Studies on the life history of some species of *Protocalliphora* (Diptera: Calliphoridae)

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The rate of development of the larvae, postfeeding larvae, puparia, and adults of 10 species of *Protocalliphora* were studied and differences noted in the rates of development and (or) survival. Larvae of *Protocalliphora* were photonegative and strongly influenced by thigmotactic stimuli. The method of feeding of the larva is described. Metamorphosis was completed more quickly at higher temperatures than at lower temperatures. Survival of purparia at temperatures of 7°C or lower was poor and indicated that this stage was not adapted to withstand low temperatures and probably did not survive the winter. Adult *Protocalliphora* females were longer lived than males, surviving for an average of 70–100 days, with some individuals surviving for over 250 days under laboratory conditions. Adults fed readily on a sugar–protein diet, crushed berries, and certain species of flowers, but most species did not feed on any form of carrion. Adults mated readily in captivity, but none of the seven species tested crossbred. Spermatozoa remained motile in the seminal receptacles for over 100 days. Eggs were laid on a few occasions, but the factors determining egg production were not studied. Adult *Protocalliphora* were strongly photopositive and became quiescent in the dark. Adult flies rarely became active until a threshold temperature of 15.5°C; different species had differing threshold temperatures. Adults successfully overwintered in a single field trial.

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Le développement des larves, des larves après un repas, des pupes et des adultes a été étudié chez 10 espèces de *Protocalliphora*; des différences ont été notées dans les taux de développement et (ou) de survie. Les larves de *Protocalliphora* étaient photonégatives et réagissaient fortement à des stimulus thigmotactiques. Le mode d'alimentation des larves est décrit ici. La métamorphose s'est avérée plus rapide aux températures élevées qu'aux températures basses. La survie des pupes à des températures de 7°C ou moins était faible, ce qui indique que ce stade n'est pas armé pour résister au froid et ne survit probablement pas à l'hiver. Les femelles adultes de *Protocalliphora* vivent plus longtemps que les mâles, en moyenne de 70 à 100 jours, et quelques femelles ont vécu plus de 250 jours en laboratoire. Les adultes ont facilement accepté une diète sucre–protéines, des petits fruits écrasés et certaines espèces de fleurs, mais la plupart ds espèces ont refusé de se nourrir de charognes. Les adultes s'accouplaient bien en captivité, mais aucune des sept espèces étudiées n'a tenté de s'accoupler avec une autre. Les spermatozoïdes restaient mobiles dans les réceptacles séminaux pendant plus de 100 jours. Des oeufs ont été pondus à quelques reprises, mais les facteurs déclencheurs de la production d'ouefs n'ont pas été étudiés. Les adultes de *Protocalliphora* étaient fortement photopositifs et cessaient leurs activités à l'obscurité. Les mouches adultes étaient rarement actives sous le seuil de température de 15,5°C; les différentes espèces n'avaient pas toutes les mêmes seuils de température. Les adultes ont tous survécu à l'hiver au cours d'une expérience tentée en nature.

[Traduit par la rédaction]

Introduction

The genus *Protocalliphora* is a Holarctic and mainly boreal group of calliphorid flies whose obligatory blood-feeding larval stages live in bird nests and feed on the blood of the nestlings. The taxonomy of the genus has been in chaos for decades but recently, Sabrosky et al. (1989), in an extensive monograph, have reviewed the group and described the adult, puparial, and larval stages of the 26 Nearctic species, of which 15 are identified as new and 2 are recognized as Holarctic. They also provided a list of the avian hosts and notes on the biology and ecology of each species, when known.

Although bloodsucking by calliphorid larvae on birds has been known in Europe since 1845 and in North America since the turn of the century, no species has had its complete life cycle elucidated. Ornithologists have frequently reported the presence of these insects in birds' nests (especially those of cavity-nesting species) and have assumed that the nestling mortality they observed was the result of parasitism by *Protocalliphora*. Bennett (1957) in Ontario and Whitworth (1971, 1976) in Utah both studied the ecology and life cycles of a number of species of the genus, with these studies forming the basis of their respective doctoral dissertations. Although they failed to study the complete life cycle of any single species, their work represented the most complete and extensive research on the topic and served as models for several smaller studies, such as those reported by Gold and Dahlsten (1984, 1989).

The Bennett and Whitworth studies were not previously published, as they dealt, in great part, with new species which had not, at that time, been described and named. The publication of the Sabrosky et al. (1989) monograph has now cleared the way to present, herein, these studies on the life history of some species of *Protocalliphora*.

Materials and methods

The field studies were carried out in the vicinity of the Wildlife Research Station on Lake Sasagewan, Algonquin Park, Ontario, Canada, during the years 1949–1956, and near Logan, Utah, during the period 1970–1975. In Algonquin Park, Bennett carried out studies on

Protocalliphora aenea, P. avium, P. bicolor, P. metallica, P. shannoni, and the eastern form of *P. sialia*, and Whitworth studied *P. asiovora, P. chrysorrhoea, P. interrupta, P. parorum,* and the western form of *P. sialia*.

In Algonquin Park, larvae of *Protocalliphora* were collected from the nests of a variety of species of birds, the majority from the nests of barn swallows (*Hirundo erythrogaster*), common grackles (*Quiscalus versicolor*), and robins (*Turdus migratorius*). To facilitate studies, nestlings of these species were often placed in substitute nests made from large tin cans filled with appropriate nesting material, and such substitute nests were moved as much as 2 m from the original nest site. The same group of nestlings were transferred as many as six times, the substitute nests being examined for *P. larvae* on each substitution. The parent birds still tended their young despite these disturbances. This technique could not be used, however, on the ground-nesting sparrows and warblers which would desert their young if disturbed early in the nestling cycle and (or) the young would leave the nest if disturbed at a later stage.

In the Utah study, most detailed observations of nests and nestlings were made on black-billed magpies (*Pica pica*), bank swallows (*Riparia riparia*), and starlings (*Sturnus vulgaris*). These birds had nests that were heavily infested and easy to examine on a regular basis, and the parents tolerated nest disturbances very well. In some cases, larvae were reared on nestlings brought into the laboratory. However, this was a difficult and somewhat frustrating process, as the nestlings required frequent feeding and the younger birds were vulnerable to hypothermia.

