EFFECT OF BODY SIZE ON THE VECTOR COMPETENCE OF FIELD AND LABORATORY POPULATIONS OF *AEDES TRISERIATUS* FOR LA CROSSE VIRUS

SALLY L. PAULSON[1] AND WILLIAM A. HAWLEY[2]

ABSTRACT. *Aedes triseriatus* females collected as pupae from 2 tireyards and one woodlot were tested for susceptibility to La Crosse virus infection and ability to transmit the virus to suckling mice. Rates of disseminated infection and oral transmission were inversely correlated with mean body size. The smallest population developed disseminated infections and transmitted virus at significantly higher rates than the 2 larger populations. Adults derived from F_1 eggs of the largest and smallest populations showed no significant differences in rates of disseminated infection and transmission, suggesting that the differences between the field populations were primarily caused by larval rearing conditions. When pupae of 2 strains of *Ae. triseriatus* were arranged into discrete size groups and tested for susceptibility to virus, an inverse relationship was observed between rate of disseminated infections and mean pupal wet weight. In contrast, *Ae. hendersoni* exhibited low transmission rates (7–10%) regardless of body size.

INTRODUCTION

Aedes triseriatus (Say) commonly breeds in artificial containers, especially discarded tires, as well as in its natural tree hole habitat (Jenkins and Carpenter 1946, Baumgartner 1988). Emerging adults from both tree holes and tires vary greatly in body size (McCombs 1980,[3] Haramis 1984). When artificial diets are used to create size variation in this species, vector competence for La Crosse virus (LACV) is inversely correlated with body size for both oral and transovarial transmission (Grimstad and Haramis 1984, Patrican and DeFoliart 1985). Thus, larval nutrition may influence vector competence. However, genetic factors may also play a role, since geographic populations vary in vector competence (Grimstad et al. 1977). In this paper, we show that field-collected populations which differ in body size also differ in vector competence and virus susceptibility and that the differences in body size and vector competence are primarily environmentally induced. We discuss our results in relation to the vectorial capacity of *Ae. triseriatus* for LACV.

MATERIALS AND METHODS

Origin of mosquito strains: The TREE HOLE strain of *Ae. triseriatus* was collected from tree holes in American beech (*Fagus grandifolia* Ehrh.) and sugar maple (*Acer saccharum* Marsh) trees in Bendix Woods County Park, St. Joseph

[1] Department of Biological Sciences, Florida Atlantic University, Boca Raton, FL 33431.
[2] Vector Biology Laboratory, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556.
[3] McCombs, S. D. 1980. Effect of differential nutrition of larvae on adult fitness of *Aedes triseriatus*. M.S. Thesis, Univ. of Notre Dame, IN, 123 pp.

County, IN. Pupae from approximately 10 tree holes were used. The TIRES-1 strain originated from tires located in a commercial auto salvage yard in South Bend, St. Joseph County, IN. This site contains thousands of discarded tires, but sampling was restricted to 10 tires situated in a partially shaded section at the northern perimeter of the tireyard. The TIRES-2 strain was collected from tires in a small tire dump, approximately 30 tires, located in South Bend, IN. These tires were located in a woodlot composed primarily of sugar maple, American beech and black oak (*Quercus velutina* Lam.), and were completely shaded. Collections were made on June 25 and 26, 1984. Pupae were held in an insectary at 21°C until adult emergence.

To obtain eggs for laboratory rearing, 3 ovitraps equipped with presoaked balsawood paddles were fixed at ground level in the Bendix Woods site. Egg paddles were collected on July 3 and August 6, 1984. At the TIRES-1 site, 3 ovitraps were also set out but few eggs were deposited. Instead, females were collected on July 14, 1984, with a battery powered aspirator (Nasci 1981), placed in cages, blood fed and allowed to oviposit.

The FORT WAYNE strain of *Ae. triseriatus* was collected as eggs from ovitraps in Fort Wayne, Allen County, IN, in August 1985. The WALTON strain of *Ae. triseriatus* was a laboratory colony established from mosquitoes collected in the Izaac Walton Preserve, St. Joseph County, IN, in June 1969. The generation tested is not known. The YANKEE WOODS strain of *Aedes hendersoni* Cockerell was collected as eggs from ovitraps in Yankee Woods Forest Preserve, Cook County, IL, in August 1983. The fifth laboratory generation was used in this study.

