary to identification by using a stereomicroscope equipped with a digital camera. Measurements were taken using PixeLINK imaging software (PixeLINK, Ottawa, Canada), and all specimens were keyed to species by using Darling and Werren (1990) and Raychoudhury et al. (2010). Specimen identifications were confirmed by members of the Werren laboratory (Rochester University, Rochester, NY). Voucher specimens have been deposited to the Ouellet-Robert entomological collection at Université de Montréal.

Statistical Analyses. Interannual comparisons in the number of *Protocalliphora* pupae per nest, per nestling and number of *N. vitripennis* and *N. giraulti* that emerged per pupa were performed using Wilcoxon rank sum tests. Interannual comparisons between infestation rates (percentages) were performed using standard chi-square tests.

Results

In total, of 202 nests from 37 farms was occupied by tree swallows in 2008 and 185 nests from 37 farms in 2009; remaining nest boxes were left empty or occupied by either eastern bluebirds [*Sialia sialis* (L.)] or house sparrows [*Passer domesticus* (L.)] and were not considered in this study. In total, 3,601 and 1,275 *Protocalliphora* pupae and puparia were collected from 33 and 24 of these farms in 2008 and 2009, respectively,

Table 1. Interannual comparison in the abundance (mean number of *Protocalliphora* per nest) and estimated parasitic burden (mean number of *Protocalliphora* per nestling) of *Protocalliphora* spp. sampled from tree swallow nests (*N* = 143 in 2008; *N* = 64 in 2009) between 2008 and 2009 within the Montérégie and Estrie regions of southern Québec, Canada

Parameter	2008		2009		W^a	P
	Mean ± SD	Range	Mean ± SD	Range		
Avg. no. *Protocalliphora* pupae/nest	25.43 ± 23.33	(1–110)	21.95 ± 43.86	(1–148)	16,390	<0.001
P. sialia pupae/nest	24.20 ± 23.38	(0–110)	19.63 ± 39.20	(0–146)	5,416	0.035
P. bennetti pupae/nest	0.99 ± 3.21	(0–27)	1.8 ± 3.59	(0–18)	4,146.5	0.15
P. metallica pupae/nest	0.23 ± 0.90	(0–7)	0.19 ± 0.38	(0–4)	4,598	0.92
Avg. no. *Protocalliphora* pupae/nestling	5.53 ± 5.61	(0.15–45)	4.66 ± 9.31	(0.2–37)	5,134	0.16
P. sialia pupae/nestling	5.26 ± 5.51	(0–43)	4.20 ± 8.38	(0–36)	5,447.5	0.029
P. bennetti pupae/nestling	0.22 ± 0.70	(0–5.4)	0.36 ± 0.73	(0–3.6)	4,163.5	0.17
P. metallica pupae/nestling	0.05 ± 0.19	(0–1.33)	0.04 ± 0.08	(0–0.8)	4,595	0.93

[a] Wilcoxon rank sum test ($\alpha = 0.05$).

with most being sampled during the winter (82.40% in 2008 and 74.82% in 2009).

Protocalliphora. The prevalence of nest infestation by *Protocalliphora* was twice as high in 2008 (70.8%, $N = 143$) as in 2009 (34.6%, $N = 64$) ($\chi^2 = 23.62$, $P < 0.0001$). A significant decrease of 15.9% was observed between the number of *Protocalliphora* pupae per nest collected in 2008 compared with those collected in 2009 (Table 1). Although the number of *Protocalliphora* pupae per nestling was 25.2% higher in 2008 compared with 2009, this difference was not significant (Table 1). The numbers of *Protocalliphora* pupae per nest and per nestling were both highly variable; however, they showed twice as much variation in 2008 as in 2009 (pupae per nest coefficient of variation, 91.7–199.8%; pupae per nestling coefficient of variation, 101.4–199.8%; Table 1). Overall, the observed within and between year levels of variation imply that most *Protocalliphora*-infested tree swallow broods experienced low-to-high parasitic burdens (Table 1).