Larval *Protocalliphora* were reared by either placing known numbers in a substitute nest in the field and recovering them at specific times thereafter or (more conveniently) by placing them in artificial nests at the field station and using young crows or robins as the blood meal source. The daily increase in length of the larvae was used as the criterion of growth and was measured by allowing the larva to crawl across a sheet of paper, marking the position of the anterior end when the posterior end crossed a mark on the paper; three to five such lengths were averaged. Puparia were maintained in small vials or bottles in dry sawdust covered with a fine mesh gauze. Containers with puparia were placed in a Stevenson screen where they encountered the ambient temperature and humidity conditions in Algonquin Park; other containers of puparia were maintained in desiccator jars at saturation humidity or lower humidities obtained by use of calcium chloride, and in incubators or refrigerators to obtain a broader range of temperatures to study duration of metamorphosis. Puparia in the Utah study were maintained at 22.2 ± 1.5°C at a relative humidity of 35–38% and a photoperiod of 15 h L : 9 h D. Adult flies were kept in 20 cm cube wooden cages, each with a glass top, one side of fine nylon mesh, and another side with a cloth sleeve to permit access.

The only species of *Protocalliphora* common to both the Ontario and Utah studies was *P. sialia*. Sabrosky et al. (1989) recognized that there were differences between the eastern and western *P. sialia*, but there was insufficient consistency of differences to postulate subspecific status. However, there also appear to be minor differences in the life cycle between the eastern and western forms, and throughout the present study, when similar experiments were carried out on both eastern and western material, the two groups are kept separate. In the absence of the designation "western," it can be assumed that the work was done on the "eastern" material of *P. sialia*.

Results and discussion

Larval and prepupal stage

Development

The larval stage of *Protocalliphora* is composed of two distinct types of larvae, i.e., the younger actively feeding forms and the older postfeeding forms.

The rate of larval development for nine species of *Protocalliphora* (Table 1) studied was determined by noting the time required for first-instar larvae to pupariate. Young larvae were obtained from nests in the field; the exact ages of these larvae were unknown, but by comparing their size with that of newly hatched larvae or freshly moulted larvae in the laboratory, age estimates were made that are believed to be accurate within 24 h. The time for development of the first-instar larvae is known approximately from observations on a substitute nest of a robin that contained 27 first-instar larvae of *P. sialia* 36 h after the nest had been substituted; these larvae passed through the first larval moult about 12 h later, indicating that development of the first-instar larvae of this species is completed in 48 h or less. There was little difference between the sizes of the first-instar larvae of nine species described by Sabrosky et al. (1989), and it is assumed that all six species studied herein had similar developmental periods for the first instar. The duration of the prepupal period (Table 2) at 35.5°C (a temperature a little lower than that of an occupied bird nest) added to the time required for larval development gives the approximate time elapsed between egg hatch and puparium formation. The data (Table 1) indicate that those species of *Protocalliphora* with large larvae have a longer developmental period than species with smaller larvae. *Protocalliphora chrysorrhoea*, with the largest larvae (16–17 mm) of those studied, had the longest developmental period, whereas *P. asiovora, P. avium, P. parorum,* and *P. sialia* (each somewhat smaller at 13–15 mm) had shorter rates of development; *P. interrupta*, by far the smallest species studied at 7–9 mm, had the shortest developmental period.

TABLE 1. Duration (days) of the larval cycle of nine species of *Protocalliphora*

	No. of larvae	Larval stage	Postfeeding stage	Total time
P. aenea	32	7–8	3–4	10–12
P. asiovora	27	8–9	1–2	9–11
P. avium	250	11–12	2–3	13–15
P. chrysorrhoea	35	9–10	3–4	12–14
P. interrupta	25	5–6	1–2	6–8
P. metallica	36	7–8	2–3	9–11
P. parorum	20	6–7	3–4	9–11
P. shannoni	17	9–10	1–2	10–12
P. sialia (eastern)	81	8–9	3–4	11–13
P. sialia (western)	18	7–8	2–3	9–11

The duration of the larval period of *Protocalliphora* may also be related to the size and (or) duration of the nestling period of the host. Larvae of *P. chrysorrhoea*, with the longest developmental period, occur in bank swallow nests where the nestlings have at least a 21-day nestling period. Larvae of *P. avium* and *P. asiovora*, which also have an extended developmental period (Table 1), normally occur in the nests of large birds (crows, magpies, hawks) whose nestlings remain for 25–45 days in the nest. Larvae of *P. aenea, P. parorum, P. shannoni,* and *P. sialia* usually occur in the nests of birds whose nestlings require only 14–21 days in the nest. The young of these birds remain longer in the nest than do the young of ground-nesting warblers and sparrows, whose nests are usually infested with *P. metallica* (in eastern North America), and of the yellow-headed blackbird (usually infested with *P. interrupta*), whose young fledge in 10 days. If the larval development of *P. metallica* and *P. interrupta* were as slow as that of *P. avium* and *P. chrysorrhoea* (Table 1), the larval development would frequently not be completed, even if oviposition occurred on the days the nestlings hatched. As it is, the development of larvae of these two species is barely rapid enough to permit completion of the larval stage in

TABLE 2. Duration of the postfeeding larval stage of seven species of *Protocalliphora* held at various temperatures and nearly saturated humidity

	Temp. (°C)	Time to pupate (h) Mean	Median	Range	No. of larvae
P. aenea	18±6	73	72	36–144	36
P. avium	35±3	42	48	19–72	18
	18±6	80	84	48–108	22
	13±1	303	312	168–456	24
P. bicolor	35±3	24	24	3–84	19
	18±6	96	84	20–228	19
	13±1	136	132	36–372	23
P. cuprina	22±2	552	528	480–672	15
P. metallica	35±3	36	24	18–84	29
	18±6	122	108	84–240	25
	13±1	327	336	156–432	21
P. shannoni	35±3	30	48	24–48	33
	18±6	45	48	24–84	8
P. sialia (eastern)	35±3	58	54	6–78	20
	18±6	102	93	32–129	18
	13±1	355	328	270–533	14
	4.5±1	700	700	650–810	7

TABLE 3. Positions taken by third-instar larvae of two species of *Protocalliphora* 24 h after being placed on the top of a 30-cm column of dry sawdust

Distance from surface (cm)	*P. sialia* Small, fed	Large, fed	Unfed	*P. shannoni*, unfed
0–2.5	0	20	17	7
2.5–5.0	5	47	10	7
5.0–7.5	2	23	2	2
7.5–10.0	2	18	0	0
10.0–12.5	1	19	1	0
12.5–25.0	7	28	0	0
25.0–30.0	0	43	0	0
Total	17	198	30	16

the nests of their hosts before the young are fledged. The presence of many undersized puparia in these nests, presumably formed by larvae that had insufficient time to feed to larval maturity, supports this conclusion.