Mosquito rearing techniques: Eggs were hatched in a nutrient broth solution (Novak and Shroyer 1978). First instar larvae were distributed 300 per pan in 1 liter of water and fed a

Table 1. Mean wing lengths and rates of infection and transmission of LACV by field-collected and laboratory-reared populations of *Aedes triseriatus*.

Source	No.	Rearing conditions	Wing length (mm)		% disseminated infections	% transmitting
			Mean	Range		
TIRES-1	29	Field	3.46 a*	(2.86–4.44)	86 e**	66 g
	30	Laboratory	3.84 b	(3.26–4.13)	67 e,f	43 g,h
TIRES-2	25	Field	3.97 b,c	(3.21–4.49)	40 f	32 h,i
TREE HOLES	19	Field	4.20 c,d	(3.67–4.69)	53 f	21 i
	22	Laboratory	4.06 d	(3.11–4.49)	59 f	32 h,i

* Means followed by different letters are significantly different (1-way ANOVA, $P < 0.001$; Duncan multiple range test, $P < 0.05$).

** Rates followed by different letters (by column) are significantly different (G-test of independence, $P < 0.05$).

standardized solution of liver powder *ad lib.* To produce mosquitoes of different size classes, first instar larvae were distributed 100 per pan in 1 liter of tap water and fed according to the dietary regime of McCombs (1980[3]) as described in Grimstad and Haramis (1984).

Size determinations: Wing length was selected as a criterion for comparing size of adult mosquitoes since it is easily measured and is positively correlated with dry weight (McCombs 1980[3]). Wings were removed with forceps, placed on a slide and covered with a coverslip. Length was measured under a dissecting microscope with an ocular micrometer. Measurements were taken from the proximal edge of the costal scales to the most distal point on the lateral margin, excluding the wing fringe.

Pupal wet weight was used to generate nonoverlapping size groups of mosquitoes before infection because it is difficult to accurately measure the wings of live specimens. Individual pupae were sexed under a dissecting microscope. Females were dried briefly on a paper towel and then weighed on an analytical balance to the nearest 0.1 mg.

Mosquito infection: Approximately 7 days after emergence, mosquitoes were allowed to feed on a virus suspension in defibrinated, LACV antibody-free rabbit or human blood contained in an artificial membrane feeder (Rutledge et al. 1964) equipped with a Baudrauche® membrane (J. Long Inc., Belleville, NJ). Individuals that failed to fully engorge after a 30-min feeding period were removed. The remainder were held for a 3-wk extrinsic incubation period at 21°C. A sample of the infectious blood was taken at the end of the feeding period, and virus content was determined by titration using the methods of Grimstad and Haramis (1983). The endpoint titers ($TCID_{50}/0.025$ ml) of the blood meals were $10^{4.6}$–$10^{4.8}$ in the experiments with the TREE HOLE, TIRES-1 and TIRES-2 strains, and $10^{5.5}$ in the WALTON, FORT WAYNE and YANKEE WOODS experiments.

The LACV used in all experiments was isolated from adult *Ae. triseriatus* collected in Muncie, IN (Pinger et al. 1983), and was in its third suckling mouse passage.

Measurement of mosquito infection and transmission: Oral transmission was demonstrated by allowing individual mosquitoes to feed on 1- to 2-day-old suckling mice (Harlan ICR strain). Mice were marked and then monitored for 7 days. Sick or moribund mice were removed and frozen at −70°C.

Disseminated infections were identified by detection of La Crosse viral antigen in head squash preparations using an indirect immunofluorescent antibody staining procedure (Paulson et al. 1989). Alternately, the virus content of the separated thorax and abdomen of individual mosquitoes was determined by plaque assay on African green monkey kidney (VERO) cells. Specimen preparation and plaque assay techniques have been described elsewhere (Paulson et al. 1989, Grimstad and Haramis 1983). Individuals that contained virus both in the thorax and abdomen were considered to have a disseminated infection. Those with virus limited to the abdomen only were determined to have a nondisseminated infection. These 2 methods yield roughly equivalent results (Grimstad and Haramis 1983).