Adults were reared from 283 of 333 collected pupae in 2008 and from 110 of 204 pupae in 2009. Three species of *Protocalliphora* were identified (Table 1). *Protocalliphora sialia* Shannon & Dobroscky was by far the most prevalent and abundant, found in 96.51% of infested nests in 2008 and 87.58% in 2009 (Table 1). *Protocalliphora bennetti* Whitworth was found in 21.11% of infested nests in 2008 and 31.29% in 2009, whereas *Protocalliphora metallica* (Townsend) was only sampled in 10.03% of infested nests in 2008 and 4.82% in 2009. Nest infestation by *P. sialia* alone was the most frequent pattern, accounting for at least two thirds of nest parasitism in 2008 and 2009 (Table 2). Mixed infestations of *P. bennetti* and *P. sialia* accounted for almost a fifth of infestations. *P. bennetti* and *P. metallica* were predominantly found in mixed nest infestations with *P. sialia* and only rarely sampled alone or in mixed infestations with each other (Table 2). Only one nest of 143 in 2008 and one nest of 64 in 2009 were simultaneously infested by the three *Protocalliphora* species.

Nasonia. Overall, more than two third of the nests containing *Protocalliphora* pupae were parasitized by *Nasonia* (Table 3). Nonetheless, the proportion of *Protocalliphora*-infested nest that incurred parasitism by *Nasonia* was 27% higher in 2008 than in 2009 (Table 3). Similarly, the level of parasitism (proportion of pupae parasitized in *Nasonia*-infested nests) was 28% greater in 2008 than in 2009 (Table 3). Although the levels of parasitism by *Nasonia* on *P. sialia, P. bennetti* and *P. metallica* were similar in 2008, *P. bennetti* experienced a level of parasitism about twice as high as those of the other species in 2009 (Table 3).

Adult *Nasonia* emerged from 52.4% ($N = 635$) of the *Protocalliphora* pupae collected after bird fledging in 2008 and from 63.6% ($N = 321$) of collected pupae in 2009. Of the pupae sampled in the winter, based on the configuration of exit holes, *Nasonia* were inferred to have emerged from 49.9% of pupae in 2008 ($N = 2966$) and 31.1% of pupae in 2009 ($N = 954$; Table 4). Two species of *Nasonia* were identified, *N. vitripennis,* by far the most common species, and *N. giraulti* (Table 4). In most cases, only *N. vitripennis* was found per nest (Table 4).

No significant interannual variation was observed ($W = 25609$, $P = 0.46$) between the mean number of

Table 2. Level (percentage) of single and mixed infestations of *Protocalliphora* spp. in tree swallow nests (*N* = 143 in 2008; *N* = 64 in 2009) in 2008 and 2009 within the Montérégie and Estrie regions of southern Québec, Canada

Parameter	2008	2009	χ^2	P
Nest with single *Protocalliphora* infestations	73.43 (N = 143)	78.17 (N = 64)	0.07	0.71
P. sialia	71.30	65.63	0.04	0.84
P. bennetti	1.40	10.94	6.66	0.01
P. metallica	0.70	1.60	0.03	0.83
Nests with mixed infestations	26.58 (N = 143)	21.95 (N = 64)	0.15	0.69
P. bennetti + *P. sialia*	17.48	18.75	0.03	0.86
P. metallica + *P. sialia*	7.00	1.60	1.45	0.23
P. bennetti + *P. metallica*	1.40	0.00	0.03	0.86
P. bennetti +*P. metallica* + *P. sialia*	0.70	1.60	0.04	0.86

Table 3. Prevalence of nest infestation and the total level of parasitism of *Protocalliphora* pupae (N = 3601 in 2008; N = 1275 in 2009) by *Nasonia* spp. in tree swallow nests in 2008 and 2009 within the Montérégie and Estrie regions of southern Québec, Canada

