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THE AGE STRUCTURE OF A POPULATION OF *AEDES PROVOCANS* (DIPTERA: CULICIDAE) IN SOUTHWESTERN ONTARIO

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ABSTRACT

In a previous paper it was shown that an eastern-Ontario population of the early-spring mosquito *Aedes provocans* had an atypical age structure: the adult-female population aged rapidly and synchronously, achieving an advanced gonotrophic age in an unusually brief period of time. The present study examined the age structure of *Ae. provocans* near Waterloo, in southwestern Ontario, at a site at which adult emergence occurred over a wider, more variable period and at which the preferred nectar sources for young adults were much less abundant. In Waterloo, the adult-female population aged more slowly and much less synchronously than in eastern Ontario. The role of resources, particularly nectar, in leading to delayed aging, is discussed.

In a study of a population of the early-spring mosquito *Aedes provocans* (Walker) in eastern Ontario, Gadawski and Smith (1992) reported rapid and synchronous gonotrophic aging of the adult females. In each of two seasons, the population achieved complete parity (all females had laid eggs at least once) in a remarkably brief period, a pattern that was unusual for *Aedes* populations, which typically age more gradually and much less synchronously. Gadawski and Smith (1992) attributed this unusual aging pattern to three features: (1) a brief emergence period; (2) the nearby abundance of important nectar sources, especially *Prunus* spp. that bloomed during and shortly after the emergence period; (3) the nearby abundance of blood-meal sources (cattle). The abbreviated emergence pattern may be a characteristic of the species, and *Ae. provocans* may be unusually efficient at finding and exploiting nectar and blood resources. Alternatively, all three features might be spatially random variables. Gadawski and Smith (1992) espoused the random-variable explanation and hypothesized that the ready availability of nectar at the study sites and the frequency with which it was used by nulliparous females were responsible, in part, for the rapid gonotrophic aging of the population. That hypothesis predicts that populations in areas that have less-abundant or less-preferred nectar sources should age more slowly and less synchronously. The present study tests that hypothesis by examining the age structure of a population of *Ae. provocans* in an area subject to intensive, modern agriculture, an area in which many of the hedgerows, so common in eastern Ontario, have been destroyed and in which, therefore, the nectar sources preferred by recently emerged *Ae. provocans* are much less abundant.

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MATERIALS AND METHODS

The 50-ha study site (43°28′ N; 80°37′ W; UTM: 17TNU307123) was situated in Wilmot Township, about 8 km west of Waterloo, ON. The site comprised a complex of mature beech-maple forest, cedar lowlands, and abandoned pastures, and contained a diversity of woodland, pasture and roadside pools, both temporary and permanent. White-tailed deer were locally abundant and a small herd of cattle was within 700 m of most of the breeding sites. *Prunus pensylvanica*, the preferred nectar source for recently emerged *Ae. provocans*, was present in the study site but at only two locations; it was not distributed equitably or commonly across the site as was the case in eastern Ontario (Gadawski and Smith 1992). *Prunus nigra*, an earlier-blooming and important nectar source in eastern Ontario (Gadawski and Smith 1992) was not present in the Wilmot study area.

Field studies were conducted from May–July in 1982 and 1983. The dates and patterns of emergence of female *Ae. provocans* were determined using floating emergence traps placed on a variety of pools and habitats (7 sites in 1982; 4 in 1983), representative of the pool types in the study area. Two traps were placed on some large pools. Traps were emptied in the morning and evening each day and monitored until no emergence had occurred for 14 d. Adults were identified using Wood et al. (1979). Population density of host-seeking females was monitored by a standardized (same host in the same clothing on moderate (temperature $\geq 10°C$), windless evenings) human-bait catch (Service 1976). Host-seeking females were aspirated from a human host situated at the edge of a deciduous-coniferous woodlot. Sampling was carried out every 1–4 evenings until *Ae. provocans* no longer came to bite. Collecting began 30 minutes before sunset and continued until sunset (1982) or for 40 min (1983). Females were refrigerated upon collection and examined within 12 h.

