THE RELATIONSHIP BETWEEN SIZE AND PARITY STATUS OF FIELD COLLECTED *CULISETA MELANURA*

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ABSTRACT. The relationship between body size and parity status of adult female *Culiseta melanura* collected from 3 locations in northeastern United States was studied by measuring wing lengths and examining ovaries of individual mosquitoes. Virus isolation was attempted from *Cs. melanura* collected in Maryland and in New Jersey. At all 3 locations, the size of *Cs. melanura* collected varied from large in the spring, to small in the summer. In New Jersey and Maryland mosquitoes collected in the fall were again large. The size of Massachusetts mosquitoes collected in the summer versus the fall was not different. In general, parous mosquitoes were larger than nulliparous mosquitoes in the spring but smaller than nulliparous ones in the fall. Eastern equine encephalomyelitis (EEE) and Highlands J (HJ) viruses were recovered from *Cs. melanura* only during the late summer when mosquitoes were small or during the fall months when larger mosquitoes were collected. We conclude that there is no detectable association between *Cs. melanura* size and parity status and that there is no obvious effect of mosquito size on EEE or HJ virus transmission.

INTRODUCTION

The size of an insect may be biologically important in that it can affect life expectancy, dispersal, fecundity, and in the case of mosquitoes that transmit arboviruses, ability to become infected and transmit a virus (Grimstad and Haramis 1984, Reisen et al. 1984, Smith 1972, Takahashi 1976). For example, small *Culex tritaeniorhynchus* Giles nutritionally deprived as larvae transmitted Japanese encephalitis virus at a higher rate than larger ones fed a normal larval diet (Takahashi 1976). Grimstad and Haramis (1984) found that in the *Aedes triseriatus* (Say)-La Crosse virus transmission cycle, nutritionally deprived small mosquitoes had a lower threshold of infection than larger ones, suggesting that the smaller members of this species may be more efficient virus vectors. Landry et al. (1988) concluded that the probability of survival for *Ae. triseriatus* infected with La Crosse virus is not affected by body size. However, other studies suggest that large females produce more eggs per egg raft (Bock and Milby 1981), have a higher parity rate (Haramis 1983, Nasci 1986a, 1986b, 1988), and live longer than smaller conspecifics (Reisen et al. 1984, Hawley 1985). Such increases in life expectancy and reproduction rates of larger females may counteract any possible decrease in the efficiency of viral transmission associated with larger mosquitoes (Patrican and DeFoliart 1985).

Therefore, results from studies of mosquito size variation and its significance to arbovirus transmission lead to conflicting predictions. On one hand, results from laboratory studies suggest that small mosquitoes are infected and transmit virus more efficiently than larger mosquitoes (Takahashi 1976, Grimstad and Haramis 1984, Patrican and DeFoliart 1985). On the other hand, field studies provide evidence that small mosquitoes may not live as long as larger conspecifics (Haramis 1983, Reisen et al. 1984, Hawley 1985, Nasci 1986a, 1986b, 1988). Thus, larger mosquitoes would have a more important role in virus transmission because their probability of becoming infected and subsequently transmitting virus is greater than that of smaller cohorts.

This study examined the relationship between size variation and parity, a measure of blood-feeding history, for an arbovirus mosquito vector. We sought to determine: 1) if large *Culiseta melanura* (Coq.) are parous more often than small cohorts, and 2) if large size is positively associated with virus infection. *Culiseta melanura* is the enzootic vector of eastern equine encephalomyelitis (EEE) and Highlands J (HJ) viruses in North America (Scott and Weaver 1989). To determine if adult body size of *Cs. melanura* is a factor in the mosquito's ability to obtain a blood meal and lay eggs, we compared size and parity status of adults collected from resting boxes in Maryland, New Jersey and Massachusetts. The effect of adult body size on the ability of *Cs. melanura* to become infected and possibly transmit EEE virus was determined by comparing trends in body size with virus infection rates of mosquitoes collected in Maryland and New Jersey.