Parameter	2008	2009	χ^2	P
Nests containing *Protocalliphora* pupae parasitized by *Nasonia* spp.	85.31 (N = 143)	67.19 (N = 64)	7.90	0.05
Protocalliphora pupae parasitized by *Nasonia* spp.	50.35 (N = 3,601)	39.29 (N = 1,275)	16.83	<0.001
P. sialia	53.88 (N = 3,380)	34.71 (N = 1,120)	12.83	<0.001
P. bennetti	47.45 (N = 137)	60.68 (N = 117)	1.10	0.29
P. metallica	46.67 (N = 14)	30.00 (N = 10)	0.07	0.79

N. vitripennis wasps that emerged per pupae in 2008 (30.66 ± 20.78 [SD]) and those that emerged in 2009 (27.83 ± 14.82 SD). Similarly, no significant interannual variation was observed (W = 67, P = 0.063) between the mean number of *N. giraulti* wasps that emerged per pupae in 2008 (29.64 ± 9.24 SD) and those that emerged in 2009 (20.63 ± 10.01SD).

Discussion

Our study presents a description of the *Protocalliphora* and *Nasonia* species composition and abundance from tree swallow nests within agricultural lands of southern Québec. We identified three species of *Protocalliphora* (*P. sialia, P. bennetti*, and *P. metallica*) and two species of *Nasonia* (*N. vitripennis* and *N. giraulti*). This is the first record of both *P. bennetti* and *N. giraulti* in the province of Québec.

Protocalliphora infestations in bird nests have been reported to be higher in western than in eastern North America (Sabrosky et al. 1989, Bennett and Whitworth 1992). In their seminal work investigating the *Protocalliphora* fauna of 73 bird species, Bennett and Whitworth (1992) documented that 52% of the 1862 nests sampled in the west (i.e., Utah, USA) were infested with *Protocalliphora*, whereas only 24% of the 2806 nests in the east (Ontario, Canada) were infested. Accordingly, in three studies on the *Protocalliphora* of tree swallows within anthropogenically modified habitats of western Canada, Rendell and Verbeek (1996), Dawson et al. (2005) and Gentes et al. (2007) reported that 93, 89, and 100% of the nests were infested, respectively. These numbers are notably higher than what was observed in our study (70.8% in 2008 and 34.6% in 2009). Not only are bird nests more frequently infested with *Protocalliphora* in western North America but also they seem to be more heavily infested with *Protocalliphora.* For example, Rendell and Verbeek (1996), Dawson et al. (2005), and Gentes et al. (2007), studying bird host species that lay clutches similar in size to that of tree swallows, reported mean abundances of *Protocalliphora* per nest that were 152, 101, and 199% higher than our study. Our findings thus provide further support for this east versus west pattern of *Protocalliphora* infestation in North America. Ecological factors accounting for this geographical difference in levels of nest infestation remain to be explored.

Several studies have investigated the effect of blood feeding by *Protocalliphora* larvae on developing nestlings. In some cases, parasitized nestlings had lower hematocrit and hemoglobin levels (Whitworth and Bennett 1992, O'Brien et al. 2001, Hannam 2006), reduced growth rates (Whitworth and Bennett 1992), decreased body temperatures and metabolic rates (Simon et al. 2005), and lower fledging survival and reduced dispersal in the first days after fledging (Thomas et al. 2007, Streby et al. 2009). Conversely, others have reported no significant effects of *Protocalliphora* ectoparasitism on any of the aforementioned parameters (Johnson et al. 1991, Rogers et al. 1991, Roby et al. 1992, Wittmann and Beason 1992, Miller and Fair 1997, Thomas and Shutler 2001). These latter studies, however, did not follow nestlings throughout their early developmental stages, when nestlings may be the most sensitive to *Protocalliphora* ectoparasitism. Negative effects on bird hosts have been observed at levels of ectoparasitism ranging from 7.2 to 13.73 pupae per nestling (Whitworth and Bennett 1992, O'Brien et al. 2001, Simon et al. 2005, Streby et al. 2009) and more specifically at 13.3 pupae per nestling for *P. sialia* on the eastern bluebird (Hannam 2006). We measured on average 5.5 and 4.7 pupae per tree swallow nestling in 2008 and 2009, respectively, but with considerable