Prepupal stage

The postfeeding larva is identical to the third-instar larva in appearance, but the intestinal tract is empty and the larva is a uniform grey or cream colour. Conditions initiating the prepupal stage are not necessarily the same for all larvae. Although placed in nests with young birds, small third-instar larvae of one-half to two-thirds normal size did not feed again once the intestinal tract was empty but passed directly into the postfeeding larval phase. The emptying of the intestine appeared to be the triggering mechanism in these cases. On the other hand, full-grown third-instar larvae did not feed after the third or fourth blood meal, even in the presence of young birds, suggesting that some other factor, perhaps size, was the triggering mechanism for these maggots.

The larval stage is passed with the young birds in the nest, and the maggots experience a temperature of 39–40°C (as measured in nests containing nestling grackles, barn swallows, robins, phoebes, and redwinged blackbirds in Algonquin Park). The postfeeding larval stage, however, is frequently passed after the young have fledged, and these larvae are subject to ambient temperatures and humidity. The effect of temperature on the duration of the postfeeding larval stage was measured for a number of species of *Protocalliphora.* Larvae were fed on young crows in an artificial nest or collected directly from nests in the field and held until fresh (red) blood was no longer visible in the crop. Larvae were placed at different temperatures in a nearly saturated humidity until puparia were formed (Table 2). Most species formed a puparium within 1–2 days at 35 ± 2.5°C in an incubator. This temperature is a little lower than would be experienced in a nest containing young birds. The average outdoor ambient temperature during the experiment was 17 ± 5°C and represents the temperatures the larvae would normally encounter after the young had fledged. Temperatures of 4.5 ± 1°C and 13 ± 5°C obtained in a refrigerator and a cellar are much below those normally encountered in Algonquin Park or Logan at this time of year. Pupation was markedly delayed at lower temperatures, and it was anticipated that under ambient outdoor temperature conditions the prepupal stage would be completed in 3–4 days. *Protocalliphora cuprina*, however, was an exception, for this species had a prolonged prepupal period; at 22 ± 2°C, the larvae required 480–672 h to pupariate.

Behaviour of larvae

All larval instars of *Protocalliphora* were strongly photonegative, reacting to light in a manner similar to that of other calliphorids. However, numerous observations indicated that the thigmotactic stimulus was also of importance and would override the photonegative response. Larvae placed in a glass container with a sheet of paper on the bottom always moved beneath the paper, whether or not the container was placed over a light. Larvae placed in a square glass container moved to the corners, where they became quiescent. If two glass plates were held at an angle that was directed into the beam of a 100-W light, the larvae touched on the dorsal and ventral surfaces moved into the angle and into the light.

The geotropic responses of larvae of *Protocalliphora* were determined by placing small third-instar (both fed and unfed), large, engorged third-instar, and prepupal stage larvae on the top of a 30 × 5 × 5 cm hinged, hollow wooden column that was filled with dry sawdust. The column was opened after 24 h and the position of the larvae noted (Table 3). The majority of the unfed larvae of *P. sialia* and *P. shannoni* were found within the top 5 cm, whereas the fed larvae of *P. sialia* were more randomly distributed throughout the column. In another experiment, larvae were placed at the bottom rather than at the top of the column and their positions noted 24 h later (Table 4). Most of the second-instar and unfed third-instar larvae of both species had migrated up the column to the top 5 cm. The second-instar and large third-instar larvae that had not moved from the bottom of the column were blood filled. It appears that *Protocalliphora* larvae, while feeding and (or) in an active growth condition, were negatively geotropic when the intestine was empty, and possibly this negative geotropism functions to keep the larvae at the top of the nest material and in close contact with the nestling. Engorged larvae, on the other hand, were relatively inactive and did not show a marked geotropism (Table 4).

Postfeeding larvae might be expected to show a marked positive geotropism, as most calliphorids burrow before forming a puparium (Hall 1948). The data (Table 5) indicated, how-

TABLE 4. Positions taken by larvae of two species of *Protocalliphora* 24 h after being placed at the bottom of a 30-cm column of dry sawdust

Distance from surface (cm)	*P. metallica*		*P. sialia*	
	No. of larvae	Description	No. of larvae	Description
0–5.0	4	Second instar	20	Unfed, small third instar
			12	Second instar
5.0–10.0	0		0	
10.0–15.0	0		2	Small, fed third instar
15.0–20.0	1	Large, fed third instar	2	Small, fed third instar
20.0–25.0	1	Large, fed third instar	6	Large, fed third instar
			5	Fed second instar
25.0–30.0	12	Large, fed third instar	0	
Total larvae	18		47	

TABLE 5. Positions taken by postfeeding larvae of five species of *Protocalliphora* 24 h after being placed on the surface of a 30-cm column of dry sawdust

Distance from surface (cm)	*P. avium*	*P. fallisi*	*P. metallica*	*P. shannoni*	*P. sialia*
0–2.5	2	7	12	21	10
2.5–5.0	9	6	70	12	7
5.0–7.5	27	2	46	8	7
7.5–10.0	33	0	19	4	11
10.0–12.5	25	0	10	0	13
12.5–15.0	13	0	4	2	6
15.0–17.5	9	0	2	0	10
17.5–30.0	82	0	8	0	57
Total larvae	190	15	171	47	121

ever, that none of the postfeeding larvae of any species showed what might be termed a pronounced positive geotropism. Although larvae of all species burrowed to some extent, larvae of *P. avium* and *P. sialia* were almost equally distributed throughout the column, whereas larvae of *P. fallisi, P. metallica,* and *P. shannoni* were grouped near the top. Presumably, the larvae pupariate anywhere they happen to be in the nest.

Larval feeding habits

The existing literature indicates that larval *Protocalliphora* spend most of their time in the nest (except *P. braueri*) and occur on nestlings only when feeding. However, observations on *P. asiovora, P. avium, P. chrysorrhoea*, and western *P. sialia* indicate that some larvae spend much of their developmental period on the nestlings themselves. The larvae of all four species have been observed in auditory and nasal cavities of nestlings, and their presence is often indicated only by scab-like accumulations of larval excreta around the cavity openings. In heavily infested nests, the young often have many small larvae attached along the rear edge of their wings, where they are usually wedged between the feather sheaths or between the folds of skin on the ventral side of the antebrachium (forearm). Since very young birds do not vigorously flap their wings (although they may shake or stretch them slightly), these larvae are apparently quite secure and have been observed to remain in the same position for several days. As the nestlings mature and start to flap their wings more vigorously, especially at feeding, this location becomes less secure, although smaller larvae can maintain position under these conditions. It is quite possible that larval survival is enhanced by their finding a secure location on the nestling and obtaining several blood meals before returning to the nest proper.