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MATERIALS AND METHODS

Maryland mosquito collections: During 1985 and 1986 *Cs. melanura* were collected from the Pocomoke Cypress Swamp, an area known for large *Cs. melanura* populations and endemic EEE and HJ virus activity (Saugstad et al. 1972, Watts et al. 1987, Scott et al. 1987). Mosquitoes were collected from 20 resting boxes (Edman et al. 1968), placed in an upland forest habitat and in CDC light traps augmented with dry ice, placed in the swamp. Resting boxes were within 100 m of the edge of the swamp and about 400 m from the light traps. Resting box collections were used for the size-parity comparisons and virus isolation was attempted from light trap collections. Some specimens collected during 1986 from resting boxes were examined for size and virus infection. Collections during 1985 were made once each week, beginning with the first week in April and ending with the first week of November. The 1986 collections were made weekly from the end of April through the first week of October.

New Jersey mosquito collections: All specimens were collected during 1987 from a 10-ha stand of mature white pine (*Pinus strobus* L.) within Belleplain State Forest, 3 km west of Dennisville. Specimens examined for size and parity were collected from resting boxes. Mosquitoes that were assayed for virus were collected from resting boxes as well as CDC light traps augmented with dry ice. Collections were made once each week from June through mid-October. Light traps were set in 3 distinct habitats: the mature white pine stand where the resting box collections were made, an adjacent hardwood forest and a nearby Atlantic white cedar (*Chamaecyparis thyoides* L.) swamp.

Massachusetts mosquito collections: Collections were made during 1987 and 1988 from resting boxes placed in areas with an open understory within the Hockamock Swamp near Raynham, MA (Nasci and Edman 1981). During 1987, mosquitoes were collected weekly from the middle of June through August 1 and during 1988 collections were made weekly from the last week of May through the third week of September. Specimens were examined for size and parity status.

Determination of size and parity status: Body size was determined by measuring wing length (Christophers 1960) and parity status was determined with the ovarial tracheole method of Detinova (1962). To determine if wing length was a sensitive measure of *Cs. melanura* size, the linear relationship between wing length and dry body weight was tested. Wing lengths and dry weights were compared for both colonized and wild *Cs. melanura.* There is no record of the number of generations for our colony, which was initiated in 1967 with material collected in Farmington, CT (Wallis and Whitman 1969). Fifteen *Cs. melanura* from our colony and a subsample ($n = 42$) collected from Maryland resting boxes from May through September 1986 were lyophilized and weighed on an electronic balance (Cahn 27, automatic electrobalance). One wing was removed from each mosquito, placed on a microscope slide and held under a coverslip, and measured from the axillary incision to the wing tip, excluding the fringe scales, using an ocular micrometer.

Ovaries were dissected into a drop of distilled water and then air-dried (Meadows 1968). Ovarian tracheoles were then examined with a compound microscope for the presence of tracheal skeins (coils); skeins become irreversibly stretched after the completion of an ovarian cycle (Detinova 1962). Mosquitoes were assumed to have completed at least one gonotrophic cycle and were graded as being parous, if tracheoles were straight and with only 1 or 2 coils (Burdick and Kardos 1963).

Statistical analysis: The size-parity comparisons for each year and study site were subjected to an analysis of covariance (SAS Institute 1985). Six variables were tested for significance in each of the models. The data was submitted to regression analysis using Proc GLM (SAS Institute 1985). In addition, the collections were divided into 2 parts (April through July and August through November) and size-parity status was analyzed separately using a test for equality of 2 percentages (Sokal and Rohlf 1969, Nasci 1986a). The first time period (April through July) is when few EEE or HJ virus isolates are recovered from *Cs. melanura* or from vertebrate hosts and the second period (August through November) is when almost all EEE and HJ virus isolations are recovered from northeastern North American (Scott and Weaver 1989). To permit inference concerning the importance of environmental stress on body size of *Cs. melanura,* we determined the coefficients of variation (CV) of wing size for each population sampled. The test used for normality was the Kolomogorov D statistic (PROC UNIVARIATE, SAS Institute 1985).

