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THE GONOTROPHIC-AGE STRUCTURE OF A POPULATION
OF THE *SIMULIUM VENUSTUM* COMPLEX (DIPTERA: SIMULIIDAE)
IN ALGONQUIN PARK, ONTARIO

Stephen M. Smith¹ and Alan Hayton^{1,2}

ABSTRACT

Eight techniques for the determination of parity and gonotrophic age were assessed for the obligatorily anautogenous blackfly-species complex, *Simulium venustum* Say. All females could be age-graded by the presence or absence of dilatations on the ovarioles. However, multiple dilatations on a single ovariole were not found and the Polovodova method could not be used to determine the number of completed gonotrophic cycles. Most females could be age-graded by the appearance of the Malpighian tubules, which undergo morphological changes, probably as a result of a bloodmeal. In some cases, the size of the fat body, the presence of retained (relict), mature ova and the presence of meconium in the gut could be used as accessory age-grading criteria. Insemination status, the volume of the esophageal diverticulum, and the stage of development of the terminal ovarian follicles could not be used to age-grade females. The literature of age-grading in blackflies is reviewed, with special reference to the interpretability of the Polovodova method.

Seasonal changes in the gonotrophic-age structure of a population of the *S. venustum* complex in Algonquin Park, ON, Canada, were examined over two years. The maximal proportion of parous females in the population was 75 and 62% in the two years, respectively. There was weak evidence that parous females were more likely to host seek in the morning and nulliparous females in the afternoon. Parity declined in mid-season, due to the recruitment of newly emerged adults to the population.

Although *Simulium venustum* Say is among the most important blackfly pests in eastern North America, knowledge of the population dynamics of the adults is poor; only two studies (Davies 1963; Magnarelli and Cupp 1977) have explored the gonotrophic-age structure (*sensu* Klowden and Lea 1980). Knowledge of the age structure of adult populations can be useful in evaluating features of the physiology and life history (e.g. autogeny, calendar aging and life span, voltinism, vector potential), and age-structure data can also be used to assess the efficacy of suppression efforts.

A wide diversity of criteria have been used to assign female Diptera to gonotrophic-age categories (Detinova 1962, Anderson 1987), almost all based on techniques originally devised for mosquitoes. Many workers have at-

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tempted to age-grade blackflies using these tools, with strong biases toward techniques based solely on assessment of ovarioles. Anderson (1987) recommended that workers should routinely monitor a suite of characters in studies of aging in wild populations, a recommendation that may be particularly relevant now, given recent work that has cast serious doubt on the interpretability of the often-used Polovodova (1949) technique for the determination of parity in multiparous mosquitoes (Lange and Hoc 1981, Hoc and Charlwood 1990, Fox and Brust 1994, Sokolova 1994).

Here we evaluate a number of criteria for their utility in age-grading females of the *S. venustum* complex and apply them to the determination of the gonotrophic age structure of a population over two field seasons. We also take the opportunity to evaluate published studies of the gonotrophic age of blackflies in the light of the findings of the "New School" of gonotrophic aging in mosquitoes (Fox and Brust 1994).

METHODS AND MATERIALS

All studies were conducted at the Wildlife Research Station in Algonquin Park, Ontario, Canada, from May to August in 1974 and 1975. Female flies attracted to a human host (AH) were captured with a standard insect net at a single site adjacent to the North Madawaska River (45°36'N; 78°20'W; UTM: 17TPA937511). The collection site was in a sheltered clearing surrounded on three sides by forest in which the most common species were white pine (*Pinus strobus*), white birch (*Betula papyrifera*), white spruce (*Picea glauca*) and sugar maple (*Acer saccharum*), and on the side adjacent to the river by speckled alder (*Alnus rugosa*). In year 1, 15 females were collected 5 h after sunrise and 15 females 2 h before sunset at 7-d intervals. In year 2, 50–80 females were collected 2 h before sunset at 3–4-d intervals. Flies were stored on ice until they were dissected (within 4 h of collecting). Flies were identified according to Davies et al. 1961.

