PAROUS RATES OF OVERWINTERING *CULEX PIPIENS PIPIENS* IN NEW JERSEY[1]

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ABSTRACT During the winters of 1974–1975 and 1975–1976, hibernating *Culex pipiens pipiens* L. were collected from a large population at Fort Mott in Salem County, New Jersey and examined for parous rates to determine the percentage which ingested blood prior to diapause. The information was used to help assess the likelihood of *Cx. p. pipiens* harboring arboviruses over the winter in New Jersey. Parity was determined by the Detinova method of ovarian tracheolation as well as the stage of ovarian development in individual ovarioles.

Results for 1974–1975 showed that of 120 mosquitoes examined, 119 were nulliparous, with the single parous specimen taken early in the hibernating period. A further indication of nulliparity was obtained from the examination of individual ovarioles since all those examined were in stage N, the earliest stage in the developmental process. During 1975–1976, only 12 of 820 *Cx. p. pipiens* were parous, 6 of which were found in October. No parous mosquitoes were found during February or March. Data suggest that some parous mosquitoes enter hibernation, but few, if any, survive the winter. Even with a low percentage of the population which might exhibit gonotrophic dissociation, *Cx. p. pipiens* seems to be an unlikely source for overwintering virus.

INTRODUCTION

Human outbreaks of St. Louis encephalitis (SLE) have occurred sporadically in New Jersey during the past 12 years. An outbreak in the Camden area during 1964 resulted in 94 cases with 8 deaths (Goldfield et al. 1965). After a single case in 1965 (Kandle et al. 1967) no SLE was recognized until 1975, when a sizeable epidemic swept across many parts of the United States. A total of 29 cases and one death was recorded in New Jersey (Anonymous 1976). The suspected epizootic and epidemic vector, as in previous outbreaks, was the common house mosquito, *Culex pipiens pipiens* L. (Goldfield et al. 1965, Luby et al. 1969).

The overwintering mechanism of SLE virus has not been determined. Possibilities include latent infections in small rodents, birds, amphibians or reptiles, infection in migratory birds which return with virus in the spring and overwintering adult mosquitoes. *Cx. p. pipiens* is a bird feeder, a known SLE vector, and it hibernates as an adult female, making the mosquito a likely host for overwintering SLE. There is,

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however, contradictory evidence in the literature (Burdick and Kardos 1963, Eldridge 1968, Jumars et al. 1969, Hayes 1973, Sanburg and Larsen 1973). To act as an overwintering agent, the mosquito would have to blood feed prior to diapause. Jumars et al. (1969) found evidence of blood feeding in *Cx. pipiens* before hibernating, but other workers have not found signs of such activity in either *Cx. p. pipiens* (Hayes 1973) or the related species *Culex tarsalis* (Bennington et al. 1958, Blackmore and Dow 1962, Burdick and Kardos 1963). During the winters of 1974–1975 and 1975–1976 parous rates of hibernating *Cx. p. pipiens* were examined to determine the percentage which had oviposited prior to entering hibernation. The information was used to help assess the likelihood of *Cx. p. pipiens* harboring arboviruses over the winter in New Jersey.

MATERIALS AND METHODS

Collections were made from a large overwintering *Cx. p. pipiens* population at Fort Mott in Salem County, New Jersey. The fort, which faces southwest on the Delaware River, was constructed in 1872 to complement batteries on Pea Patch Island in mid-river and Fort DuPont in the State of Delaware. The fort was closed in 1943 but was reopened as a state park in 1951.

The fort is a concrete structure built into a hillside with about 30 individual shell storage rooms approximately 12 ft wide by 20 ft long by 10 ft high. Early in the season mosquitoes can be found in most rooms. Later in the winter adult *Cx. p. pipiens* females seem to retreat to the rooms farthest from the entrances, where temperature and humidity are high enough to assure survival. The mosquitoes are found on the walls and ceilings, especially in areas where the white plaster is missing and the gray concrete subceiling is exposed.

During 1974–1975, 20 mosquitoes were collected once each month from November to early April. In 1975–1976, at least 120 specimens were taken each month at intervals of 1–2 weeks and sampling extended from early October through March.

Mosquitoes were collected from the walls and ceilings of the fort by hand aspiration, transferred to pint containers and transported to the laboratory. They were temporarily immobilized in a freezer and transferred to a 10 cm petri dish lined with damp filter paper. The petri dish was taped shut, labeled and placed in an inflated, sealed plastic bag which kept the humidity high and prevented desiccation. The mosquitoes were then frozen and subsequently dissected.

Dissections were performed under a stereoscopic microscope at 10X. The ovaries were removed from the mosquito and placed in a drop of distilled water, rinsed thoroughly and placed in a final drop to dry. A template was used to allow even spacing of 20 pairs of ovaries per slide.