Table 4. Percentage of parasitism of *Protocalliphora* pupae by *Nasonia* spp. as determined from pupae collected during the first sampling effort (N = 333 pupae for 2008; N = 204 for 2009) in tree swallow nests in 2008 and 2009 within the Montérégie and Estrie regions of southern Québec, Canada

Parameter	2008	2009	χ^2	P
Nests containing parasitized *Protocalliphora* pupae infested with *N. vitripennis*	63.13 (N = 57)	89.20 (N = 28)	5.1	0.024
Nests containing parasitized *Protocalliphora* pupae infested with *N. giraulti*	8.80 (N = 57)	25.00 (N = 28)	2.85	0.09
Protocalliphora pupae parasitized by *N. vitripennis*	93.01 (N = 333)	94.61 (N = 204)	0	1
Protocalliphora pupae parasitized by *N. giraulti*	3.90 (N = 333)	2.96 (N = 204)	0.1	0.75
Single *N. vitripennis* infestation	90.01 (N = 333)	92.65 (N = 204)	0.02	0.89
Single *N. giraulti* infestation	0.90 (N = 333)	1.00 (N = 204)	0.14	0.71
Mixed *Nasonia* infestations	3.00 (N = 333)	1.96 (N = 204)	0.14	0.71

Nasonia Infestation rates do not sum to 100% because some *Protocalliphora* pupae contained parasitoids at larval stages that we were not able to key to species.

variation among infested broods. For example, in 2008 and 2009, 38 of the 143 and 10 of 64 infested nests had levels of ectoparasitism >7.5 pupae per nestling, the highest being 47 pupae per nestling. Although not intended to assess the effect of *Protocalliphora* on tree swallows, our data suggest that some birds are exposed to high levels of ectoparasitism that could influence breeding success.

We observed considerable interannual variation in the prevalence of *Protocalliphora* infestation in 2008 (70.8% of infested nests) and 2009 (34.6%). This variation should be interpreted with caution because the study was conducted over only two field seasons. Yet, Johnson et al. (1991), Bennett and Whitworth (1992), Roby et al. (1992), and Germaine and Germaine (2002) also reported differences in prevalence of infestation between sampling years of 16, 75, 22, and 38%, respectively. When studying the conditions favoring parasitism by *Protocalliphora* within Algonquin Park in Ontario, Canada, Bennett and Whitworth (1992) suggested that the experimental removal from the field of immature stages of *Protocalliphora* before adult emergence decreases the rate of nest infestation the following year. This might partly explain the observed interannual variability described in the previously referenced studies, because they all removed nest material and *Protocalliphora* pupae within days of bird fledging. However, our sampling protocol was designed to minimize the removal of adult *Protocalliphora* from the field. Indeed, only 16.8% of the pupae were removed from the field in 2008, and it is therefore unlikely that the interannual variation we observed resulted from oversampling. Another possibility lies in the interannual differences in nest occupation and destruction by mice and voles after tree swallow fledging, which was slightly higher in 2009 than in 2008 (S.P.D., unpublished data). Long-term monitoring would be necessary to elucidate the underlying mechanisms governing the population dynamics of *Protocalliphora.*

Of the seven *Protocalliphora* and the one *Trypocalliphora* species known to parasitize tree swallows in eastern North America, we sampled three (*P. sialia, P. bennetti,* and *P. metallica*). This is not entirely surprising because the other three species are seldom found in tree swallow nests. The predominance of *P. sialia,* both in terms of nest infestation and abundance (Table 1), is consistent with other studies on tree swallows from across North America (Roby et al. 1992, Wittmann and Beason 1992, Smar 1994, Dawson et al. 2005), as is the low prevalence and abundance of the two other species collected within our study system, *P. bennetti* and *P. metallica* (Bennett and Whitworth 1992, Whitworth 2002). Bennett and Whitworth (1992) and Whitworth (2002) suggested that females of some species of *Protocalliphora* have either a "nest site preference" or a "host specificity" and possibly an interaction of the two, when ovipositing. *P. bennetti,* for example, are host specific, being most frequently found in cavity nests of either wrens or chickadees but rarely in nests of tree swallows (Bennett and Whitworth 1992, Whitworth 2002). In contrast, *P. metallica* seem to prefer habitats with open canopies rather than forested areas (Sabrosky et al. 1989, Bennett and Whitworth 1992). In addition, *P. metallica* have been shown to preferentially oviposit in open nests such as those of ground nesting birds but rarely in cavity-nesting birds (Sabrosky et al. 1989, Bennett and Whitworth 1992). Thus, the pattern of *Protocalliphora* species collected from tree swallow nests within the agricultural lands of southern Québec conforms to those reported in other studies on tree swallows.