Females were dissected in a saline solution (Lum 1961) to which a small quantity of liquid detergent had been added. The abdominal cuticle was torn along the pleuron from the first abdominal segment to the genitalia and the viscera exposed. The volume of the ventral esophageal diverticulum ("crop") was assessed on an 3-level ordinal scale (0=empty, no visible fluid; 1=a small quantity of fluid present, crop wall wrinkled; 2=large quantity of fluid present, crop distended, surface unwrinkled). The quantity of abdominal fat also was judged on a 3-level ordinal scale ("small", "medium", and "large"). The color and morphology of the Malpighian tubules were recorded, as well as the color of any material in the gut. The ovaries were inspected for relict eggs.

The female reproductive tract was removed by severing the tracheal connections to the ovaries and oviducts and the spermathecal connections to the oviduct. The ovaries were gently teased apart with *minuten nadeln* and examined under a compound microscope; phase-contrast microscopy was used in year 2. The stage of development of the terminal follicles was scored according to the scheme in Watts and Smith (1978). The ovarioles were inspected for dilatations and, if present, the color of follicular relics was recorded. The spermatheca was examined for spermatozoa, also under compound microscopy; if sperm could not be seen emerging from the severed spermathecal duct, then the spermatheca was crushed.

Based on the results in year 1, flies in year 2 were scored only on the basis of the presence or absence of dilatations, relict eggs, and the morphology of the Malpighian tubules.

Statistical analysis. A between-year comparison of the stage of develop-

ment of the terminal ovarian follicles was made by an exact, 2-tailed Jonckheere-Terpstra (J_T) test of the 2×2 contingency table (Hollander and Wolfe 1973); this test is appropriate for contingency tables in which both the row and column classifications have a natural ordering. An exact, 2-tailed likelihood-ratio test (G) (Agresti 1990) was used to examine the frequency of follicular stages as a function of parity. All exact tests were computed with *Stat-Xact* (Mehta and Patel 1992). Asymptotic chi-squared tests were used to examine seasonal fluctuations in the parity rate of the population. The frequency distributions of relict eggs were tested for randomness by fitting Poisson and negative-binomial distributions. Goodness-of-fit of the Poisson distribution was assessed by a chi-square procedure, with classes pooled to yield expected frequencies ≥ 5 . The negative-binomial distribution was fitted by maximum likelihood (Bliss and Fisher 1953); goodness-of-fit was assessed by the statistics in Evans (1953). Both Poisson and negative-binomial models were fitted and evaluated in *MATLAB* (Matlab 1990). A log-linear model and the Mantel-Haenszel test, computed in *Systat* (Wilkinson 1990), were used to examine the hypothesis that the proportion of parous females was independent of time of day; the log-linear model was fit by backward selection. Exact confidence limits for proportions were computed in *Systat* (Wilkinson 1990) using the F distribution (Zar 1984). All hypothesis tests were 2-tailed and, for each, the maximal probability of a type-1 error was set at 0.05.

RESULTS

Ovariolar morphology. Parous females were easily diagnosed by the presence of distensions of the ovariolar intima that comprised three morphological types ($n=94$ females): (1) elongate, sacculate dilatations with pale, translucent follicular relics (51.1%); (2) small, nodular dilatations with yellow, optically dense, follicular relics (37.8%); (3) dilatations intermediate between (1) and (2) (above) (11.1%). In most females all ovariolar dilatations were of a single type, but occasionally females were found that had both sac-like and small dilatations. Multiple, nodular dilatations on individual ovarioles were not found.

Developmental stage of terminal follicles. Discrimination between follicular stages Ib and IIa in living ovarioles was not possible without phase-contrast microscopy; therefore, the results presented here relate to year 2 only. Follicular stage was independent of parity ($J_T=3.6 \times 10^4$; $p=0.15$; $n_{\text{nulli-par}}=400$; $n_{\text{par}}=194$); 18.9% of females were at stage Ib and 79.8% were at stage IIa. A small number (1.3%) of nulliparous females, all with traces of blood in the midgut, had follicles at stage IIIa.

Relict eggs. Apparently normal, chorionated eggs were not uncommon in females diagnosed as parous by the presence of ovariolar dilatations; 14.5% ($n=117$) of the parous females in year 1 had from 1 to 12 retained eggs and 11.6% ($n=481$) of the parous females in year 2 had from 1 to 27 relict eggs (Fig. 1); the proportion of parous females with relict eggs was independent of year ($G=0.70$, $p=0.43$). Parous females with relict eggs appeared soon after the first appearance of parous females in the population (Fig. 2, 3). The proportion of parous females with relict eggs and the mean number of relict eggs per parous female increased as the population aged, but the rates of increase were not uniform (Fig. 2). In both years, the mean number of relict eggs per parous female remained relatively constant during June and then abruptly increased in mid-July.