Mosquitoes were dissected in a saline solution (Hagedorn et al. 1977) to which a small quantity of liquid detergent had been added to facilitate wetting. The ventral esophageal diverticulum (crop) was removed and the nectar quantity it contained was scored on the following ordinal scale: large (>2 μl); moderate (0.5–2 μl); small (<0.5 μl) and none (0 μl). Gonotrophic age was assessed by the Polovodova (1949) technique. The developmental stage of the terminal ovarian follicle was scored using phase-contrast microscopy according to the scheme in Watts and Smith (1978).

Exact 2-sided confidence limits for proportions were computed with the F distribution, using the technique described in Pollard (1977). Age structures and nectar ranks within years were compared using a log-likelihood-ratio G test (Sokal and Rohlf 1981). A hierarchical log-linear model was used to explore the relationships of crop volume, follicular stage and parity; models were fit hierarchically using backwards selection. The maximal probability of a type-1 error was set at 0.05 for all hypothesis tests.

RESULTS

The immature stages of *Aedes provocans* did not occur in pasture or permanent pools; they were found only in temporary pools in deciduous or mixed, deciduous-coniferous forest, or in roadside pools bordering forest. Emergence was variable across these sites, both in the time of onset and the variance; the 1983 season was somewhat later and much more variable than the 1982 season (Fig. 1). In 1983, one of the sites produced a late second brood (Fig. 1). In many sites the emergence pattern was markedly right skew, with small numbers of individuals emerging many days after most (95%) of the

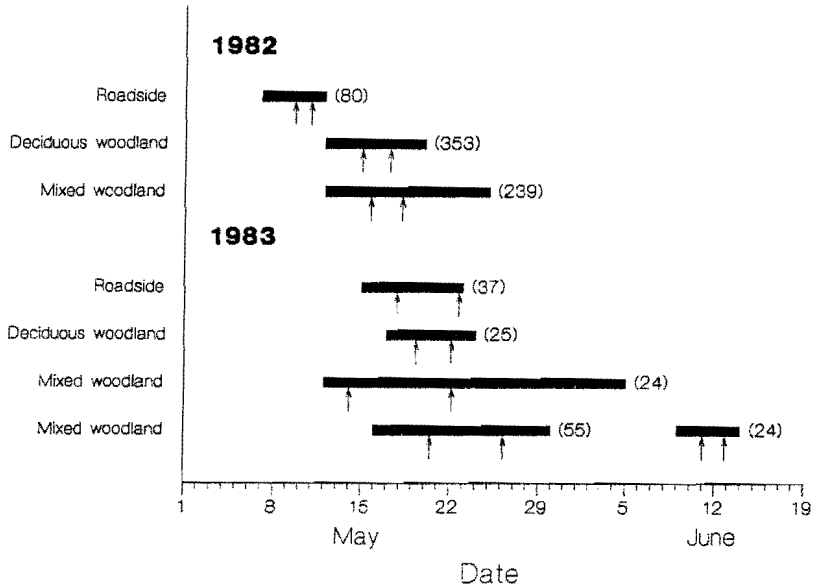

Figure 1. Emergence periods of female *Aedes provocans* in several breeding sites in 1982 and 1983. The solid bar portrays the range of emergence dates in a pool; the left arrow beneath a bar indicates the median emergence date; the right arrow indicates the 95th percentile. Sample sizes are given in parentheses. Mixed woodland is deciduous-coniferous forest.

population had emerged. In 1982, over all sites, 95% of the emergence was completed in about 11 d; in 1983, ignoring the small second brood, 95% of the emergence was completed in about 14 d (Fig. 1).

In 1982, blood-seeking females were first observed on 13 May, 6 d after the onset of emergence; females continued to seek blood for 54 d (Table 1). In 1983, blood-seeking females were first observed on 21 May, 9 d after emergence had begun; biting females were encountered for 51 d (Table 1). All early-season host-seeking females were nulliparous. Parous females were first encountered 13 d (1982) and 23 d (1983) after the onset of host seeking, but complete parity of the population was not achieved for 31 d (1982) and 32 d (1983) (37 and 41 d after the beginning of emergence) (Table 1). In both years, biparous females were encountered while nulliparous females were still present in the population, and nulliparous females reappeared after the population had achieved 100% parity (Table 1). Uniparous females were encountered until the end (1982) or almost the end (1983) of the season. Some late-season host-seeking females were biparous, but triparous females were rare (Table 1).