Statistical analysis: Rates of infection and transmission by groups within experiments were compared using the G-test with Williams' correction (Sokal and Rolhf 1981). The wing length distributions were analyzed by one-way ANOVA followed by Duncan's multiple range test (Steel and Torrie 1980).

RESULTS

Size and vector competence of field-collected Ae. triseriatus: The 3 populations collected as pupae had significantly different mean wing lengths; the 2 tireyard populations were smaller than the tree hole population (Table 1). The

field-reared tree hole population showed a significantly lower rate of disseminated infection than the smallest field-reared tireyard population ($P < 0.05$) (Table 1). The smaller tireyard population transmitted virus at a significantly higher rate than the other 2 field-reared populations ($P < 0.05$); the rates of transmission by the tree hole population and the second tireyard population did not differ significantly ($P > 0.10$) (Table 1).

When the TREE HOLE and TIRES-1 larvae were reared in the laboratory on a 1× diet (McCombs 1980[3]), the resulting mosquitoes were intermediate in size compared with the field-reared populations (Table 1). The larger, laboratory-reared TIRES-1 mosquitoes developed disseminated infections at a lower rate than the smaller, field-collected individuals (67 vs. 86%) and transmitted at a correspondingly lower rate (43 vs. 65%). A decrease in size in the TREE HOLE mosquitoes resulted in an increase in infection and transmission rates (Table 1). None of these differences were statistically significant ($P > 0.10$). However, when the mosquitoes from these 5 populations were placed in 10 non-overlapping groups based on wing length, an inverse relationship between rate of transmission and size was observed ($P < 0.005$) (Fig. 1).

Size and vector competence of laboratory-reared Ae. triseriatus *and* Ae. hendersoni: Larvae of the WALTON and FORT WAYNE strains of *Ae. triseriatus* were reared on the McCombs diet to generate a variety of size classes. For each strain, female pupae were arranged into 5 discrete groups based on 1-mg increments of wet weight. Rates of infection and transmission for each size class are shown in Table 2. By regression analysis, both strains

showed an inverse relationship between rate of disseminated infection and mean pupal wet weight ($P < 0.05$) (Fig. 2). The 2 smallest size classes of WALTON transmitted LACV at a significantly higher rate than the larger groups ($P < 0.05$).

When pupae of the YANKEE WOODS strain of *Ae. hendersoni* were placed into 3 non-overlapping size classes based on pupal wet weight, the rate of disseminated infection declined with increasing size, but not significantly so (Table 2). The rate of transmission of virus by *Ae. hendersoni* remained low (7–10%) regardless of the size of the population tested.

DISCUSSION

Our results show that environmental conditions in natural larval habitats have a dramatic effect on the vector competence of *Ae. triseriatus* mosquitoes. A previous study (Grimstad and Haramis 1984) showed that size and vector competence are inversely correlated for laboratory populations of this species reared on specialized laboratory diets. Our study confirms this general result and extends it to the field. In addition, our study shows that the relationship between vector competence and size is linear (Fig. 1), as is the relationship between the proportion of disseminated infections and size (Fig. 2). In marked contrast, *Ae. hendersoni* exhibited low transmission rates irrespective of larval diet and adult size.

The findings of this study have implications in 2 major areas: 1) the mechanics of virus dissemination in *Ae. triseriatus* and *Ae. hendersoni*, and 2) the epidemiology of La Crosse encephalitis.

Mechanics of virus dissemination: The primary barrier to LACV dissemination in *Ae. triseriatus* is the midgut escape barrier; cells of the midgut almost always become infected, but a portion may fail to develop disseminated infections (Paulson et al. 1989). Disseminated infections occur only if the virus escapes from the midgut. Our results indicate that the "strength" of the midgut escape barrier is linearly related to size of the adult mosquito. In more concrete terms, the weakening of the barrier may be related to an alteration of the midgut ultrastructure due to nutritional stress. Grimstad and Walker (in press) have shown that the basal lamina on the serosal side of the gut epithelium in large mosquitoes is 10–16 layers thick while only 4–6 layers are present in small mosquitoes. Our results imply that the thickness of the basal lamina should be linearly related to adult size.