Older nestlings begin standing in the nest and are, therefore, less "available" to the larvae. At this time, larval feeding is concentrated on the nestling's lower, naked abdomen, which becomes covered with scabs in heavily infested nests. Heavy feeding is usually less evident in younger nestling birds because of the more scattered distribution of the feeding spots. In addition, although the blood wells up after a larva stops feeding, the resultant scab soon flakes off, leaving little evidence of bloodsucking. Only when repeated feeding in the same site occurs do the feeding spots become conspicuous. Feeding spots casused by *Carnus hemapterus* (Diptera: Carnidae) in magpies, starlings, kestrels, and woodpeckers may also be conspicuous in heavily infested nestlings and can be confused with those of *Protocalliphora* (Capelle and Whitworth 1973). Feeding by *C. hemapterus* is usually most concentrated under the wings, where the whole undersurface may become covered with dry blood.

It was difficult to watch the larval feeding process because of the strong photonegative response of the maggots. Nevertheless, four larvae were observed feeding on a young bronze grackle. The four larvae placed their posterior ends against the wing and the cephalic annuli against the side of the bird. The cephalo-

TABLE 6. Effect of temperature and humidity on the rate of metamorphosis

	Mean temp. (°C)	No. of puparia	Days (±2) to complete development at: saturation	10–15% RH	ambient (50–80%) RH
P. aenea	15.5±2	35	21.5	25.0	21.5
	13±2	40	34.0		35.5
P. asiovora	22±2	32			10.5*
P. avium	20±5	85	15.0	15.0	15.5
	16±2	79	21.0	20.0	20.0
P. bicolor	15±2	21	21.5	22.0	23.0
P. chrysorrhoea	22±2	35			10.5*
P. interrupta	22±2	25			9.5*
P. metallica	15.5±2	45	22.0	22.5	20.0
P. parorum	22±2	20			12.5*
P. shannoni	17.0±2	30	18.0	17.5	16.5
P. sialia (eastern)	19.0±5	75	20.0	19.0	19.5
	17.0±2	78	20.0	20.0	20.5
P. sialia (western)	22±2	20			14.5*

*These puparia were maintained at a relative humidity of 35–38%.

pharyngeal mechanism then rasped a small hole in the skin and then the anterior end was briefly withdrawn while the larva maintained position by use of the ventral pads and pressure with the posterior end. The prothoracic segment was then contracted to form a narrow cylinder that was thrust into the hole in the skin; the segment was then expanded, which presumably set the spines of the prothoracic fringe into the flesh of the wound. At the conclusion of this action, the larvae were firmly anchored to the bird. The cutting, rasping, and resetting of the prothoracic fringe continued until blood flowed from the wound. Then the rasping ceased and the muscular pharynx pumped the blood into the crop. After a brief period, the blood clotted in the wound and the rasping resumed to start a fresh flow; apparently no anticoagulant was used by the larvae, and the blood flow was maintained by mechanical means. One larva required 35 min to penetrate the skin and fill the crop to about half of capacity. The remaining three larvae fed for 40–45 min, but their crops were not as full as those seen in larvae that had been in a bird nest overnight. Presumably the larvae normally need at least an hour to fully engorge. In the nest, larvae presumably feed on the most accessible portions of the bird, i.e., feet, legs, and belly. Similar observations were made on four larvae of *P. avium* which fed between the fingers of one of us.

Puparial stage

The puparia of *Protocalliphora* are the most commonly encountered of the stages because nests are usually collected after the young birds have fledged. Puparia are usually found within the nest, their position depending on the nest structure. In solid mud nests, the puparia are usually found in the bottom of the cup, the larvae frequently passing through the mud and pupariating against the nest support. In nests such as those of robins, however, the puparia are frequently found on the lip of the nest or in crevices on the exterior of the nest. Presumably some larvae crawling on the external surface fall off and pupariate in the ground under the nest. In bulky nests, such as those of crows, magpies, and hawks, puparia may be found anywhere deep in the mass of fibres, sticks, and mud that usually compose such nests. Larvae from loosely woven or flimsy nests, such as those of catbirds, mourning doves, or some of the warblers and flycatchers, frequently burrow through the nest and pupate in the ground under the nest, even though the nest may be some height above the ground. Neff (1945) made numerous observations on *Protocalliphora* in dove nests, and although many were infested with larvae while the young birds were present, he never found a puparium in such nests. Larvae infesting nests of chickadees, wrens, goldfinches, and some warbler species often wound themselves up in the nest material, forming a sort of "coccoon." Larvae of *P. aenea, P. halli, P. hirundo, P. parorum*, and *P. sialia* often encase themselves in the downy feathers frequently found in tree and barn swallow nests. *Protocalliphora hirundo* larvae had the unique habit when pupating in cliff swallow nests of burrowing into the hard-baked mud and pupating against the nest support. The puparia were so firmly embedded in the mud they could not be easily removed without damage to them. It appeared that the larvae must have secreted–excreted moisture to soften the mud sufficiently to permit their penetration. Puparia in the nests of sparrows and other ground-nesting birds can be found in the soil up to the 3 in. (1 in. = 25.4 mm) below and around the nest. Presumably such behaviour affords the puparium a modicum of protection against predators and parasites. The puparium is initially cream coloured and soft to the touch. It changes colour slowly to a dusty cream, reddish cream, light red to orange–red, and finally to a deep brown or black, when the cuticle has hardened. The whole process of puparium formation requires about 24 h at 18°C.

The effect of temperature on the rate of metamorphosis of species of *Protocalliphora* was tested by placing puparia at different temperatures and noting the times of pupation and their eclosion to produce adults. The duration of the puparial period was computed separately for males and females, but as the time for development did not differ, these values were combined (Table 6). Generally, the puparial stage was completed in a shorter period at high temperatures than at lower ones. However, the highest of the high temperatures were not usually encountered in the environment unless the nestlings were still utilizing the nest. Since most puparia form and develop after the young have been fledged, development essentially takes place at the ambient environmental temperature about the vacated nest. Therefore, the rates of development at the average ambient environmental temperatures of 15–17°C (Algonquin Park) or 21–24°C (Utah) are particularly significant (Table 6) and show that adult flies can emerge in 14–21 days. At 13°C, which is lower than would be experienced continuously under natural

TABLE 7. Survival of pupae of six species of *Protocalliphora* maintained at different relative humidities but at the same temperature (15.5 ± 2°C)

	Ambient outdoors (50–80% RH)		Desiccator (10–15% RH)		Desiccator (saturation)	
	Total	% dead	Total	% dead	Total	% dead
P. aenea	124	29	54	13	81	44
P. avium	203	21	154	10	409	37
P. bicolor	30	16	4	0	6	0
P. metallica	118	20	54	17	11	36
P. shannoni	15	7	16	0	55	40
P. sialia	80	25	45	36	55	40

conditions, development of all species, especially *P. avium*, was markedly delayed. At an average temperature of 7°C, *P. sialia* required 50 days to complete metamorphosis and, furthermore, adults emerged from only 5 of 50 (10%) puparia. Since the survival of the puparia at this low temperature was so poor, and viable puparia were never found during the fall and winter months, it was concluded that species of *Protocalliphora* do not overwinter in the puparial stage. Only minor differences in the duration of the puparial stage were noted among the different species, and it appeared that development was consistent across the genus when the puparia were maintained at the same temperatures. The puparial stage of *Protocalliphora* is of longer duration than quoted by Hall (1948) for any other species of calliphorid.