In each year, the density of host-seeking females was noisily multimodal (Figs. 2, 3). With the exception of the first peak in 1982 and the first 2 peaks in 1983, which comprised only nulliparous females, the biting population at any one time was of mixed gonotrophic age, and the peaks in biting activity could be only coarsely related to the successive appearance of nulli-, uni- and bipa-

rous females. In each year, the first peak in biting activity coincided closely with the flowering time of *Prunus pensylvanica* (Figs. 2, 3). In 1982, the initial peak of biting by nulliparous females occurred near the end of the emergence period (Fig. 2) whereas in 1983, the two initial peaks of biting by nulliparous females occurred many days after most emergence was completed (Fig. 3).

Host-seeking females were predominantly at follicular stage IIa or IIb (Fig. 4). Females with early-stage follicles (Ib) were rare, and few females in advanced stages of oogenesis (III-V) were found, and only in 1983. Most females came to bite with the terminal ovarian follicles in stage II; evidently, a single blood meal was usually sufficient for oogenesis in *Ae. provocans*.

In exploring the relationships among parity, ovarian stages and stored carbohydrates, the frequency of sparse cells was reduced by excluding females in ovarian stages III–V and by pooling nectar ranks 2 and 3 (few females contained >2 μl of stored carbohydrate in the crop). In the resulting 4-way table ($2\times2\times3\times3$; year, parity, ovarian stage and crop rank), the 4-way interaction was highly significant ($G_4=43.2$, $p\ll0.0001$), due to marked differences

Table 1. Gonotrophic age structure of a population of *Aedes provocans* near Waterloo, Ontario.

Date	Day[a]	n	% in each gonotrophic cycle[b]			
			0	1	2	3
1982						
13 May	6	10	$100.0^{+0.0}_{-30.8}$	0.0	0.0	0.0
15 May	8	37	$100.0^{+0.0}_{-9.5}$	0.0	0.0	0.0
17 May	10	17	$100.0^{+0.0}_{-19.5}$	0.0	0.0	0.0
20 May	13	21	$100.0^{+0.0}_{-16.1}$	0.0	0.0	0.0
25 May	18	22	$100.0^{+0.0}_{-15.4}$	0.0	0.0	0.0
26 May	19	50	$96.0^{+3.5}_{-6.6}$	$4.0^{+9.7}_{-2.7}$	0.0	0.0
27 May	20	34	$70.6^{+14.3}_{-14.9}$	$29.4^{+18.1}_{-12.0}$	0.0	0.0
30 May	23	34	$47.1^{+17.8}_{-14.6}$	$52.9^{+17.3}_{-15.1}$	0.0	0.0
1 June	24	52	$30.8^{+14.3}_{-10.4}$	$69.2^{+12.1}_{-12.3}$	0.0	0.0
3 June	26	31	$22.6^{+18.5}_{-10.7}$	$77.4^{+13.0}_{-14.9}$	0.0	0.0
6 June	29	90	$13.3^{+8.8}_{-5.4}$	$85.6^{+6.5}_{-7.7}$	$1.1^{+4.9}_{-0.8}$	0.0
8 June	30	43	$23.3^{+15.4}_{-9.7}$	$76.7^{+11.5}_{-12.8}$	0.0	0.0
10 June	32	40	$12.5^{+14.3}_{-6.8}$	$87.5^{+8.3}_{-11.2}$	0.0	0.0
13 June	35	17	0.0	$100.0^{+0.0}_{-19.5}$	0.0	0.0
16 June	38	6	0.0	$100.0^{+0.0}_{-45.9}$	0.0	0.0
20 June	42	6	$16.7^{+47.5}_{-12.3}$	$83.3^{+16.2}_{-29.3}$	0.0	0.0
21 June	43	21	$28.6^{+23.6}_{-14.0}$	$66.7^{+18.7}_{-18.8}$	$4.8^{+19.1}_{-3.6}$	0.0
23 June	45	24	0.0	$100.0^{+0.0}_{-14.2}$	0.0	0.0
26 June	48	22	0.0	$86.4^{+10.7}_{-15.5}$	$9.1^{+20.1}_{-6.2}$	$4.5^{+18.3}_{-3.4}$
27 June	49	4	0.0	$100.0^{+0.0}_{-60.2}$	0.0	0.0
29 June	51	1	0.0	$100.0^{+0.0}_{-97.5}$	0.0	0.0
1 July	53	6	0.0	$83.3^{+16.2}_{-29.3}$	$16.7^{+47.5}_{-12.3}$	0.0
5 July	57	6	0.0	$66.7^{+29.0}_{-30.8}$	$33.3^{+44.4}_{-21.5}$	0.0
7 July	59	1	0.0	$100.0^{+0.0}_{-97.5}$	0.0	0.0