Virus isolations: The isolation and identification of virus from mosquitoes collected in Maryland were carried out in baby hamster kidney and African green monkey cell cultures following the methods of Scott et al. (1987). *Culiseta melanura* collected during 1985 and 1986 in light traps were assayed for virus in pools of 50 or fewer mosquitoes. Six-hundred mosquitoes collected during 1986 in resting boxes were assayed individually for virus after their wings had been removed and measured.

Culiseta melanura collected in New Jersey were grouped in pools containing 50 or fewer mosquitoes and inoculated into White Leghorn chicks less than 12-h-old using the methods of Crans et al. (1986). Brain suspensions of chicks that became ill were inoculated into fresh chicks, suckling mice and duck embryo cell culture. Virus identification was made by fluorescent antibody assay of the cell culture preparations.

Temperature: Water temperatures in the Pocomoke Cypress Swamp, Maryland, were recorded hourly using a Datapod (Digital recorder Omnidata) from May 2, 1985, to October 28, 1985. Temperatures were taken from a known *Cs. melanura* larval habitat located in an area approximately midway between the light traps and the resting boxes.

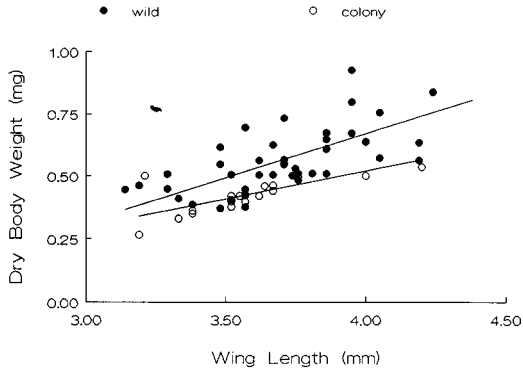

Fig. 1. The relationship between dry body weight and wing length for female *Culiseta melanura* from a laboratory colony (y = −1.1431+0.4397x, r = 0.92, *n* = 15) and from collections made at the Pocomoke Cypress Swamp, Maryland (y = −0.8120+0.3728x, r = 0.52, *n* = 42).

RESULTS

The relationship between dry body weight and wing length was found to be linear for both colonized and wild caught *Cs. melanura* (Fig. 1). Thus wing length is a reliable measure of body size.

In Maryland and New Jersey during all 3 seasons the size of *Cs. melanura* collected varied from large in the spring, to small in the summer, to large again in the fall (Fig. 2). Massachusetts mosquitoes did not show the fall increase in size; the 1987 Massachusetts collections were made only from June through the first week of August and the 1988 collections stopped during the 3rd week of September. Mosquitoes collected in the fall did not attain the size of mosquitoes collected during the early spring from any of the study sites or years sampled. The coefficient of variation of mean wing length for all locations and years ranged from 7.07 to 9.40% (Table 1). For specimens collected in Maryland during 1985, New Jersey during 1987, and Massachusetts during 1987, there was a significant interaction ($P < 0.05$) between parity status and the collection date (Fig. 2). Therefore, the relationship between size and parity varies with time, and parity status does not respond to size in the same way throughout the year. Consequently, no direct comparisons can be made between size and parity status for specimens from those 3 collections and dates. The data from mosquitoes collected in Maryland during 1986 and Massachusetts during 1988 showed no such interaction, but there was no significant difference in mosquito size by parity status (Fig. 2).

Regression analyses showed that models of *Cs. melanura* size variation over time vary within and between sites of collections. Depending on the year and collection site, the following variables had an effect on *Cs. melanura* size: date, date squared, date cubed, parity status, date × parity, and date squared × parity.

