ABSTRACT: The abundance and structure of urban autogenous and anautogenous populations of *Culex pipiens* mosquitoes were documented systematically in Boston, MA, during three successive years. Autogenous larvae become abundant mainly in enclosed sites and anautogenous larvae in sites that provide free access and egress. Both populations begin to proliferate when the water temperature exceeds 15°C during June. Larval anautogenous mosquitoes increase in abundance 10-fold in two weeks and autogenous in three weeks. Although anautogenous larvae rapidly disappear after mid-August when winter diapause commences, the abundance of autogenous larvae continues to increase until mid-October. The forms generally are reproductively isolated in nature but occasionally hybridize during August and thereafter. Anautogenous females feed mainly on birds; autogenous females generally never feed on blood; and hybrid females appear to feed indiscriminately on avian or mammalian hosts. Such northern *C. p. pipiens* mosquitoes range as far south as 33°N. Taken together, these observations suggest that *C. p. pipiens*-borne pathogens may proliferate in the northern United States until mid-August and affect human hosts thereafter. Intensity of transmission decreases toward the south.

**KEYWORDS:** West Nile virus; *Culex pipiens* populations; autogenous populations; larval anautogenous mosquitoes

INTRODUCTION

The recent introduction of West Nile virus into urban sites in eastern North America has directed public health attention toward the members of the *Culex p. pipiens* complex of mosquitoes,¹ the main presumed vectors of this infection. These insects appear to transmit this arboviral pathogen among birds because they are ornithophilic, competent as hosts for the pathogen,² frequently infected in nature, and are the main species of mosquito present where and when human infection is most frequent.³ Efforts to protect the public health frequently involve intensive insecticidal applications directed against these mosquitoes.

The environmental factors that influence the abundance and host-seeking behavior of mosquitoes in the *C. p. pipiens* complex of populations were subjects of in-
tense study during the mid-twentieth century. Mattingly assembled a series of reports on the subject in 1951 that inspired numerous additional studies, including my own early contributions, and an international conference on this subject that was convened by the World Health Organization in 1964. Interest in these insects subsequently declined because their populations in the temperate zone appeared to feed on people only rarely and because their public health importance seemed minimal. Vinogradova’s recent monograph on these insects, for example, cites relatively few publications dating to the 1990s. This treatment focuses on the Eurasian experience, while devoting little attention to the structure and seasonal dynamics of *C. p. pipiens* populations in North America, where West Nile virus has become endemic. Although it attracted much media attention, a 1998 English study on the structure of these populations omitted discussion of the relevant North American experience. A biological rationale for antivector interventions against this emergent disease in northeastern North America, therefore, is lacking.

To provide a public health rationale for anti-West Nile interventions in this newly endemic Nearctic region, I shall synthesize information that I published nearly a half century ago concerning the structure and seasonal dynamics of *C. p. pipiens* populations infesting a site in Boston, MA, located 2 km from the epicenter of the outbreak of West Nile virus infection that occurred in 2000. In particular, I shall identify conditions that appear to lead toward virus amplification and transmission to human hosts. At the time that this work was performed, these mosquitoes appeared to be harmless to the inhabitants of this urban environment. This experimental design cannot now be reapplied because these insects no longer can be considered innocuous.

**GENERAL METHODS**

The study site was located on the Harvard Medical School campus in an urban section of the city of Boston. Larvae were sampled systematically at least once a week throughout a three-year period from three sites, designated as the “catch basin,” “air shaft” and “pit.” Larval density and water temperature were recorded. A sample of the larvae were reared through to the adult stage and the resulting females individually confined over water without food. Those that failed to oviposit were dissected in order to determine the degree of ovarian development. Adults were sampled from the nearby “phone booth” and “tunnel” sites. The contents of their abdomens was judged by their external appearance and classified as “distended with blood” or “hypertrophic fat,” and those that were slender were considered to be “flat.” A sample of flat females was induced to gorge from capillary tubes on defibrinated chicken blood. Others were dissected to ascertain the developmental stage of their ovaries. A sample of blood-gorged females were dissected, the gut contents smeared on a microscope slide and stained with Giemsa stain.