In both years, the frequency distribution of relict eggs in parous females was highly non-random ($p < 0.0001$), fitting a negative-binomial distribution

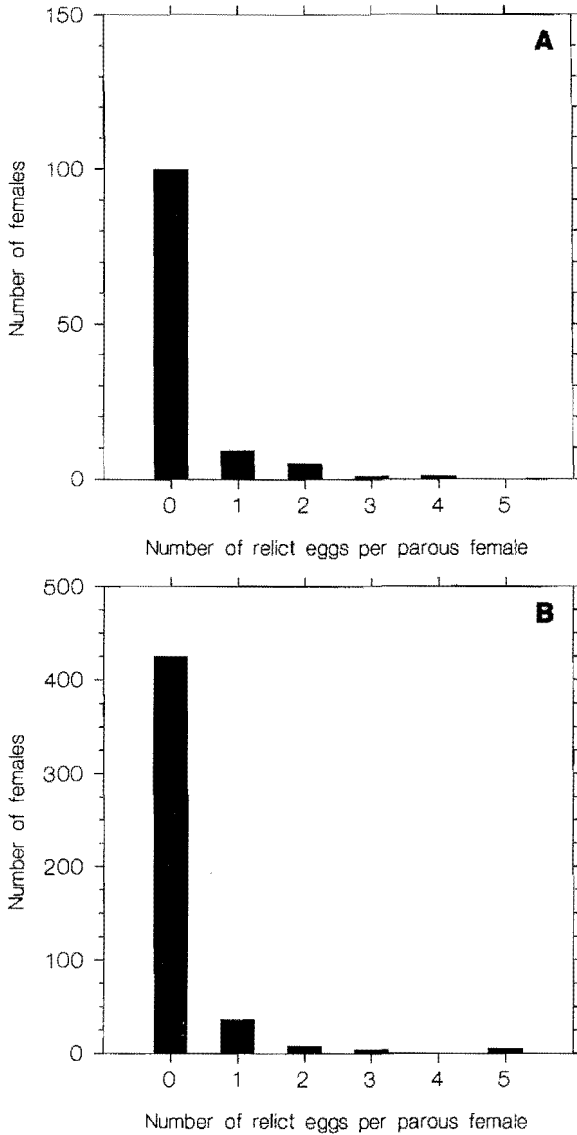


Figure 1. Frequency distribution of the number of relict eggs in parous females of *Simulium venustum* in year 1 (A; n=117) and year 2 (B; n=481). One female in year 1 with 12 relict eggs and one female in year 2 with 27 relict eggs have been omitted.