When mosquitoes were analyzed within years and with the year divided in 2 parts using the test for equality of 2 percentages, larger mosquitoes had a significantly higher parity rate than smaller ones in the spring of 1985 in Maryland and the spring of 1987 in Massachusetts. While not significantly different, larger mosquitoes had a higher parity rate in the spring of 1986 in Maryland and 1987 in New Jersey. In the fall the reverse happened: smaller mosquitoes had a significantly higher parity rate during 1985 in Maryland and 1987 in New Jersey. In the fall large mosquitoes from 1986 in Maryland and 1988 in Massachusetts tended to have a higher parity rate, but not significantly greater than the smaller ones (Table 2). The sample size from Massachusetts in the fall of 1987 was too small to analyze with this method.

During 1986, 5 viral isolates were recovered from pools of *Cs. melanura* (2,713 mosquitoes in 83 pools) collected in Maryland; all isolates were from mosquitoes collected during late September through late October (Table 3). Two of these isolates were EEE virus and 3 were HJ virus. No viral isolations were made from the 600 individual mosquitoes collected from resting boxes in Maryland during 1986. Data concerning virus isolations from mosquitoes collected during 1985 in Maryland are presented by Scott et al. (1987).

During 1987, 11 viral isolates (6 EEE and 5 HJ) were recovered from *Cs. melanura* (7,884 mosquitoes in 308 pools) collected in New Jersey (Table 4). A single HJ isolation was recovered in June, 2 HJ and 1 EEE virus strains were recovered in July, 2 HJ and 2 EEE virus strains