Table 1. Continued.

Date	Day[a]	n	% in each gonotrophic cycle[b] 0	1	2	3
1983						
21 May	9	10	$100.0^{+0.0}_{-30.8}$	0.0	0.0	0.0
24 May	12	20	$100.0^{+0.0}_{-16.8}$	0.0	0.0	0.0
28 May	16	32	$100.0^{+0.0}_{-10.9}$	0.0	0.0	0.0
2 June	21	54	$100.0^{+0.0}_{-6.6}$	0.0	0.0	0.0
5 June	24	42	$100.0^{+0.0}_{-8.4}$	0.0	0.0	0.0
7 June	26	17	$100.0^{+0.0}_{-19.5}$	0.0	0.0	0.0
8 June	27	45	$100.0^{+0.0}_{-7.9}$	0.0	0.0	0.0
10 June	29	50	$100.0^{+0.0}_{-7.1}$	0.0	0.0	0.0
13 June	32	46	$87.0^{+8.1}_{-10.5}$	$13.0^{+13.2}_{-6.7}$	0.0	0.0
15 June	34	60	$68.3^{+11.4}_{-11.5}$	$31.7^{+13.3}_{-10.0}$	0.0	0.0
17 June	36	50	$2.0^{+8.6}_{-1.5}$	$98.0^{+1.9}_{-5.1}$	0.0	0.0
20 June	39	50	$6.0^{+10.5}_{-3.8}$	$92.0^{+5.8}_{-8.5}$	$2.0^{+8.6}_{-1.5}$	0.0
22 June	41	50	0.0	$92.0^{+5.8}_{-8.5}$	$8.0^{+11.2}_{-4.7}$	0.0
24 June	43	47	0.0	$91.5^{+6.1}_{-9.0}$	$8.5^{+11.9}_{-5.0}$	0.0
26 June	45	14	$14.3^{+28.5}_{-9.6}$	$64.3^{+23.0}_{-22.4}$	$21.4^{+29.4}_{-13.0}$	0.0
28 June	47	40	$2.5^{+10.7}_{-1.9}$	$67.5^{+13.9}_{-14.0}$	$27.5^{+16.4}_{-10.9}$	$2.5^{+10.7}_{-1.9}$
1 July	51	16	0.0	$100.0^{+0.0}_{-20.6}$	0.0	0.0
3 July	53	3	0.0	$66.7^{+32.5}_{-37.4}$	$33.3^{+57.2}_{-23.9}$	0.0
6 July	56	11	0.0	$72.7^{+21.3}_{-24.5}$	$27.3^{+33.7}_{-16.3}$	0.0
8 July	58	2	0.0	0.0	$100.0^{+0.0}_{-84.2}$	0.0
10 July	60	2	0.0	0.0	$100.0^{+0.0}_{-84.2}$	0.0

[a]Number of days after initial emergence.
[b]Numbers following the percentages are the values to derive upper (superscript) and lower (subscript) confidence limits for the percentages.

between the two years, so further analysis was restricted to within-year tables. In 1982, the best-fitting model ($G_8=11.3$, $p=0.18$) included the interactions parity × ovarian stage and parity × crop rank). In terms of the stage of the terminal ovarian follicle, parous females were at significantly earlier stages of ovarian development than were nulliparous females ($G_{10}=59.5$, $p<0.0001$) and parous females had significantly larger carbohydrate stores ($G_{10}=21.9$, $p=0.016$) (Fig. 3). The relationship among these 3 variables was more complex in 1983; the 3-way interaction was highly significant ($G_4=83.8$, $p<0.0001$). Thus, for example, the quantity of stored carbohydrates differed between parous and nulliparous females in a manner that differed across ovarian stages (Fig. 4). Ignoring parity and ovarian stage, females in 1983 had significantly smaller nectar stores than did females in 1982 ($G_2=72.1$, $p<0.0001$), largely attributable to the very high frequency in 1983 of females with empty crops (Fig. 3).