Density of larvae was estimated by dipping a pair of 300 mL containers alternately into the water in a breeding site in a manner calculated to derive the greatest possible number of larvae. After each dip, the contents of the dipper was compared to that of the previous dip. The dipper containing fewer larvae was then discarded and the other retained. The process was repeated 10 times, and the larvae in the last remaining dipper were counted and the total recorded.
FIGURE 1. Seasonal abundance and proportion of autogenous larval *C. p. p. pipiens* breeding in the air shaft and catch basin sites in Boston, MA (part of a figure in Ref. 9; reproduced with permission). Larvae were reared to the adult stage and tested individually.

**OBSERVATIONS**

*Characteristics of Autogenous Populations*

Samples derived from laboratory colonies of autogenous *C. p. p. pipiens* differ genetically from those comprised of anautogenous mosquitoes. Genes regulating autogenous development are located on both autosomes. When isolated in a container containing a little water, virtually all female pupae taken from an autogenous colony develop into adults that develop mature eggs when deprived of food. Virgin females would generally fail to oviposit. The ovaries of females derived from pupae isolated from an anautogenous colony fail to develop beyond the yolk-competent stage of development. Such an ovarian follicle contains an oocyte that is covered by a well-developed oolemma, including a mature microvillar coat. F1 hybrid progenies include similar numbers of females that (1) produce mature eggs (stage V), (2) commence
vitellogenesis but cease egg maturation before the eggs are mature (stage III or IV), or (3) cease ovarian development in the yolk-competent stage (stage IIb). Genetically autogenous females can thereby be distinguished from anautogenous females as well as from hybrid females by examining their offspring.

**Seasonal Abundance**

The density of larvae in the air shaft and catch basin sites was recorded as well as the proportion of the derived adults that commenced vitellogenesis in the absence of food. Larvae first became evident in the catch basin and air shaft sites in June or July when the water temperature came to exceed 15°C and were most abundant in August. They were no longer evident in these exposed sites after the beginning of October, when the water cooled to less than 15°C (Fig. 1, illustrating the second of three similar years of observation). Larvae in the air shaft were consistently more autogenous than those in the catch basin, and their density rose more rapidly. Larvae in the relatively autogenous catch basin became most abundant in September, more than a month after those in the air shaft began to decline. More autogenous larvae develop in the relatively open air shaft site than in the enclosed catch basin site; the density of anautogenous larvae begins to wane on 10 August at 42° latitude, when day length decreases to about 14 h 15 minutes.

The rate of increase of autogenous larvae was compared to that of anautogenous larvae by analyzing the ascending segments of the curves representing the growth of the populations infesting the catch basin and air shaft sites in each of three years of observation. The anautogenous components in each of these sites during each of these years multiplied far more rapidly than did the autogenous populations (Fig. 2). Anautogenous larvae increased 10-fold in two weeks while the autogenous larvae did so in three weeks.

The density and degree of autogeny in adult mosquitoes sampled by aspirator from the phone booth site were recorded throughout the three-year period of study. The first mosquitoes that appeared there were noted in June, and autogenous mosquitoes predominated among them (Fig. 3, illustrating the second of three similar years of observation). Anautogenous females and somewhat fewer males (autogenous status not determined) were most abundant at the end of July and early in August. Autogenous mosquitoes became most numerous there in September. The abundance of mosquitoes in this semisheltered resting site reflects the emergence of mosquitoes in the nearby air shaft site.