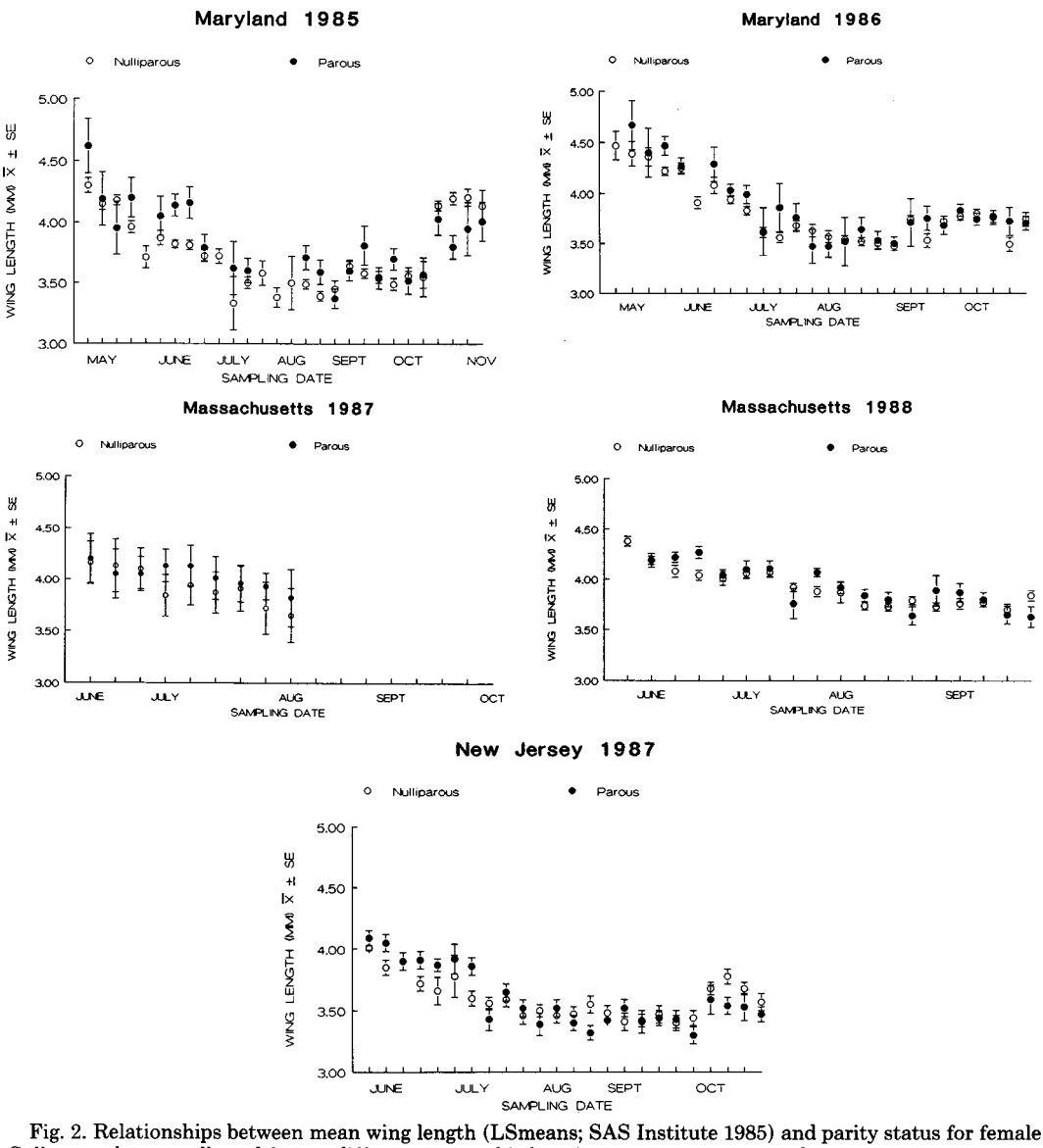

Fig. 2. Relationships between mean wing length (LSmeans; SAS Institute 1985) and parity status for female *Culiseta melanura* collected from 3 different geographic locations over a 4-year period.

Table 1. Coefficients of variation (CV) of mean wing length for *Culiseta melanura* collected from 3 different geographic locations during a 4-year period. MD = Maryland, NJ = New Jersey, and MA = Massachusetts.

Year and location	No. mosquitoes examined	Mean wing length ± SE (mm)	CV
1985 MD	531	3.80 ± 0.015	9.40
1986 MD	797	3.80 ± 0.013	9.31
1987 NJ	761	3.65 ± 0.012	9.02
1987 MA	386	3.91 ± 0.012	7.40
1988 MA	512	3.93 ± 0.012	7.07

were recovered in August, and 2 EEE virus strains were recovered in September.

Water temperature monitored in Maryland during 1985 varied from a low of 13°C in May to a high of 19°C in September and back down to 15°C at the end of November (Fig. 3).

DISCUSSION

Seasonal variation in size of *Cs. melanura* (Fig. 2) is not surprising because of the great number of physical parameters that affect mosquito development (Christophers 1960) and be-

Table 2. Percent parity of *Culiseta melanura* collected during 1985–1988 from 3 different locations in northeastern United States, by class size and time of collection (transmission of eastern equine encephalomyelitis and Highlands J virus is rarely detected during the spring but is regularly detected during the fall).

Year	Class size group (Z score)	No.	% of population in group	No.	% parous
Spring (April–July)					
1985 MD	+2,+3	29	12.7	9	31.0[a]
	−1,+1	180	78.6	14	7.8
	−2,−3	20	8.7	1	5.0
1986 MD	+2,+3	122	47.8	21	17.2
	−1,+1	121	47.5	15	12.4
	−2,−3	12	4.7	1	8.3
1987 NJ	+2,+3	108	29.0	43	39.8
	−1,+1	237	63.5	79	33.3
	−2,−3	28	7.5	9	32.1
1987 MA	+2,+3	50	14.3	18	36.0[a]
	−1,+1	258	73.9	73	28.3
	−2,−3	41	11.8	3	7.3
1988 MA	+2,+3	57	22.4	21	36.8
	−1,+1	183	72.0	60	32.8
	−2,−3	14	5.5	3	21.4
Fall (Aug–Oct)					
1985 MD	+2,+3	48	15.9	1	2.1[a]
	−1,+1	179	59.3	43	24.0
	−2,−3	75	24.8	15	20.0
1986 MD	+2,+3	19	3.5	5	26.3
	−1,+1	409	75.5	87	21.3
	−2,−3	114	21.0	13	11.4
1987 NJ	+2,+3	14	3.6	2	14.3[a]
	−1,+1	271	69.9	111	41.0
	−2,−3	103	26.6	50	48.5
1987 MA	+2,+3	1	2.7	1	100.0[b]
	−1,+1	19	51.4	10	52.6
	−2,−3	17	46.0	6	35.3
1988 MA	+2,+3	6	2.3	2	33.3
	−1,+1	139	53.9	42	30.2
	−2,−3	113	43.8	23	20.4