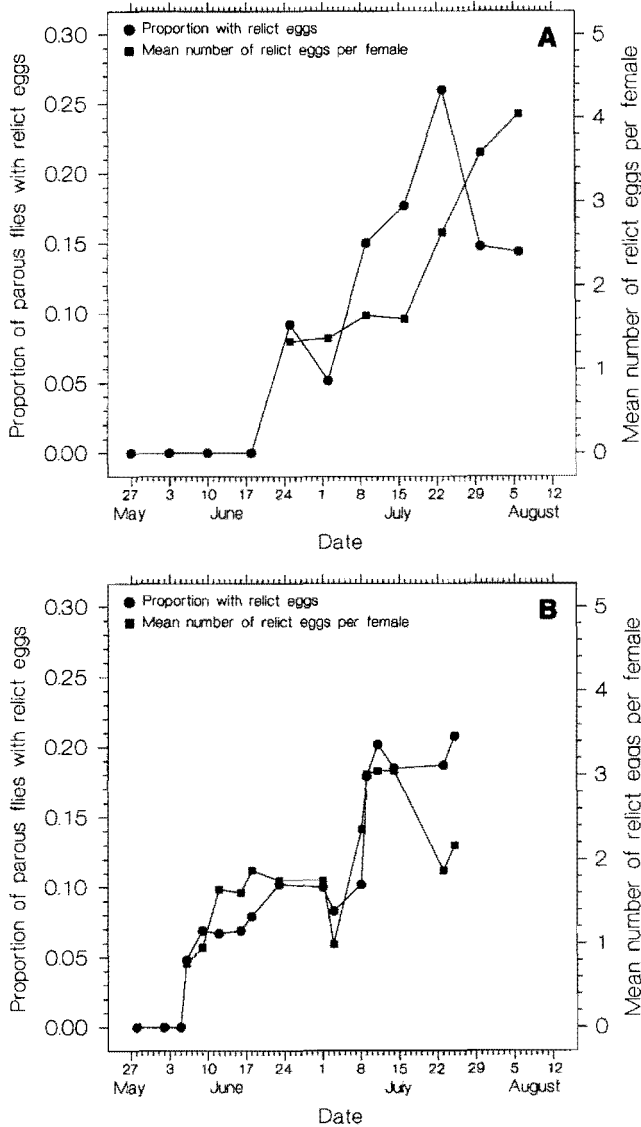


Figure 2. Seasonal change in the proportion of parous females of *Simulium venustum* with relict eggs and the mean number of relict eggs per parous female in year 1 (A) and year 2 (B).

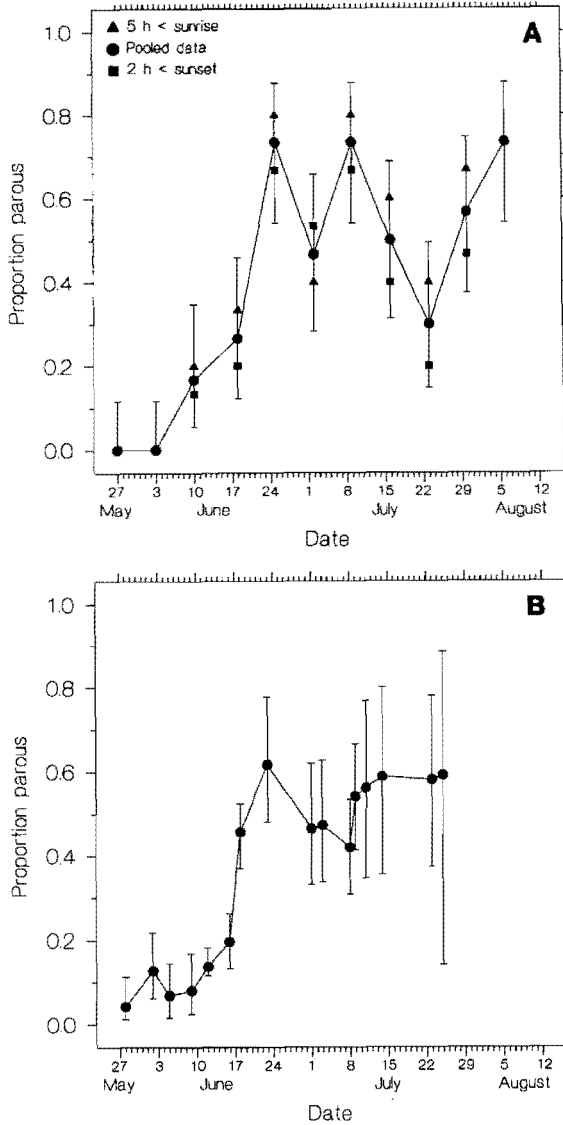


Figure 3. Seasonal change ($\pm 95\%$ confidence limits) in the proportion of parous females in samples of *Simulium venustum* attracted to man in year 1 (A) (sample n=30) and year 2 (B) (n=50-80).

but with values of k_3 approaching zero (i.e. approaching a logarithmic series) (year 1: $k_3=0.12\pm 0.044$, $U=0.038\pm 0.37$; year 2: $k_3=0.11\pm 0.022$, $U=0.053\pm 0.11$).

Malpighian tubules. In recently emerged flies the Malpighian tubules were densely packed with opaque material and individual cells were swollen, giving the tubules a coarse, moniliform appearance. The tubules in young flies were bicolored, with the distal portion (free end) light brown and the proximal three-quarters yellow-brown to orange-brown. In parous flies the tubules were no longer densely packed with opaque material and they no longer had a moniliform appearance. The little remaining opaque material was red to red-brown and transparent sections were visible in the tubules. In some cases, especially late in the season, the amount of opaque material in the tubules decreased even more, so that large sections of the tubules were transparent.