Puparia of *Protocalliphora* are subjected to a wide variety of humidities, as the nests in which they occur are alternately saturated with rain and dried by sun and (or) wind; puparia in exposed nests are subjected to wider fluctuations than those occurring in sheltered sites such as cavities or in buildings. The effects of extremes of humidity on the survival and rate of development of the puparia of different species were investigated by maintaining puparia in screened vials in desiccator jars under ambient temperature conditions. One desiccator contained wet sawdust that maintained a relative humidity close to saturation and the other contained anhydrous calcium chloride, which maintained a relative humidity of 10–15%, considerably drier than the 50–80% range of humidity encountered in Algonquin Park during the course of the experiment. There was no difference in the rate of development of the adults due to humidity (Table 6). However, with the exception of *P. bicolor*, all species had a higher mortality under conditions of saturated humidity than under the ambient and low humidities (Table 7).

Adults

Preliminary observations suggested that adult *Protocalliphora* were long-lived. To determine their longevity, adult flies of four species (Table 8) were maintained in wooden cages and fed on a diet of a mixture of brown and white sugar, powdered whole milk, and bacto-yeast extract, suspended in cheesecloth bags. The cages were sprayed with water daily. The flies were maintained in two cage arrangements, viz. (*i*) a mixture of both sexes in the same cage, and (*ii*) the sexes maintained in separate cages. All the species of *Protocalliphora* studied were long-lived, the females living longer than the males (Table 8). The ovaries were not developed in most females, which may have contributed to their longevity. Males lived for an average of 28 days or more, longer than reported for most other calliphorids (Hall 1948).

The longevity of *P. avium* (Table 8), as shown by the maximum survival, is especially significant. This species usually occurs in the nest of large birds that nest only once in a year, and therefore the single generation of the parasite must survive until the following year. The adult *P. avium* were maintained in the laboratory for over half of their lives at an average temperature of 23°C and a relative humidity of 35 ± 10%, abnormally harsh conditions compared with the natural environment. Conceivably, in nature, the flies would hibernate during the months of November and April inclusive and thus readily survive for a year. The observed longevity strongly supports the hypothesis that it is the adult stage of the *Protocalliphora* that overwinters.

To confirm this hypothesis, about 50 adults of *P. sialia* (western) collected as puparia on July 23, 1972, were retained in a cage out-of-doors until October 28. Several females were dissected during this interval, but none were gravid. On October 28, about 15 adults were still alive, and sawdust was placed in the cage to serve as some protection from the elements. This cage was left outside, fully exposed to the elements, through the winter of 1972–1973, when temperatures dropped to −29°C. On March 8, 1973, when the high was 7°C, three adult *Protocalliphora* (two males, one female) were seen moving about within the cage. Dissection of the female showed that no ovarial development had occurred. The study clearly demonstrated that adult *Protocalliphora* can overwinter.

Adult feeding habits

As *Protocalliphora* were so closely similar in many respects to other calliphorids, it was ancitipated that their food and feeding behaviour would also be similar. Adult flies were offered a variety of fresh and decaying meats, including blood, liver, and whole carcasses as well as meat extracts. However, no flies were observed to be attracted to even investigate these different materials. Since most of these flies had been previously supported on a sugar – powdered milk diet, it was possible they were already sufficiently fed and thus not interested in further feeding. This possibility was tested by offering newly emerged, unfed *P. avium* and *P. sialia* the same foods. No flies were attracted to the carrion (fresh or decayed), and all flies were dead in 7 days. It was quite apparent that adult *Protocalliphora* were not carrion feeders. However, K. J. Capelle (personal communication) caught a number of *P. interrupta* in bait traps in Utah using carrion; most of these flies were female.

Further attempts were made to discover the natural foods of these flies. Seven groups of 25 *P. sialia* were placed in small, screened, wooden cages and provided with water. These flies, taken at random from a large cage where they had been supported on a composite sugar – powdered milk diet, were approxi-

TABLE 8. Longevity (±2 days) of adults of four species of *Protocalliphora*

	P. aenea	*P. avium*	*P. metallica*	*P. sialia*
Total flies (♂ and ♀)	264	329	112	157
Mean longevity	56	77	84	76
Maximum longevity	215	281	173	170
Median longevity	49	84	85	78
Males only (no. of flies)	23	84	10	36
Mean longevity	28	56	10	69
Maximum longevity	51	195	51	128
Median longevity	20	39	8	78
Females only (no. of flies)	42	115	34	46
Mean longevity	75	83	80	76
Maximum longevity	215	281	173	170
Median longevity	51	89	82	79

NOTE: For the first 40–50 days, flies were maintained outdoors in Algonquin Park at a mean temperature of 17.5°C (2–26.5°C) and a relative humidity of 40–95%. The balance of the period was passed under indoor city conditions at an average temperature of 23.5°C (19–35.5°C) and relative humidity of 25–45%.

TABLE 9. Survival of *P. sialia* fed on a variety of foods (water provided)

	Total no. of flies	Survival (days) Mean	Median	Max.
Sugar and powdered whole milk	22	68	83	125
Flowers (variety of Compositae)	21	35	47	62
Bird feces and nest debris	25	18	12	62
Powdered whole milk	24	28	17	62
Variety of native berries and fruits	24	49	55	80
Fresh and decomposing meat	25	19	17	50
No food	25	21	17	55

NOTE: Twenty-five *P. sialia* were placed in each of seven 20 cm cube cages at the start of the experiment. Several flies in some cages were killed by accident or by spiders. The flies were 40 days of age when the experiment was started.

mately 40 days of age. The composite sugar – powdered milk diet supplied in cage 1 (Table 9) maintained life for the longest period, and the flies were frequently observed feeding on it. The flies feeding on flowers and berries survived longer than the flies in the remaining four cages. The flies particularly fed on blueberries (*Vaccinium* spp.) and sarsaparilla (*Aralia nudicaulis*); flowers visited most frequently were goldenrod (*Solidago* spp.) and *Hieracium* spp. The flies appeared to be feeding on the pollen. Species of *Protocalliphora* have been recorded five times on *Solidago* and once each on *Angelica* and *Heracleum maximum* (collection labels of the United States National Museum). D. M. Wood (personal communication, in a letter), who captured a number of *P. shannoni* on *Solidago* in Quebec in late August, reported that "... the flies seem to crawl right into the head of the flowers and are sometimes overlooked." Some flies survived for 62 days without further food, although food had been available for 40 days prior to the experiment. However, 33 adult *P. avium* and *P. sialia* were dead within a week when given only water following emergence, and it appears that the adults must feed shortly after emergence, following which they can survive without food for some time.