[a] Significant differences ($P < 0.05$) in % parous mosquitoes in large (Z = +2,+3) vs. small (Z = −2,−3) class size groups as determined by test for equality of two percentages (Sokal and Rohlf 1969).
[b] Sample size too small for meaningful analysis.

Table 3. Virus isolations from *Culiseta melanura* collected in Maryland during 1986.

Month	Resting box collections			Light trap collections		
	No. mosq.	No. pools	No. virus isolations	No. mosq.	No. pools	No. virus isolations
May	74	2	0	105	6	0
June	2	1	0	86	5	0
July	0	0	0	145	7	0
Aug.	12	2	0	451	14	0
Sept.	364	10	2[a]	1,260	27	0
Oct.	63	3	2[b]	151	6	1[c]
Total	515	18	4	2,198	65	1
Grand total	2,713	83	5			

[a] Both isolates were Highlands J (HJ) virus.
[b] Both isolates were eastern equine encephalomyelitis virus.
[c] Virus isolate was HJ virus.

Table 4. Virus isolations from *Culiseta melanura* collected in New Jersey during 1987.

Month	Resting box collections			Light trap collections		
	No. mosq.	No. pools	No. virus isolations	No. mosq.	No. pools	No. virus isolations
June	517	20	0	601	27	1[a]
July	2,637	64	2[b]	1,278	57	1[a]
Aug.	457	18	2[b]	924	50	3[c]
Sept.	1,102	37	1[d]	322	31	1[d]
Oct.	46	4	0	0	0	0
Total	4,759	143	5	3,125	165	6
Grand total	7,884	308	11			

[a] Virus isolate was Highlands J (HJ) virus.
[b] One isolate was HJ virus and the other was eastern equine encephalomyelitis (EEE) virus.
[c] One isolate was HJ virus and 2 were EEE virus.
[d] Virus isolate was EEE virus.

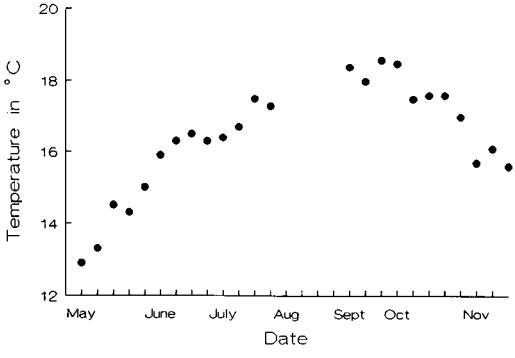

Fig. 3. Weekly mean water temperatures calculated from hourly readings during 1985 in a known *Culiseta melanura* larval habitat in the Pocomoke Cypress Swamp, Maryland.

cause variation in size has been reported for many other mosquito species (Fish 1985). Temperature of larval habitats (Fig. 3) alone might account for much of the size variation in adult *Cs. melanura* by altering the duration of larval development. Leprince and Bigras-Poulin (1988) found that the size of *Tabanus quinquevittatus* Wied. decreased during each of 2 years studied. They attributed this decrease in size to a specific period of emergence that is controlled by environmental factors. Larvae that develop and emerge during one summer were small because of their short development period. Larvae with protracted development through the winter emerged the following spring as larger adults. Similarly, Mullens (1987) showed that the size of adult *Culicoides variipennis* (Coq.) in California was inversely related to ambient air temperature. He concluded that cool temperatures prolonged larval development and resulted in the emergence of large adults.