From late May, when adult females of *S. venustum* first appeared, until the end of June, most females could be readily age-graded as parous or nulliparous based solely on the appearance of the tubules. A small number of nulliparous females misdiagnosed as parous on the basis of the tubules showed evidence of having taken a partial bloodmeal—there were small quantities of blood residue in the midgut and the terminal ovarian follicles had progressed beyond stage IIa. By the end of June, changes in the tubules of nulliparous flies were apparent; there was a reduction in the amount of opaque material in the tubules but not enough to produce transparent sections; the remaining opaque material was light red. These changes were similar to those that appeared in the tubules of parous flies but, in most cases, females could still be graded on the basis of the tubules. Tests of the reliability of the Malpighian tubules as a diagnostic character were carried out in year 2; females were independently scored for parity on the basis of the appearance of the Malpighian tubules and on the basis of ovariolar dilatations. During June there was an exact correspondence between the two methods. In July, 90.1% ($n=172$) of females were correctly age-graded on the basis of the tubules. One nullipar was erroneously scored as parous and only 16 females (9.3%) could not be diagnosed because of the indeterminate nature of the tubules; of these, 12 were nullipars and 4 pars.

The Malpighian tubules of several other species of blackflies were examined to aid in the interpretation of the age-related changes that occurred in *S. venustum*. Obligately anautogenous (Crosskey 1990) species (*Eusimulium aureum* Fries and *S. parnassum* Malloch) exhibited the same physiological-age-related changes in the Malpighian tubules as did *S. venustum*. However, in *S. vittatum* Zetterstedt, a species autogenous for the first gonotrophic cycle (primiparous autogeny), the females showed no age-related changes in the morphology of the Malpighian tubules when compared to newly emerged individuals.

Meconium. In year 1 the presence or absence of meconium was not scored until 10 June, by which time parous females were present. At this time, the midgut of both parous and nulliparous flies was empty. In year 2, a pulse of females in early July (6–9 July) had yellow or yellowish-green material and yellow fluid in the midgut. Thereafter, midguts were clear, as in year 1.

Fat. From late May until early June, most nulliparous females had rank-2 fatbodies. Thereafter, fat stores declined. Parous females were always rank 1.

Insemination. Without exception and irrespective of gonotrophic age, host-seeking females were inseminated.

Crop. Most females, regardless of gonotrophic age, had large quantities of translucent, viscous fluid, probably carbohydrates, in the ventral crop; there was no relationship between quantity of stored fluid and gonotrophic age.

Seasonal changes in parity. In year 1, female *S. venustum* were first collected on 27 May but host seeking had begun several days earlier. The first

parous flies were encountered on 10 June (Fig. 3), more than 2 weeks after the initiation of host seeking. Over the following 2-week period, parity increased to nearly 75% (Fig. 3) and then declined significantly ($p < 0.05$) on 2 July, a decline that coincided with the re-appearance in the population of females with rank-2 fatbodies. About 2 weeks later, parity again peaked. From 9 July to the end of season, parity fluctuated from a low of about 30% on 23 July to a seasonal high of 75% on 6 August. Density of host-seeking females declined after 9 July and females were not encountered after 6 August.

With one exception (1 July), the observed proportion of parous females was higher in morning than in afternoon collections (Fig. 3). As expected, given the seasonal changes in parity, a log-linear model showed that most changes in parity were a function of day rather than time of collection (goodness of fit for the model $\text{PARITY_DAY_OF_YEAR}$: $G_{16} = 6.73$, $p = 0.98$). However, there was weak evidence that the proportion parous differed in morning and afternoon collections ($G_1 = 3.29$, $p = 0.07$), a result that was confirmed by a Mantel-Haenszel test of the $\text{PARITY_TIME_OF_DAY}$ interaction stratified by DAY_OF_YEAR ($C^2 = 3.76$, $p = 0.05$).

In year 2, host-seeking flies were first collected on 28 May, by which time 4% of the females were already parous. As in year 1, parity peaked in late June and then declined, coincident with the appearance in the population of females with rank-2 fatbodies and meconium in the midgut. The proportion of presumably newly emerged flies was 0.14 ($n = 171$) on 8–9 July. After 9 July, apparently newly emerged females were not encountered and parity rose and remained steady at about 60%.