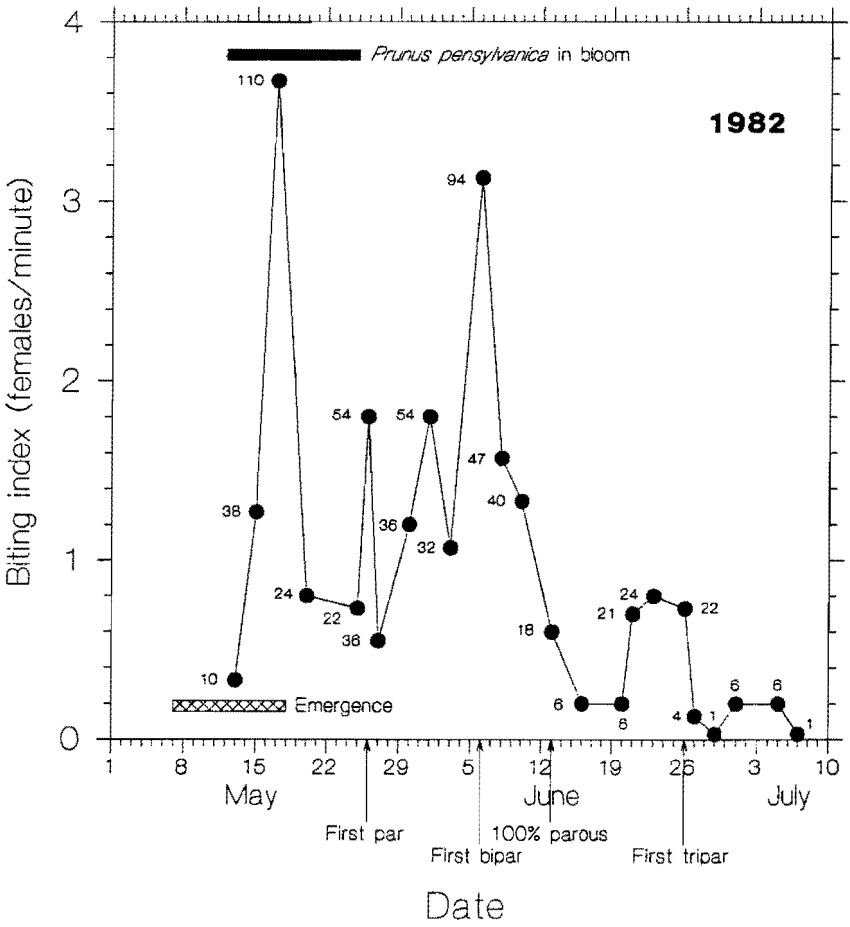

Figure 2. Biting index of *Aedes provocans* at Waterloo in 1982. Integers next to data points are sample sizes. Days on which the sample size was zero are not shown (3 collections before 13 May 1982; 4 after 7 July 1982). The solid bar indicates the flowering period of the important nectar source *Prunus pensylvanica*. The hatched bar indicates the period over which 95% of the emergence occurred.