We speculate that because *Culiseta melanura* overwinter as larvae (Joseph and Bickley 1969), the first adults emerging in the spring would be from larvae which have a protracted development period during the winter months and an opportunity to grow to a large size. As the mosquito season progresses these large adults die and are replaced by smaller adults that have a shortened larval development period, due to increasing water temperatures (Fig. 3). As water temperatures decrease during late summer and fall, larval development is again drawn out and adult size increases correspondingly. This increase in size was not detected in Massachusetts presumably because of the shorter period of time during the fall that adult mosquitoes emerge and are active.

There are at least two explanations for differences among results in the comparison between parity rate and size that we found with *Cs. melanura* and results published for other mosquito species (Haramis 1983, Nasci 1986a, 1986b, 1988). First, we sampled and analyzed specimens throughout the mosquito season. By conducting our analysis throughout the season that adults were active, we were able to find the date-size interaction which would have been missed if mosquitoes were collected during only part of the season or if the data analysis was based on specimens that had been grouped across collection dates. The second explanation for the disparity between our study and those of others is the mosquito species we studied. *Culiseta melanura* is a multivoltine permanent water species that overwinters as larvae. Its relatively low CV for wing length (Table 1) implies that this species is less limited by larval food than other species (Fish 1985). Differences in parity rates may be more easily detected in species with shorter larval development times or more crowded larval populations such as *Ae. triseriatus* and *Ae. aegypti* (Linn.), which exhibit greater intraspecific size variation than *Cs. melanura.*

The CV values we found for *Cs. melanura* were lower than any that Fish (1985) found while analyzing 10 species of North American mosquitoes. Environmental constraints placed on *Cs. melanura* may not be great enough to

result in detectable negative effects on the development of this mosquito.

For some of our collections, we found that parous *Cs. melanura* were larger than the nulliparous mosquitoes in spring and early summer, but smaller than nulliparous mosquitoes in the late summer and fall (Fig. 2). An explanation for this seasonal size variation in the parous versus nulliparous *Cs. melanura* is the age structure of the population at the time of collection. Parous mosquitoes are more likely to be older than nulliparous mosquitoes. Parous mosquitoes collected during late spring and early summer probably emerged during the time period when the individual members of the population as a whole were large. They would, therefore, be larger than mosquitoes that had recently emerged during the summer. In the fall the reverse would be true, the older mosquitoes would be from a time period when the smaller summer mosquitoes had emerged and were most abundant.

Even though mosquitoes were small when virus isolations were made, we were unable to determine if the size of *Cs. melanura* is an important factor in the transmission of EEE or HJ virus. Because we did not isolate virus from the individual mosquitoes we examined nor did we test the hypothesis in the laboratory that smaller *Cs. melanura* are more susceptible to EEE virus infection (Grimstad and Haramis 1984), we were unable to analyze the association between mosquito size and virus transmission. However, because of the low coefficients of variation for *Cs. melanura* (Table 1), it is doubtful there is enough variation in the size of this mosquito for size to be a major factor in the dynamics of EEE or HJ virus transmission.

We conclude that size is not a determining factor for the parity status of *Cs. melanura.* This trend was found during 4 consecutive years and in 3 geographic regions (Fig. 2). We used parity status as a measure of mosquito blood-feeding success and survival. Mosquito size, therefore, did not account for variation in these important parameters of vectorial capacity (Macdonald 1957). We also conclude that there is no obvious cause and effect relationship between the size of *Cs. melanura* and EEE or HJ virus transmission.

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In conducting the research described in this report, the investigators adhered to the *Guide for the care and use of laboratory animals*, as promulgated by the National Research Council.

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