DISCUSSION

Ovariolar dilatations. The presence of dilatations on the ovarioles of parous *S. venustum* had been noted by Davies (1963). In the present study, ovariolar "dilatations" were either large and sac-like, indicating relatively recent oviposition, or small and compact. Few flies were found with dilatations in an apparently intermediate stage. There are a number of competing hypotheses to explain this observation: (1) dilatations arise from post-oviposition sacs but females do not host seek when the dilatations are at an intermediate stage of contraction; (2) contraction of the sac-like dilatations is rapid (i.e. requiring less time than the normal interval between oviposition and resumption of host seeking); (3) dilatations do not arise from sacs. If we assume that dilatations arise from sacs, then data on the rate of contraction of the follicular sac are contradictory; Arkhipova (1966) found that the sacs contract within a few days following oviposition whereas both Lewis (1960) and Cupp and Collins (1979) reported that the sacs in were fully contracted in about 24 h.

Multiple dilatations were not found on the ovarioles of *S. venustum*, so the Polovodova (1949) technique could not be used to subclassify the parous component of the population. Only a few workers (Detinova and Bel'tyukova 1958, Shipitsina 1962, Magnarelli and Cupp 1977, Gryaznov 1993) have reported multiple (up to 5) dilatations in blackflies, interpreting each set of dilatations as evidence of a gonotrophic cycle. All other workers have reported at most a single ovariolar dilatation (see review in Anderson 1987) or occasional multiple dilatations in females held for oviposition in the laboratory (Rühm 1970).

The premise of the Polovodova (1949) technique for age grading blood-sucking insects is that, following each oviposition, the stretched ovariolar sheath (the "sac") contracts to form a dilatation on the ovariolar stalk and that each successful gonotrophic cycle leaves a permanent dilatation, so that the number of dilatations is equal to the number of gonotrophic cycles completed.

In mosquitoes it is now established that the sac does not contract to form a sequential and permanent dilatation; rather, each new sac that is formed following oviposition in successive gonotrophic cycles merges with the remnants of previous sacs at the ovariole-calyx junction (Sokolova 1983, 1994). Multiple follicular dilatations in mosquitoes arise only as a result of abortive or abnormal oogenesis. If one uses only dilatations as an age-grading tool, then an ovariole that is reliably diagnostic of the gonotrophic age of the female is a normal ovariole (Sokolova 1994) in which the terminal follicle aborted in *each* gonotrophic cycle; the number of such diagnostic ovarioles, not surprisingly, declines with age (Hoc and Charlwood 1990). By using both ovariole dilatations and the neutral-red-stainable granulation zones that form as a result of lysis and apoptosis of the sacculus tissue (the follicular epithelium and trophocyte remnants), it is possible to reliably diagnose the gonotrophic age and to retrospectively assess the fertility of mosquito females up to the end of the third gonotrophic cycle (Sokolova 1983, 1994). The lesson from this work on mosquitoes is that reliable age grading requires painstaking dissection, usually employing oil-injection techniques (Lange et al. 1981, Sokolova 1994) and that large numbers of normal ovarioles must be examined in each female, numbers that may have to increase with age because of the progressive rarity of diagnostic ovarioles. As well, there are significant species-specific differences in ovariole dynamics, requiring individual approaches for each species (Sokolova 1983, 1994).

In part, these studies of mosquitoes may suggest an explanation for the common observation in blackflies that only single, terminal dilatations are found, even in females whose gonotrophic age is thought to be >2 . However, aborting follicles are common in blackflies and, if the ovariole morphology and dynamics are similar to those of mosquitoes, diagnostic ovarioles with multiple dilatations should be formed (see Gryaznov 1993). It may be that in some species of blackflies the likelihood of a single ovariole aborting in each gonotrophic cycle is rare; alternatively, workers, especially western workers, may have failed to examine sufficient numbers of ovarioles in each female, thereby overlooking diagnostic ovarioles, or the dissection techniques employed have been so crude that most diagnostic ovarioles are destroyed during dissection by mechanical and osmotic stress (Sokolova 1994).