Two or more species of *Protocalliphora* were found in 24.5% of the infested nests (data from both years combined); this value is above what was reported in Bennett and Whitworth (1992) who found an average mixed infestation rater of 12.5% in Algonquin National Park, Ontario, Canada. The determinants governing this phenomenon have not yet been elucidated.

During the first sampling effort, the collected pupae were parasitized by two species of *Nasonia* (*N. vitripennis* and *N. giraulti*). *N. vitripennis* was the most prevalent and abundant of the two for each of the three *Protocalliphora* species. *N. giraulti* was usually found in association with *N. vitripennis.* These data are consistent with those reported by Grillenberger et al. (2009) who identified *Nasonia* wasps emerging from *Protocalliphora* pupae collected from tree swallow and eastern bluebird nests in upper New York state; the majority of the nest contained single *N. vitripennis* infestations (67%), whereas 29% of nest contained mixed infestations of both *N. vitripennis* and *N. giraulti.* No single infestations of *N. giraulti* were observed. Historically, this pattern of relative abundance between *N. vitripennis* and *N. giraulti* might have been different as *N. giraulti* had once been more common within its range (J. H. Werren, personal communication). A recent study proposes that the appearance of a new species, *N. oneida* in New York state, as well as the introduction of *N. longicornis* into eastern North America, could play a role in the decrease of *N. giraulti* populations within that region (Raychoudhury et al. 2010). Whether this is the case for southern Québec remains unknown as there is a complete lack of historical data for this area.

To our knowledge, only three field studies have been conducted on *Nasonia.* Grillenberger et al. (2008) (Europe), Grillenberger et al. (2009) (upper New York state), and Bennett and Whitworth (1991) (across North America) observed that 60, 91, and 72% of bird nests contained *Protocalliphora* pupae that were parasitized by *Nasonia* with levels of parasitism (i.e., parasitized pupae) of 46.8, 48.0, and 22.1%, respectively. Similarly, 85.3 and 67.2% of our nests contained parasitized pupae in 2008 and 2009, respectively, with levels of parasitism of 49.8 and 35.7%.

Nasonia do not seem to discriminate between *Protocalliphora* species, because *P. sialia, P. bennetti,* and *P. metallica* experienced comparable levels of parasitism (Table 4). The higher value observed for *P. bennetti* in 2009 was due to an exceptionally high level of parasitism in a single nest, where all pupae from a single infestation of *P. bennetti* were parasitized.

Acknowledgments

We thank Arnaud Ghilain, Renaud Baéta, Audrey Robillard, and all members of the Tree Swallow group at Université de Sherbrooke for support and hard work. We also thank R. Raychoudhury for aid. This work would not have been possible without the field help of Josée Doyon, Brian Mader, Krista Giguère, Simon Legault, Mathieu Bélanger-Morin, Fanny Maure, Simon Laurin-Lemay, Louis Laplante, Jeremy Daoust, Christian Daoust, Jean-François Limoges, and Sylvain Daoust. Financial support was provided to J.S., M.B., and J.B. from the National Sciences and Engineering Research Council of Canada. S.P.D. was supported by scholarships from the Fonds québécois de la recherche sur la nature et les technologies and the Québec Center for Biodiversity Studies.

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Received 17 September 2011; accepted 24 February 2012.