**Winter Diapause**

In a series of laboratory experiments, the degree of ovarian development was described in mosquitoes exposed to a short diel. Specimens from autogenous as well as anautogenous laboratory colonies isolated in the catch basin and air shaft study sites were used in these experiments. Mature larvae were reared at 18°C and cohorts subjected to a graduated series of diels. The ovaries of virtually all anautogenous females that were limited to 8 h of light per day developed only to the previtellogenic stage. Primary follicles are about as long as secondary follicles. The oolemma of the oocytes in such females is devoid of microvilli. Further development (to the yolk-competent stage) requires juvenile hormone stimulation. By contrast, the ovaries
FIGURE 2. Development of larval autogenous (A) and anautogenous (N) C. p. pipiens breeding in the air shaft and catch basin sites in Boston, MA (part of a figure in Ref. 9; reproduced with permission). Adults were fed blood from capillary tubes and permitted to deposit eggs; their larvae were reared, and a sample of the resulting adults were tested individually.

TABLE 1. Degree of ovarian development in food-deprived adult C. p. pipiens mosquitoes derived from autogenous or from anautogenous larvae that were reared under short day length (8 h/day) and under long day length (16 h/day) conditions.

<table>
<thead>
<tr>
<th>Kind</th>
<th>No. of hours light/day</th>
<th>No. females</th>
<th>Percent in diapause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anautogenous</td>
<td>8</td>
<td>33</td>
<td>97.0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>36</td>
<td>8.3</td>
</tr>
<tr>
<td>Autogenous</td>
<td>8</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>14</td>
<td>0</td>
</tr>
</tbody>
</table>

of almost all females exposed to 16 h of light per day developed to the yolk-competent stage (Table 1). About a third of the adults that emerged from larvae held at 14 h 15 m developed into adults whose ovaries remained in the previtellogenic (Fig. 4). Ovaries of diapausin C. pipiens mosquitoes ceased development in response to a short diel and had yolk-incompetent ovarioles that had not been stimulated by juvenile hormone.

External evidence of hibernation was sought by examining the abdomens of adult females captured while they were resting in the phone booth site. Resting adults were most abundant there late in October (Fig. 5). Freshly blood-gorged females ini-
FIGURE 3. Seasonal abundance and proportion autogenous adult C. p. pipiens resting in the phone booth site located in Boston, MA (part of a figure in Ref. 9; reproduced with permission). Adults were fed blood from capillary tubes and permitted to deposit eggs; their larvae were reared, and a sample of the resulting adults were tested individually.

FIGURE 4. Effect of photoperiod on the development of gonoactive ovaries in larval C. p. pipiens derived from an anautogenous colony isolated in Boston, MA.^{10}
FIGURE 5. Condition of adult C. p. p. pipiens resting in the phone booth site located in Boston, MA (part of a figure in Ref. 13; reproduced with permission).

Initially predominated, and some were present throughout October. A few females whose abdomens were distended with hypertrophic fat bodies became evident in October. Evidence of diapause was more evident in females taken in the underground tunnel site in October (data not shown). Half of the fat-containing females were dissected and their ovaries examined microscopically. The ovaries of these females were invariably arrested in stage 0. Blood-gorged females are most abundant early in the fall and diapausing females late in the fall.

Evidence of ovarian diapause was sought in mosquitoes captured in the tunnel site. Female mosquitoes with previtellogenic ovaries first appeared in August, and the proportion of females expressing this sign of diapause increased with the onset of winter (Fig. 6). The proportion of females in this site whose abdomens were distended with fat increased rapidly during the month of September, and virtually all mosquitoes contained hypertrophic fat bodies by the end of that month. The ovaries of many of these mosquitoes contained scars that suggested previous deposition of eggs. Ovarian diapause is characterized by previtellogenic ovaries and hypertrophic fat bodies, and many females appear to enter diapause after developing and depositing their first clutch of eggs.

To determine whether autogenous C. p. pipiens mosquitoes would breed continuously throughout the winter, larvae were sampled systematically from the pit site. Temperature of the water contained in this subterranean reservoir of stagnant water remained above 18°C between October and April. Adults reared from larvae sampled from this site generally produced eggs autogenously (Fig. 7). Anautogenous females were reared from larvae that were sampled mainly before the onset of winter.
FIGURE 6. Proportion of female C. p. pipiens captured in the tunnel site whose ovaries were considered to be in the diapause stage (stage 0) (part of a figure from Ref. 13; reproduced with permission13).