Gryaznov (1993)³ has recently modified the Sokolova technique for use in blackflies. Instead of an oil-injection technique, which he found to be ineffective with the relatively more robust ovaries of blackflies, the whole ovary is gently removed from the female, avoiding stretching and distortion; in a 0.9% saline solution to which a minute quantity of neutral red has been added (to stain necrotic tissue) the ovary is opened by slitting it lengthwise and then inverting it to lie calyx-side up; the preparation is *very* gently stretched using *minuten nadeln* and then spread by the application of a drop of paraffin oil to the surface of the saline; this preparation is subsequently viewed under a compound microscope in a hanging-drop preparation. These gentle procedures result in the preservation of a considerable amount of the ovarian structure, including the crucial ovariole-calyx junction, enabling a more powerful resolution of the ovariole architecture. The combination of ovariole dilatations and granulation zones can permit the recognition of 1- and 2-parous and possibly even gonotrophically older females (Gryaznov 1993). Studies of the age structure of North American blackflies and especially of vector species here and elsewhere, employing these new techniques, are clearly needed. Recently, Hoc

³An English translation of Gryaznov's diagnostic table is available from the first author.

and Wilkes (1995) have employed an oil-injection technique to diagnose the age structure of *Simulium woodi*, an East African vector of onchocerciasis.

Developmental stage of terminal follicles. In agreement with the observations of Davies (1963), the developmental stage of the terminal ovarian follicles did not differ between nullipars and pars. This character, therefore, has no value for age grading *S. venustum*.

Relict eggs. In comparison to other groups of blood-sucking insects, relict eggs in parous blackflies are common (Davies 1957, 1963; Lewis 1960) and are therefore useful in a differential-diagnosis procedure. Up to 25% (15% in the present study) of a parous population can be diagnosed as parous solely on the basis of the presence of relict eggs, a procedure that requires only a few seconds per individual. In common with the findings of other workers (Davies 1963; Rühm 1970), the proportion of parous *S. venustum* with relict eggs increased with time and possibly gonotrophic age, a feature that can make relict eggs increasingly valuable as a diagnostic tool as a population ages. In year 2 of the present study, the larger sample sizes showed that the proportion of females with relict eggs and the mean number of relict eggs per female followed a step function, suggesting that the probability of egg retention following oviposition increases with parity (see also Davies 1963). A definitive test of this hypothesis will require large samples and modern age-grading techniques. The reasons why relict eggs are so common in simuliids and why their frequency increases with age are not known but Davies (1963) speculated that ovulation might "become slightly less efficient with increasing age", a hypothesis that does not account for the relatively high frequency of relict eggs in young, parous simuliids.

The frequency distribution of relict eggs in parous females was highly contagious, fitting a negative-binomial distribution. This distribution can arise in a number of contexts so the model is more useful as an empirical descriptor rather than a hypothesis test of the factors leading to the observed distribution. With respect to relict eggs in females, a negative-binomial distribution could arise because of true contagion resulting from individual-based differences in the population—some females are inherently more likely to retain eggs than are others. On the other hand, the distribution could be *Poisson-stopped logarithmic* (Douglas 1980); if the distribution of relict eggs is a logarithmic series and the distribution of females with relict eggs is Poisson, then a negative-binomial would result. Some observations of *S. venustum* in the present study suggested that the distribution of relict eggs *within* an ovary might be non-random.

Malpighian tubules. Age-related changes in the Malpighian tubules were useful in age-grading *S. venustum*. As for relict eggs, Malpighian tubules offer advantages in a differential diagnosis because of the speed with which the tubules can be assessed. Early-season parous females had tubules with little granular material so that transparent sections were apparent; this loss of granularity was also noted by Lewis (1957) and Rühm (1970). The color of the granular material that remained changed from light yellow-brown in nullipars to red-brown in pars. The loss of granular material appears to be progressive with age.

The age-related changes in the extent and color of the granulation of the Malpighian tubules is clearly related to the blood meal and the red granules are probably hematin (Anderson 1987). Anautogenous parous flies and partially blood-fed nulliparous flies exhibit changes in the tubules, whereas parous flies that have completed an autogenous gonotrophic cycle do not show changes. This has important implications for aging studies of vector populations. The changes in the Malpighian tubules can be used to determine the proportion of a population returning for at least a second blood meal but not

necessarily the proportion of the population that has oviposited. Use of the Malpighian tubules alone for species exhibiting primiparous autogeny could result in an underestimate of the proportion of a population that had laid eggs.