For *Ae. hendersoni*, the primary barrier to LACV transmission is a salivary gland escape
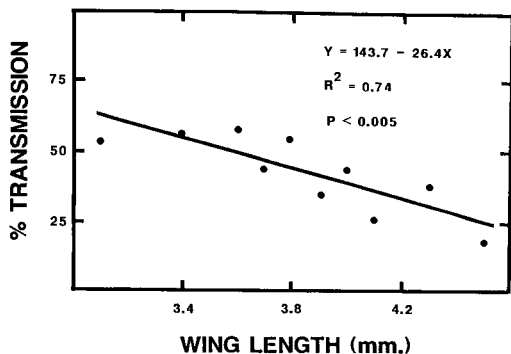

Fig. 1. The relationship between rate of transmission (%) of LACV and size as measured by wing length (mm) for *Aedes triseriatus*. Each point represents the transmission percentage for 10–15 mosquitoes.

In the figure:
Y = 143.7 − 26.4X
R^2 = 0.74
$P < 0.005$

Table 2. Rates of infection and transmission of LACV by different size classes of strains of laboratory-reared *Aedes triseriatus* and *Ae. hendersoni*.

Species/strain	Pupal weight (mg)		No.	% disseminated infections	% transmitting
	Mean	Range			
Ae. triseriatus					
WALTON	1.9	(1.5–2.2)	30	93 a*	87 c
	2.8	(2.5–3.2)	30	97 a	93 c
	3.8	(3.5–4.2)	30	77 b	47 d
	4.9	(4.5–5.2)	30	67 b	40 d
	5.8	(5.5–6.2)	30	70 b	50 d
FORT WAYNE	2.3	(1.7–2.8)	24	67 e	—**
	3.5	(3.1–3.9)	30	40 f	—
	4.5	(4.2–4.8)	30	27 f,g	—
	5.5	(5.1–5.9)	30	27 f,g	—
	6.6	(6.2–7.2)	30	17 g	—
Ae. hendersoni					
YANKEE WOODS	2.3	(1.8–2.8)	15	93 h	7 i
	3.5	(3.3–4.3)	20	80 h	10 i
	5.0	(4.8–5.5)	20	75 h	10 i

* Rates followed by different letters are significantly different (G-test of independence, $P < 0.05$).
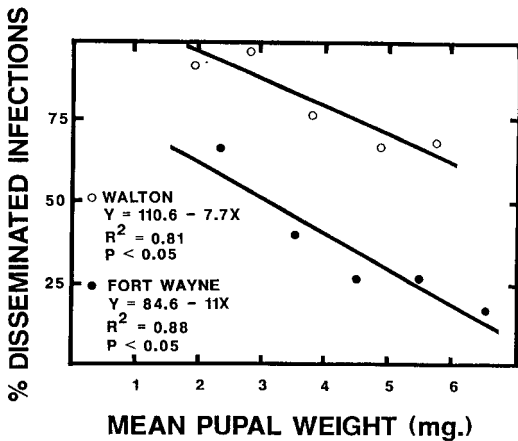** No data.

Fig. 2. The relationship between the rate of disseminated infections (%) and size as measured by mean pupal wet weight (mg) for the WALTON and FORT WAYNE strains of *Aedes triseriatus*. Each point represents the percentage of infection of 30 mosquitoes.

barrier; individuals with infective virus in their salivary glands fail to transmit by bite (Grimstad et al. 1985, Paulson and Grimstad 1989). Our study shows that larval nutrition does not affect this barrier. *Ae. hendersoni* of all size classes transmitted LACV at low rates.