Mixed progenies containing autogenous as well as anautogenous individuals were discovered mainly during midwinter. These were considered to be the progeny of heterozygous females. The ovaries of these females commenced vitellogenesis but ceased maturation before their eggs had matured (stage V). In sheltered sites, winter-breeding mosquitoes tend to be autogenous or heterozygous for this characteristic.

Blood-feeding Behavior

To determine whether these mosquitoes were parasitizing human hosts, blood-gorged females collected in the phone booth and tunnel sites were subjected to serological testing.13 Of 184 females tested in this manner, only five contained human blood. These human-feeding mosquitoes were found during one week of observation (in September) when a guard stationed nearby complained of an episode of mosquito bites. In addition, fresh blood found in the midguts of 40 additional specimens was smeared on slides, stained with Giemsa, and examined microscopically. Each specimen contained nucleated, avian blood, and many contained red cells infected by Plasmodium sp. Birds provide the main source of vertebrate blood for these mosquitoes.

An additional episode of human biting occurred during the course of this study, in the pit during midwinter.14 Females that fed on a human host were permitted to engorge and to produce progenies. Of these 13 blood-gorged mosquitoes, seven produced wholly autogenous progenies, and six produced progenies that appeared to be heterozygous. Some of the resulting first generation females matured their eggs, while others failed to undergo vitellogenesis or produced partially developed eggs. These mosquitoes appeared to be autogenous-anautogenous heterozygotes. Epi-
sodes of human biting appear to be associated with episodes of interbreeding between these *C. pipiens* variants.

**Population Structure**

To determine whether adult mosquitoes found resting in the phone booth and tunnel sites were derived from panmictic but separate autogenous or anautogenous populations, a sample of flat adult mosquitoes found resting there during the summer and fall were fed blood from capillary tubes and their progenies reared through to the adult stage. Of the 353 females that were subject to progeny tests, most were homozygous anautogenous (304) and some homozygous autogenous. Only a few (6) gave rise to families that included autogenous as well as anautogenous mosqui-
FIGURE 8. Effect of successive generations of brother-sister inbreeding on the embryonic development of autogenous and of anautogenous *C. p. pipiens* captured in various sites located in Boston, MA (part of a figure in Ref. 15; reproduced with permission).

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FIGURE 9. Conceptual model of the pattern of increase in the abundance of larval autogenous and of anautogenous C. p. pipiens in Boston, MA. The model assumes that anautogenous mosquitoes increase 10-fold in two weeks, that autogenous mosquitoes do so in three weeks, that development commences when the water temperature exceeds 15°C, and that anautogenous females fail to reproduce when day length is less than 14 h.
Although autogenous *C. p. pipiens* mosquitoes generally coexist sympatrically with anautogenous mosquitoes, and they intermate readily in the laboratory, they generally remain genetically isolated in nature. In part, this isolation is due to differences in their breeding sites. In addition, anautogenous mosquitoes are said to be "eurygamous," meaning that their mating requires a space large enough to permit unrestricted flight. "Stenogamous" autogenous mosquitoes can mate in containers that are so small that opportunity for flight activity is denied. Genetic isolation between these populations is further insured by the reproductive inhibition that is evident in the progeny resulting from hybridization events. About a third are functionally autogenous and a third anautogenous. Vitellogenesis commences in the remaining third of hybrid females but without formation of mature eggs. Such mosquitoes are functionally sterile.

New infestations of autogenous *C. p. pipiens* mosquitoes appear to be founded by fewer females than are anautogenous infestations. They are capable of mating and producing eggs without leaving the site and are likely to produce clonal populations. Indeed, the relative insusceptibility of autogenous mosquitoes to inbreeding depression confirms their essentially inbreeding mode of perpetuation. The effective population size of anautogenous mosquitoes, on the other hand, appears to be much larger. We have applied allozyme technology to populations of these mosquitoes and find that loci of autogenous mosquitoes are far more likely to be fixed than are those of anautogenous *C. pipiens* (unpublished). Each autogenous infestation would be unique.