DISCUSSION

There are many accounts of the age-structure of mosquitoes but few studies of the geographical or temporal variability of age structure within a species. The age structure of *Ae. cinereus* Meigen at a site in Byelorussia (Shlenova and Bei-Bienko 1962) differed from that of the same species in Ivanovo, only a few hundred km distant (Volozina 1958). In contrast, Magnarelli (1977) concluded that the age structure of three *Aedes* species in New York and Connecticut did not differ; however, a statistical reexamination

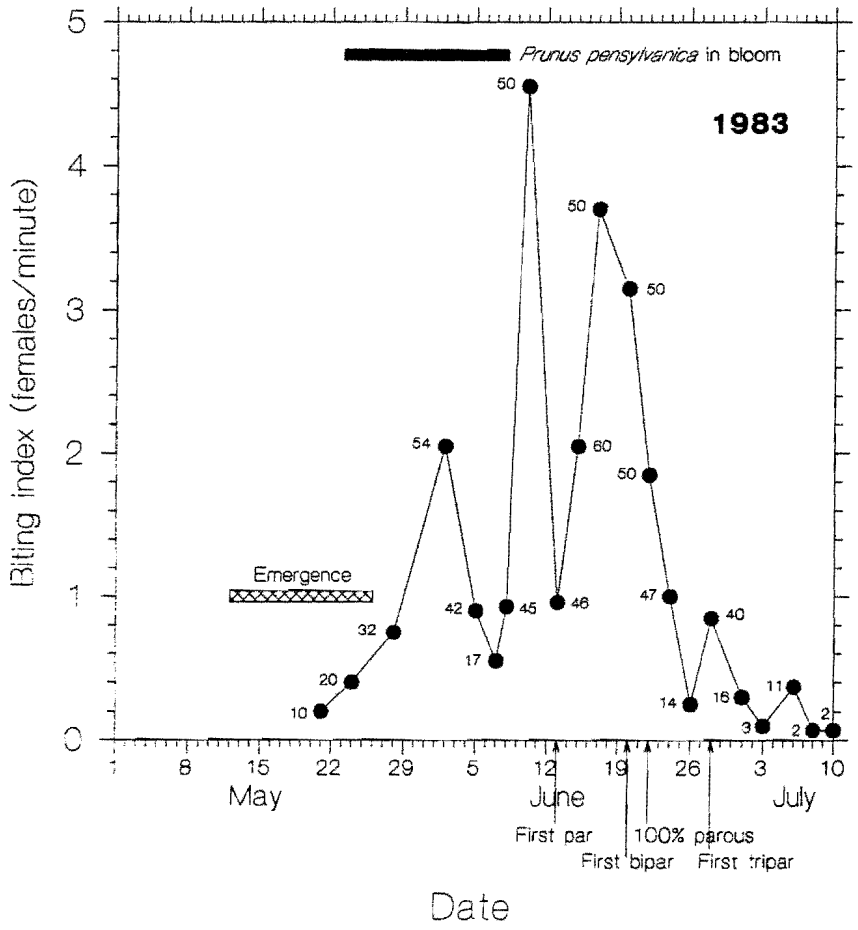

Figure 3. Biting index of *Aedes provocans* at Waterloo in 1983. Integers next to data points are sample sizes. Days on which the sample size was zero are not shown (3 collections before 21 May 1983; 2 after 10 July 1983). The solid bar indicates the flowering period of the important nectar source *Prunus pensylvanica*. The hatched bar indicates the period over which 95% of the emergence occurred.

of the data for two species (*Ae. canadensis* [Theobald] and *Ae. stimulans* [Walker]), suggests that the age structures might have differed across locales. *A priori*, temporal and spatial variability in age structure would be expected, but there may be important species-dependent factors that could dampen spatial or temporal variances. In eastern Ontario, an *Ae. provocans* population aged rapidly and highly synchronously in each of two years (Gadawski and Smith 1992). Only three peaks of biting activity were detected and each peak corresponded almost perfectly to a single gonotrophic age (nulli-, uni-