Mating

Species of *Protocalliphora* mated readily in captivity; mating would occur shortly after emergence as soon as the wings were dry and the flies had attained full coloration and hardening. Mating continued intermittently until the death of the flies some months later. The number of matings could be increased, especially on hot, dry days, by spraying the cages with water. Copulation was frequently preceded by what might be termed a mating dance. A male would approach a female and start to circle around her, walking stiffly on all legs. The female, if receptive, would raise the middle leg on the side closest to the male and extend the other middle leg when the male circled to that side. This activity occurred for 15–30 s after which copulation took place. Duration of copulation was variable and the longest observed time was 125 s. If the female did not raise her middle legs, presumably she was not receptive, for the male flew away without trying to copulate.

The viability of the spermatozoa was assessed by noting their motility in preparations made from the seminal receptacles of *P. avium*. The females may have been mated on several occasions, but the time of the last mating was known. Spermatozoa observed 119 days after insemination were apparently as motile as those examined 10 days after insemination, and possibly only a single insemination is required during the life of a female.

The possibility of the occurrence of hybrid *Protocalliphora* was suggested by the considerable numbers of species of the genus found in the relatively limited area of Algonquin Park. A variety of specific crosses were attempted. Ten males and 10 females (segregated from emergence from the puparium) of each of *P. aenea, P. avium, P. metallica, P. shannoni,* and *P. sialia* were used in each experiment. Reciprocal crossing was attempted with *aenea* × *sialia, aenea* × *metallica, aenea* × *shannoni, avium* × *metallica, avium* × *sialia*, and *shannoni* × *sialia*; each species was mated normally as a control. The flies were left together in 25 cm cube cages for 8 days and were under observation on several occasions each day. Water was sprayed into the cages on hot days. No cross matings were observed, although mating was frequent in the control cages. Once a male *P. metallica* started its mating dance when it approached a female *P. avium*, the female flew away in about 5 s. The different species segregated themselves in the cages by species, mixing only on the food. After 8 days, the seminal receptacles were examined for the presence of motile spermatozoa; none was seen in the cross-mating experiments but were present in all within-species crosses. Further evidence that these species, especially the closely similar *shannoni–sialia* group, are not hybrids is given by the studies of Boyes (1961) and Boyes and Brink (1965), who showed that there were significant differences in the chromosome structure of the following species of *Protocalliphora*: *avium, hirundo, metallica, shannoni,* and *sialia*.

Egg production

The production of eggs by most species of calliphorids is dependent on a balanced, protein-based diet (Hall 1948). Rasso and Fraenkel (1954) showed that on diets of sugar or proteins alone, *Phormia regina* did not produce eggs, but eggs were produced when the diet consisted of a mixture of powdered whole milk, liver extract, and brewer's yeast. In Algonquin Park, a similar diet was fed to various species of *Protocalliphora*, and eventually one or two females of each of *P. aenea, P. avium, P. metallica*, and *P. sialia* laid eggs. However, as the females were virgins, the eggs did not hatch. The eggs were laid 20–280 days after the females had emerged from the puparium. Females of most calliphorids lay eggs within 1 or 2 weeks of emergence (Hall 1948; Rasso and Fraenkel 1954) and die after 3–4 weeks. Most of these *Protocalliphora* females, except a single *avium* which was 280 days old when she laid eggs, lived for 40–60 days following oviposition. Fertile eggs were laid by a single *P. sialia* captured near the entrance of a bank swallow burrow. The fly laid 15 eggs on the wall of the container, which hatched within 24 h. The number of eggs laid by the reared *Protocalliphora* ranged from 14 by *P. metallica* to 22 by *P. aenea*. On dissection, 45 eggs were found in the ovaries of a *P. avium*.

Considerably more success was enjoyed at Logan, Utah, in obtaining gravid females and viable eggs. Most viable eggs were obtained from gravid, field-caught females that subsequently laid eggs in the laboratory. However, a single laboratory-reared *P. asiovora* laid viable eggs, but this result was never repeated. Eggs were obtained from field-caught *P. asiovora* and *P. chrysorrhoea*. A female *P. asiovora* was captured in the vicinity of a magpie nest and placed in a small, capped vial; within 1.5 h, she had laid 76 eggs, which were scattered randomly over the surface to which they were attached securely. The eggs were examined and breathed on at 24-h intervals, since it is known that the carbon dioxide in exhalation will stimulate the hatching of some Diptera such as the Cuterebridae. After 72 h, breathing on the eggs stimulated the hatching of most of them. Another female *P. asiovora* was captured in the field but failed to lay eggs; on dissection, 37 mature eggs were found in the ovaries.

A female *P. chrysorrhoea* was captured and placed in a plastic vial furnished with diluted honey and raw liver; it laid 50 eggs some 32 h later. Unlike those of *P. asiovora*, these eggs were laid in clumps, and some were inserted under the small watering vial placed with the fly. After 24 h, the eggs were exposed to the warmth of sunlight and breathed on, but they did not hatch. After a total of 38–43 h, the eggs hatched without any special stimulus. Dissection of the fly upon its death revealed an additional 25 developed eggs. These limited data indicate that the incubation period of the eggs is shorter for *P. chrysorrhoea* than for *P. asiovora*.

Three additional wild *P. chrysorrhoea* were captured and fed honey and raw liver. Although they lived for 5–7 days, none oviposited. Dissections of two flies yielded slightly over 100 mature eggs each, and 40 mature eggs were found in the third. Six additional *P. chrysorrhoea* were captured and dissected immediately; four had 77–104 developed eggs and two had undeveloped ova. A single female *P. hirundo* was captured but on dissection was found to have undeveloped eggs. These data suggest that *Protocalliphora* lay about 100 eggs at one time. On dissection it was found that each mature egg had three immature ova attached to it, suggesting that the maximum fecundity of the flies is about 400 eggs.

As it had been shown that adult *Protocalliphora* can overwinter, it was decided to test the effect of cold temperatures on ovarian development. Initially, adults obtained during midsummer were placed directly into a cooler at 7.8°C for 2 months after they had been fed and watered and mating had occurred. However, these flies suffered a high mortality, and the ovaries were undeveloped in the few females that did survive. The fact that the flies had not been acclimatized before their sudden cooling may have accounted for much of the mortality.