Parity was determined by the Detinova method of ovarian tracheolation (Detinova 1962). The presence of tightly coiled tracheolar skeins was considered an indication of nulliparity; uncoiled tracheoles indicated a prior oviposition and a prior blood meal. As an added indication of parity or nulliparity, each mosquito collected during 1974–1975 was also examined for the stage of gonotrophic development according to Christophers (1911). Individual ovarioles from one of the ovaries were separated and examined for yolk deposition. Stage N through stage I–II were considered an indication of nulliparity in overwintering specimens; ovarioles in stages II through V indicated a possible oviposition and the chance of a prior blood meal (Detinova 1962, Clements 1963).

RESULTS AND DISCUSSION

Of the 120 mosquitoes which were examined during 1974–1975 (Table 1) 119 were nulliparous indicating that almost all of the overwintering *Cx. p. pipiens* had not oviposited before diapause. The single parous specimen was taken in early December near the beginning of the hibernation period; only nulliparous mosquitoes were collected from January through April. Added proof of nulliparity was obtained from the examination of individual ovarioles for yolk deposition. Table 2 shows that all of the specimens collected during the winter months had ovarioles in stage N, the earliest stage in development. A single specimen collected in April had its ovarioles in stage I–II. Data indicated that none of the specimens examined had oviposited prior to entering hibernation. Data also suggest that if parous specimens did enter hibernation, few or none survived the winter.

During 1975–1976, 820 overwintering females were examined and only 12 parous specimens were detected. Six of the 12 were collected in October; 2 were found in November, and the remaining 4 were taken in December and January. Collections in February and March yielded only nulliparous mosquitoes. Data suggest that some parous *Cx. p. pipiens* do enter hibernation, but few, if any, survive the entire winter.

Eldridge (1968) reported gonotrophic dissociation in *Cx. p. pipiens* under laboratory conditions which questioned the validity of basing blood feeding solely on parity. His studies suggested that a small percentage of the population might blood feed in the fall and develop fat body instead of eggs with the protein from the blood meal. Since the eggs do not develop, the tracheolar skeins of the ovaries would not uncoil even though the mosquitoes had blood-fed. Mosquitoes exhibiting gonotrophic dissociation would appear nulliparous even though they may have ingested virus particles with a prior blood meal. The importance of gonotrophic dissociation in the overwintering maintenance of mosquito-borne viruses has not yet been demonstrated. Gonotrophic dissociation after a blood meal can be induced under laboratory conditions (Eldridge 1968) but its frequency of occurrence in natural populations of *Cx. p. pipiens* is not known. Prehibernating female *Cx. p. pipiens* are known to enter a condition of ovarian diapause

Table 1. Parous rates of *Culex pipiens pipiens* L. collected from hibernation during the winters of 1974–1975 and 1975–1976.

	1974–1975		1975–1976	
Month	P	N	P	N
Oct.	–	–	6	134
Nov.	0	20	2	158
Dec.	1	19	1	159
Jan.	0	20	3	117
Feb.	0	20	0	120
Mar.	0	20	0	120
Apr.	0	20	–	–
	1	119	12	808
Total	.8%	99.2%	1.5%	98.5%

Table 2. Christophers' stage of ovarian development in *Culex pipiens pipiens* L. collected from hibernation during the winter of 1974–1975.

	Christophers' Stage			
Month	N	I	I–II	II–V
Nov.	20	0	0	0
Dec.	20	0	0	0
Jan.	20	0	0	0
Feb.	20	0	0	0
Mar.	20	0	0	0
Apr.	19	0	1	0
	119	0	1	0
Total	99.2%	0	.8%	0

in late summer where a combination of cool temperatures and shortened daily photophases result in cessation of ovarian development (Spielman and Wong 1973). Blood feeding by *Cx. p. pipiens*, however, is also drastically reduced under these same conditions in the laboratory (Eldridge 1968) and evidence leads one to suspect that blood feeding may also be reduced in nature. Spielman and Wong (1973) reported that *Cx. p. pipiens* rarely fed on blood in nature after the 1st week of August in the Boston area and suggested that the mosquitoes entered diapause without dissociated feeding.

If prehibernating female *Cx. p. pipiens* do feed on blood, gonotrophic dissociation could assume an important role in the overwintering mechanism of mosquito-borne viruses. Current research, however, has not yet indicated that the phenomenon is common in the overwintering behavior of *Cx. p. pipiens*.

The present study showed that small numbers of parous females enter hibernation and few, if any, survive an entire overwintering season. The vast majority of the overwintering population is nulliparous, thus, unless future research indicates that ovarian diapause is preceded by blood feeding, *Cx. p. pipiens* does not seem to be a likely source for overwintering virus.

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