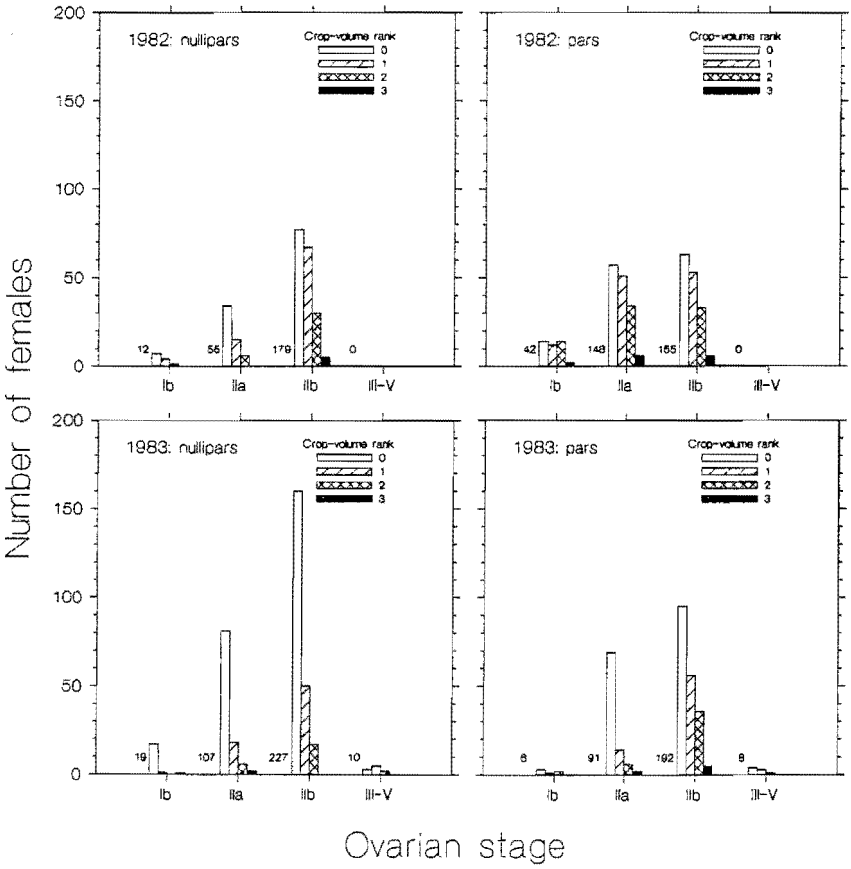

Figure 4. Crop volume of female *Aedes provocans* in relation to parity and stage of ovarian development; 0: empty; 1: <0.5 μl; 2: 0.5–2.0 μl; 3: >2 μl. Integers next to bars are the subgroup sample sizes.

and biparous females) (Gadawski and Smith 1992). The time between the appearance of the first parous female and the attainment of 100% parity in the population was very nearly equal to the duration of the emergence period, suggesting that virtually the entire population was aging gonotrophically at the same rate and that, therefore, females encountered no difficulty in obtaining resources (nectar, mates, blood, oviposition sites). The present study has shown that this pattern of gonotrophic aging in *Ae. provocans* is site and possibly time specific.

In the present study, the adult-female population of *Ae. provocans* aged more slowly and more asynchronously than was found in eastern Ontario, and, overall, the population did not attain the same advanced gonotrophic age (Table 2). As well, there were marked differences in the gonotrophic-aging profile between the two years. 100% parity was not achieved until 37–41 d

Table 2. Some features of gonotrophic aging in two populations of *Aedes provocans*.

Location	Year	Duration (d) of		Day[a] when		n	Parity[b] (%)		
		Emergence[c]	Host seeking	First parous	100% parous		0	1	2
Belleville[d]	1978	5	38	11	17	352	28.4	54.5	17.1
	1979	7	41	17	27	907	35.4	53.2	11.4
Waterloo	1982	11	51	19	37	595	42.4	56.3	1.3
	1983	14	54	32	41	661	54.2	41.1	4.8

[a]Days after the first day of emergence.
[b]Proportion of the entire season's catch of host-seeking females of a given gonotrophic age.
[c]Time to 95% emergence.
[d]From Gadawski and Smith (1992).

after the beginning of emergence, in contrast to 17–27 d observed in eastern Ontario (Gadawski and Smith 1992). In the present study, biparous females were encountered at a time when nulliparous females were still present in the population (Table 1), a situation not found in eastern Ontario. In the Waterloo population, multiple peaks of biting activity occurred within a single gonotrophic cycle whereas, in eastern Ontario, each gonotrophic cycle appeared as a single peak in the hostseeking population. In both years at Waterloo, lengthy periods (19 and 32 d) elapsed between the beginning of emergence and the appearance of the first parous female in the population; in 1982, a further 18 d then elapsed before the entire population was parous whereas in 1983, the population became 100% parous in only 9 d (Table 2). To what extent are these differences attributable to phenologies and resource availability?