Generally, the studies in both Ontario and Utah failed to have reared, caged females produce viable eggs. Although dietary problems may be responsible, they were fed diets that had proved successful for other calliphorids. Possibly the failure to have females produce viable eggs is due to the lack of a specific environmental stimulus or behavioural cue. All of the studies completed to date have been carried out on adult flies kept in relatively small cages and exposed to a single environment. Perhaps if the flies were released into a much larger cage or holding area such as a greenhouse where environmental conditions might start to duplicate the field conditions, both ovarian development and oviposition would occur.

It is assumed that flies oviposit only in nests occupied by nestling birds. Some experimental nests of barn swallows, common grackles, house wrens, and robins were examined and replaced daily from the time the nestlings hatched until the nest was found to be infested with *Protocalliphora*. Eighteen nests of barn swallows were infested when the young were 5–7 days of age; six nests of common grackles were infested when the young were 5–6 days of age; one robin and two house wrens were infested when the young were 5–8 days old. These limited data suggest that the nestlings were attacked when they were one-quarter to one-third grown. However, oviposition must occur when the nestlings of warblers and ground-nesting sparrows are much younger if the *Protocalliphora* are to mature in the short nestling period that these birds have.

Reaction to light and temperature

All species of *Protocalliphora* are markedly photopositive, with activity increasing with light intensity and ceasing in the dark. At dusk, flies grouped together in three to four masses in the corners of their cages and remained there until the following morning when the masses eventually broke up. Even on a hot night (21–23°C), flies remained inactive unless a bright light was shone on them. On the other hand, flies were inactive on cold mornings (7–10°C), even though the light intensity was high. Activity was thus controlled by both light and temperature. The lower temperature threshold for adult fly activity was determined by placing cages containing 50 or more adults of *P. aenea, P. avium, P. metallica, P. shannoni*, and *P. sialia* outdoors in the shade of a tree on three clear, cloudless, and windless days. The temperature at the start of each experiment was 12°C or less. The air temperature in the cages was recorded every 10 min. Some *P. avium* and *P. shannoni* started to move sluggishly when the temperature reached 17°C, but none of them flew about the cage. At 18°C, some individuals of each of the species attempted to fly but fell to the floor of the cage. At 18.5°C, most of the flies flew about the cage, but most *P. aenea* did not fly until the temperature was 19–20°C. *Protocalliphora avium* and *P. shannoni* were active at the lowest temperatures.

The species of birds usually attacked by *P. avium* (crows, hawks) have young in the nest by the middle of May in Algonquin Park, and the robins and grackles, which are the frequent hosts of *P. shannoni*, have their first brood of young in the nest by the end of May. Thus, a lower threshold temperature may be of value to these two species of flies. On the other hand, *P. aenea* required the highest threshold temperature; this species normally attacks birds nesting in buildings or under highway bridges in Algonquin Park where the temperatures, through shelter from the wind and radiation from the sun, are 2–5°C warmer than normal external ambient temperatures. Furthermore, the usual hosts of *P. aenea* in Algonquin Park are barn swallows and phoebes, which do not have young in the nest until mid-June or later. The threshold temperature for activity of all these species of *Protocalliphora* was remarkably high and indicates an adaptation to a temperature that will maximize their potential to find and oviposit in bird nests. Unfortunately, similar studies were not carried out on the species of *Protocalliphora* that occurred in Utah, but it is assumed a similar pattern of adaptation to environmental conditions would be found.

The adults of most species of *Protocalliphora* are rarely seen in the field. In fact, in Algonquin Park, no more than two wild flies were observed in the field during the course of the study. In the Utah study, more success in observing wild flies was enjoyed. In particular, in bird species that are colonial and experience a high rate of infestation (e.g., bank and cliff swallows), adults can usually be observed around the colony. Since bank swallows were studied in depth, adult *P. chrysorrhoea* were frequently observed. During the day, some adults were usually seen resting on the vertical wall above the nest cavities. Early in the season, before adult *Protocalliphora* had developed in bank swallow nests, most of the flies around the colony were gravid females, and adult males were rarely encountered. Later in the season, newly emerged flies of both sexes were observed in abundance near the nests in the early morning. By midday, most of these flies had gone and only a few gravid females would be observed around the nests. Gravid females seemed to be most active when temperatures were above 27°C. Then they could be seen making short, hopping flights from one burrow to another. They would usually land near the edge of a nest cavity, hestitate, then walk or fly into the burrow. Adults were observed entering burrows on many occasions, but attemps to observe the activity of the flies in the nest with the aid of flashlight always failed, with the fly flying out immediately. On one occasion, an adult was observed in a nest, walking from one nestling to another while dipping its abdomen. Examination of the nestlings revealed eggs laid in small clumps and attached to the feathers. No eggs were found on the nest material.

Summary of the life cycle of Protocalliphora

The life cycle of species of *Protocalliphora* can be summarized as follows. The female flies oviposit from late May through July (Algonquin Park) and April through July (Utah). The eggs probably hatch within 38–72 h of being laid in or around an occupied nest. The larvae feed on the nestlings and pupate in 9–14 days, the adults emerging in 14–21 days at the normal ambient temperatures. A new generation can thus be produced in 23–38 days, which would allow some of these flies to oviposit in the second-brood nests of those birds that have more than a single breeding cycle in a summer and thus produce a second generation of flies in each year.

A viable puparium was never found in the period September–November in Algonquin Park, where the last of the nestling birds usually fledge from the nest no later than the last week of August. The time between this fledgling and the onset of winter temperatures is approximately 70 days, whereas the time required for metamorphosis at 7°C is about 50 days. Furthermore, only about 10% of the puparia of *P. sialia* produced adult flies at this low temperature, and no puparia of *P. aenea, P. avium, P. metallica,* or *P. shannoni* survived at continuous temperatures of 7°C. Pupae of *Protocalliphora* cannot tolerate low temperatures and obviously cannot overwinter in this stage.

It is difficult to accept the hypothesis that *Protocalliphora* produce eggs that overwinter. This method of overwintering would necessitate the adults ovipositing in nests that would be reused the following year. However, most birds do not reuse the old nest, and the chances of the larvae from such eggs finding suitable hosts would be virtually nonexistent. Experimentally, it has been demonstrated that *Protocalliphora* overwintered as adults, confirming Johnson's (1927) hypothesis that "... the females apparently hibernate, probably in hollow trees and other sheltered places, and in the spring, deposit their eggs in the nests of birds."