Meconium and Fat. In *S. venustum*, the presence or absence of the meconium was of little value for age grading. Early in the season, the presence of meconium may be used to rapidly identify some nulliparous females but the meconium was apparently evacuated quickly, certainly before the appearance of parous flies in the population. The presence of meconium was valuable in detecting the arrival of a new cohort of nulliparous females into the population.

The fat body was useful for age grading some flies, particularly for recognizing nulliparous females and detecting waves of mid-season, presumably recently emerged, nullipars. However, problems similar to those encountered by other workers (e.g. Crosskey 1958, Rühm 1970) would be encountered if all flies were graded on the basis of this character alone; the amount of fat stores in blackflies may vary not only as a function of gonotrophic age but as well to differences in nutritional or environmental factors among breeding sites.

Anderson and Shemanchuk (1987) found that in a small sample of females of *S. arcticum* Malloch, there was little or no fat body remaining in the abdominal hemocoel and no meconium in the midgut by the time the ovarian follicles had reached stage I-II or II. No meconium was found in any female >20 h old.

Insemination status. Every host-seeking female in the present study was inseminated. Other workers report that variably small proportions of the host-seeking population are apparently uninseminated. In a collection of 466 female *S. venustum* from New York State, Magnarelli and Cupp (1977) found 19 uninseminated females; the proportion of uninseminated females in the population is not known because these workers reported only that they had dissected "at least 75% of the captured females". Wenk (1965) reported that 98% of mammalophilic host-seeking females were inseminated but much lower rates (53%) for the ornithophilic species *Eusimulium latipes*. It is difficult to reconcile these findings with those of the present study, but the most parsimonious hypothesis relates to the techniques used to assess insemination.

Seasonal change in age composition. The population of *S. venustum* aged similarly in each year of the study. Following the initial detection of parous flies in early June, parity rose steadily in each year, peaking at 60–75% parous in late June. The proportion of the population that succeeded in laying eggs at least once was slightly lower than reported for *S. venustum* in New York State (Magnarelli and Cupp 1977) and Ottawa (Davies 1963). A second period of recruitment in late June resulted in declining parous rates, followed by rebounds (Fig. 3), patterns that were not detected in either New York (Magnarelli and Cupp 1977) or Ottawa (Davies 1963) where the *S. venustum* season did not extend beyond the end of June. It is possible that the multiple recruitment periods reflect large habitat-associated variance in developmental rates but it is more likely that more than a single cytotype was involved.

The *Simulium venustum* complex in North America is known to consist of several reproductively isolated, virtually isomorphic cytospecies (Rothfels et al. 1978; Rothfels 1981). Although we were confident of separating *S. venustum* females from those of *S. verecundum*, we were unaware of the isomorphic species *S. truncatum*, which has host-seeking habits similar to those of *S. venustum* (Hunter et al. 1993). In Algonquin Park, host-seeking *Simulium truncatum* is earlier than *S. venustum*, so the late-June pulse of nullipars may

have been females of *S. venustum*. It is now possible to identify the cytotypes of adult blackflies based on polytene-chromosome banding patterns in cells from the Malpighian tubules (Procunier and Post 1986). Inasmuch as almost all regional simuliid populations comprise isomorphic species complexes, cytological typing should be required in future age-grading studies.

Periodicities in host-seeking behavior. Host-seeking behavior in many species of blackflies is strongly periodic, often bimodal (Crosskey 1990). In some species, the host-seeking periodicity is dependent on the gonotrophic age of the fly, the most common pattern being one in which young, nulliparous flies are more abundant in morning collections and older, parous (often soon after oviposition) flies predominating in afternoon or evening collections (Crosskey 1990). The year-1 collections in the present study suggest a pattern considerably at odds with that norm, parous *S. venustum* predominating in morning collections. The differential age-related periodicities are often a function of times of emergence and oviposition and might therefore be expected to show geographic and weather-dependent variation; certainly populations are known in which nullipars predominate in the late-afternoon collections (Duke 1968, Disney 1972).

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