In eastern Ontario, the emergence period of *Ae. provocans* was brief (95% complete within 5–7 d). In western Ontario, in contrast, emergence of *Ae. provocans* was both more prolonged (11–14 d) (Table 2) and variable across sites (Fig. 1). As compared to the host-seeking population of *Ae. provocans* near Belleville (Smith and Gadawski 1994), the Waterloo population had significantly smaller nectar stores; the difference was particularly marked in 1983 (1982: $G_3=29.0$, $p\ll0.0001$; 1983: $G_3=108.2$, $p\ll0.0001$). In the Belleville study, some females with nectar rank 0 were subsequently moved to nectar rank 1 as a result of a cold-anthrone test, but the site difference in nectar stores persists even if the comparison is restricted to females with nectar ranks 2 and 3 (1982: $G_1=24.47$, $p\ll0.0001$; 1983: $G_1=33.75$, $p\ll0.0001$). Nectar resources may therefore have been more limiting in western as compared to eastern Ontario; certainly, one of the important early-spring nectar sources (*Prunus nigra*) was not present in the Waterloo site and *P. pensylvanica* was relatively uncommon. It is reasonable to assume that oviposition sites were not limiting in either locale; pools were abundant and close by in both sites. We have no quantitative data on the relative availability of blood-meal sources in the two sites but cattle and deer were locally abundant in each site. Thus, the western-Ontario sites were characterized by a longer and more variable emergence period and, especially in 1983, may have exhibited a marked, relative shortage of nectar resources.

At least some of the noise in the biting cycles at the Waterloo site is undoubtedly attributable to the greater duration and variance of emergence in western as compared to eastern Ontario. Whereas the breeding sites for *Ae. provocans* in Belleville were snowmelt pools in deciduous woodland, at Waterloo the species was found in pools in both deciduous and coniferous woodland as well as in roadside pools bordering wooded areas. Not unexpectedly, this greater diversity of breeding sites contributed to a more heterogeneous emer-

gence pattern. The multiple peaks within a gonotrophic cycle may have been due to the host-seeking activities of cohorts of females derived from breeding sites with different phenologies.

The emergence pattern, however, does not adequately account for the generally longer period of time for the Waterloo populations to age gonotrophically and it cannot account for the very large between-year difference in aging pattern. In 1983 particularly, gonotrophic aging was severely retarded, 32 d elapsing between the beginning of emergence and the appearance of the first parous female. Thereafter, however, gonotrophic aging was exceedingly rapid. It is possible that the relative nectar shortages detected in the host-seeking population in 1983 were reflected in a failure of females to find blood-meal sources as rapidly as they did in 1982, or as rapidly as was the case in eastern Ontario.

Although the average western-Ontario female of *Ae. provocans* was gonotrophically younger than a female from eastern Ontario, in terms of calendar age, the opposite condition obtained. The flight season for females in western Ontario was considerably longer than that of females in eastern Ontario (Table 2). This suggests that important mortality events are associated with blood-feeding, during the act of blood-feeding itself, during post-blood-meal oogenesis, or at oviposition.

Although the western-Ontario population of *Ae. provocans* aged more slowly than a population in eastern Ontario, compared to other *Aedes* mosquitoes, the population nevertheless aged in a relatively rapid fashion. Most populations of north-temperate *Aedes* mosquitoes age slowly and sometimes erratically (see discussion in Gadawski and Smith 1992); some populations never attain 100% parity in spite of lengthy flight seasons (Packer and Corbet 1989). In contrast, *Ae. provocans* populations always achieved 100% parity and substantial portions of the population completed more than one gonotrophic cycle. That differences in aging patterns of *Aedes* mosquitoes are not exclusively a function of the local availability of blood-meal sources is illustrated by studies in which populations aged slowly in the presence of abundant hosts (Service 1977). It may be that *the* critical resource that determines the rate at which populations of snow-melt *Aedes* age is nectar and not blood. Studies of the mechanisms by which mosquitoes find and utilize nectar resources and the impact of the varying efficiencies of those mechanisms could be very rewarding.

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