A model designed to represent these autogenous-anautogenous relationships is presented in Figure 9. The representation is based on the observation that eggs fail to embryonate when the water temperature falls below 15°C, that anautogenous mosquitoes generally fail to produce eggs when day length falls below 14 h 15 m, that anautogenous density generally increases 10-fold in two weeks and autogenous density in three weeks, and that intermating between the populations commences mainly when anautogenous males most outnumber autogenous males. Episodes of human biting appear to accompany episodes of interbreeding, and such events might be most likely when anautogenous males most outnumber autogenous males. If these considerations are correct, a complex of *C. p. pipiens* vector populations at latitude 42° would provide a seasonal "window" for amplifying West Nile virus among birds mainly during mid-May through mid-August, assuming favorable weather conditions. The same complex of mosquitoes would provide the "bridge vector" population that would carry the agent between its avian reservoir population and human hosts. The model (Fig. 9) suggests that anti-mosquito interventions or flushing rains occurring during June or July would reduce the force of transmission among birds while also reducing the likelihood that intermating events might induce the resulting mosquitoes to feed on people.

These considerations also suggest that latitude may profoundly limit the force of transmission of West Nile virus in North America. When reared at a short diel, the ovaries of anautogenous *C. p. pipiens* lack the juvenile hormone signal that stimulates formation of a yolk-competent oolemma. To the extent that anautogenous *C. p. pipiens* mosquitoes enter diapause in response to a short diel, the duration of the permissive breeding season will become progressively shorter with proximity to the equator. The length of the period that includes days that are 14 h 15 m long decreases sharply in the Middle Atlantic states and becomes nil at 32°N (Fig. 10). The duration of the permissive
period, however, decreases as latitude increases. This consideration suggests that the force of transmission of West Nile virus may increase toward the north of the New York City epicenter of this outbreak and decrease toward the south. An extension of the range of West Nile virus into more southern parts of North America remains in doubt.

Byrne and Nichols' allozyme analysis confirms, without citation, my prior finding that autogenous populations tend to perpetuate by inbreeding, anautogenous populations by outbreeding, and that interbreeding is infrequent. I demonstrated that individual breeding sites frequently contain autogenous as well as anautogenous larvae and that autogeny predominates as the season progresses. In my opinion, their observations are flawed because they characterized their study subjects by mass-examining samples of larvae from various breeding sites, without regard to season. They considered an entire population to be autogenous if any mosquito reared from the site at any time of year produced even a single egg raft. If the sampled mosquitoes deposited no eggs, the site would be characterized as anautogenous. Without evidence, they assumed that any autogenous mosquito would be anthropophilic and incapable of diapause.

In the course of this work, I established the biological basis for the hormonomimetic insecticide that is now used most extensively for eliminating sources of C. pipiens breeding. Mosquitoes, for the first time, were exposed to the preparation that later served as the model for synthesizing methoprene, then known as the Williams-Law mixture. I discovered that mosquitoes were vulnerable to this insecticide during their fourth larval instar, that nonviable juvenilized pupae would develop from treated larvae, and that adult ecdysis and terminalic rotation would be inhibited. The first semifield trials were conducted, and slow-release, plaster of paris formulations were field tested.
Populations of *C. p. pipiens* mosquitoes are structured in a complex manner, with genetically isolated autogenous populations coexisting sympatrically with sibling anautogenous populations. Anautogenous populations enter diapause in response to a photoperiodic cue and are almost exclusively ornithophilic. These vector mosquitoes and the force of transmission of West Nile virus may increase as latitude increases and may wane at lower latitudes. Transmission of West Nile virus may increase as anautogenous *C. p. pipiens* proliferate, and occasional episodes of interbreeding between these mosquitoes may provide the epidemiological bridge that results in human infection and disease.

ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health Grant #RO1 Al 44064.

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