Possibly some adults flies from first-brood nests infest second-brood nests of the hosts of the same summer and then overwinter to infest nests the following spring. Adult *P. avium*, and possibly *P. fallisi*, survive for the entire year, since these parasites occur in the nests of birds that nest only once a year in May and early June. Adults from the second-brood nests of the hosts probably also overwinter and oviposit in the first-brood nests in the following spring, and if they live as long in nature as in the laboratory, they may also oviposit in the second-brood nests of that same year. Thus, the adults produced from both broods of birds in one year probably survive to oviposit in the first-brood nests of the following year, thus ensuring survival of the species. It must be noted, however, that there is no hard or fast demarcation in time between broods of birds, nor is there necessarily only two broods. Some robins in Algonquin Park, for example, will produce as many as four broods per year, although this is uncommon. This type of nesting with one species of bird followed in time by another species of bird leads to a continuous source of nestling food sources and a continuous emergence of *Protocalliphora* over the course of a summer. Adults produced by such a continuous emergence could survive to infest nests throughout the next year, with probably no more than two full generations per year from any given cohort of flies.

Parasites of Protocalliphora

Puparia of *Protocalliphora* are frequently parasitized by *Nasonia vitripennis* (Walker), a pteromalid wasp that attacks a wide variety of species of muscoid and calliphorid flies (Sabrosky et al. 1989). This formerly monotypic genus has been reviewed by Darling and Werren (1990), who now recognize two additional species (one in eastern and one in western North America), with two species able to coexist in the same puparium. In this present study, the term *N. vitripennis* is used in the broadest sense to include all three species.

Whitehead (1933) recorded that 92.7% of 121 puparia of *P. "splendida"* in a purple martin's nest at MacDonald College, Quebec, were parasitized by this wasp. Johnson (1927, 1930, 1932) recorded that 624 (36%) of 1735 puparia of *P. sialia* were parasitized by *N. vitripennis*. In the present study, puparia of *Protocalliphora* were also frequently parasitized by *N. vitripennis*. In a sample of 335 nests, 105 (29.5%) contained parasitized puparia (Table 10). The number of puparia parasitized per nest was extremely variable, ranging from 1 to 100%; in all,

TABLE 10. Hyperparasitism of species of *Protocalliphora* by the pteromalid wasp *N. vitripennis*

	P. aenea	*P. avium*	*P. hirundo*	*P. metallica*	*P. shannoni*	*P. sialia*	Total
			Brood 1 (before July 14)				
Total nests examined	63	10	22	62	38	72	267
Nests with hyperparasitism	8	5	4	7	8	30	62
% with hyperparasitism	12.7	50.0	18.2	11.3	21.1	41.7	23.2
Total puparia examined	3555	2807	739	886	484	2106	10577
Puparia hyperparasitized	156	340	77	126	65	482	1246
% puparia hyperparasitized	4.4	12.1	10.4	14.2	13.4	22.9	11.8
			Brood 2 (after July 14)				
Total nests examined	15		5	21	20	27	88
Nests with hyperparasitism	12		0	4	9	18	43
% with hyperparasitism	80.0		0	19.1	45.0	66.7	48.9
Total puparia examined	830		107	298	78	1128	2441
Puparia hyperparasitized	306		0	37	26	433	802
% puparia hyperparasitized	36.9		0	12.4	33.3	38.4	32.9

	Colonial nests	Noncolonial nests	Cavity nests
Total nests examined	269	86	29
Nests with hyperparasitism	86	19	21
% with hyperparasitism	31.9	22.1	72.4
Total puparia examined	10 595	2423	2344
Puparia hyperparasitized	1 740	308	519
% puparia hyperparasitized	16.4	12.7	22.1

nearly 16% of the 13 000 puparia involved were parasitized. It was noted that the wasps could overwinter as pupae within the puparium or emerge as adults in late August, with both conditions occurring in a single puparium. A puparium with one or more wasp exit holes was not necessarily empty of all the pupal parasites. Preliminary observations suggested that puparia obtained late in the summer were more frequently parasitized than those in the spring of the year. The data were reexamined with this time frame in mind, and the summer was arbitrarily divided into nests examined before July 14 (brood 1) and those examined after July 14 (brood 2).

The data (Table 10) were derived only from those nests which contained a single infestation of one of the six commonly occurring species of *Protocalliphora* in Algonquin Park. The percentage of nests and number of puparia attacked by *N. vitripennis* was nearly twice as great and three times the number in nests, respectively, after July 14 than before. As there were many more birds nesting before July 14 than after this date, the higher rate of brood 2 parasitism may merely reflect that there were fewer *Protocalliphora* available to be hyperparasitized. The proportion of the puparia attacked by *N. vitripennis* varied among the different species of *Protocalliphora*; *P. avium* and *P. sialia* were attacked most frequently, *P. metallica* the least. The rate of attack in the latter species did not differ between the two broods. Within the *P. avium* sample, 50% of the brood 1 nests contained parasitized puparia, but only 12% of the puparia in the nest were actually parasitized; 23% of the puparia in 40% of the brood 1 nests containing *P. sialia* were parasitized. Generally, *P. sialia* infests nests that can be considered as either cavity and (or) colonial nests, and the high rate of puparial parasitism of this species may reflect the nest density. A comparison of the rate of hyperparasitism in puparia in colonial and noncolonial nests (Table 10) indicated that puparia in each category were attacked at roughly the same rate but that more colonial nests showed evidence of hyperparasitism. Species of *Protocalliphora* in colonial cavity nests (especially those in stumps in marshes or lakes) were the most heavily hyperparasitized, with 75% of the puparia containing the pteromalid. Colonial and (or) cavity nesting birds frequently use the same nesting site year after year and brood after brood. Such behaviour enhances the probability of infestation by *Protocalliphora* and the survival of *N. vitripennis*, which is assured of a constant supply of host calliphorids on which to feed. Possibly this type of bird nest serves as a reservoir for this pteromalid wasp. If this is true, then the practice of cleaning out nesting boxes in the late fall or early spring of each year (Mason 1944) would limit the following year's supply of the hyperparasite and limit its value as a biological control agent.

Similar studies were not carried out on the Utah material. However, it was generally noted that the rate of hyperparasitism (primarily *N. vitripennis*) in nests prior to mid-June was lower than in nests after this time, a pattern completely reminiscent of that in Algonquin Park. Different species of *Protocalliphora* were attacked with greater or less frequency than others; *P. interrupta* was the most frequently parasitized species, and its major host, the yellow-headed blackbird, is colonial and tended to nest much later than other birds, usually in June–July. Possibly the hyperparasites had less choice of targets at this time and concentrated on the most readily available host species.

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