Mosquito size and the epidemiology of LACV: Our data show that vector competence of natural populations of *Ae. triseriatus* is greatly influenced by environmentally determined size variation. Small field-reared parentals have high vector competence; when eggs are obtained from

the field, hatched in the laboratory and provided with excess food, large offspring with low vector competence result. In general, the vector competence of the smallest females is 2–3 times greater than that of the largest females (Figs. 1 and 2). Though within population variation in vector competence is primarily environmentally determined, genetic differences between populations are also important (Grimstad et al. 1977). The WALTON and FORT WAYNE strains both exhibit a clear inverse correlation between size and rate of disseminated infection, but the strains also show clear inherent differences (Fig. 2).

Though it is tempting to hypothesize that tires produce smaller mosquitoes which transmit LACV at higher rates, leading to higher incidence of this disease, we are hesitant to do so for several reasons. First, though tree hole mosquitoes were larger than tireyard mosquitoes in the study of Haramis (1984) and corroborated for the same sites in our study, a broad correlation between mosquito body size and habitat has not been confirmed. Indeed, the TIRES-2 strain was nearly equal in size to the TREE HOLE strain with a correspondingly low transmission rate. The 3 habitats studied are separated by 10–15 km of residential area and farmland. Though there is enough gene flow to homogenize allozyme frequencies (Matthews and Craig 1980, Matthews 1983), factors affecting population size and food levels in the 3 habitats may operate independently. When the size of pupae yielded by tires and tree holes in the same woodlot was compared, no differences were observed (W. A. Hawley, unpublished data). Thus, though we

have demonstrated the importance of larval food in natural habitats as a factor affecting vector competence, we have not shown that the quality (or quantity) of such food is correlated with habitat type.

Second, vector competence is correlated with vectorial capacity only if all other things are equal. Vector competence is the proportion of mosquitoes ingesting a pathogen that, after a given extrinsic incubation period, transmit the pathogen. Vectorial capacity is a complex field parameter that provides a true measure of the ability of a vector to sustain natural transmission of a pathogen. If size affects the vector competence, it might also affect other parameters that determine vectorial capacity such as blood feeding behavior, duration of the gonotrophic cycle, intrinsic incubation period or, especially, longevity. For *Ae. triseriatus*, size and parous rate are positively correlated (Nasci 1988, Haramis 1983), suggesting that larger adults live longer. However, a mark-release-recapture study failed to show size-related differences in longevity in this species (Walker et al. 1987). The extent to which higher survivorship in larger mosquitoes would counteract, or outweigh, the greater vector competence observed for smaller mosquitoes would depend on the quantitative relationship between size and survival rate. This relationship is unknown, and might also vary between habitats and populations.

Though we wish to avoid a simplistic model of LACV epidemiology where tires = smaller mosquitoes = increased vector competence = greater incidence of La Crosse encephalitis, we do not wish to underestimate the importance of tires as producers of large numbers of *Ae. triseriatus*. Given an EPA estimate that 200 million tires are discarded each year (Deese et al. 1981), it is a factor of increasing importance. In La Crosse, WI, Thompson and Gunderson (1983) found the highest rates of virus isolation from larvae collected from tires, and Parry (1983) reported a reduction in the cases of encephalitis in La Crosse county following an eradication program that included the removal of thousands of tires. Discarded tires were the predominant breeding sites in Ohio and, based on larval counts, surpassed tree holes in production of *Ae. triseriatus* (Berry 1983). Also important in the production of mosquitoes are other man-made water-holding containers. Berry (1983) estimates that water-holding trash, including tires, increases the *Ae. triseriatus* population around human habitations by a factor of 7.7. In a Minnesota study, *Ae. triseriatus* was collected from various artificial containers located in close proximity to 78% of the human La Crosse en-

cephalitis cases where exposure was presumed to have been at their residence (Hedberg et al. 1985). Vectorial capacity depends to a large extent on simple numbers of biting vectors—and carelessly discarded tires most definitely multiply this number.

ACKNOWLEDGMENTS

REFERENCES CITED

Baumgartner, D. L. 1988. Suburban accumulations of discarded tires in northeastern Illinois and their associated mosquitoes. J. Am. Mosq. Control Assoc. 4:500–508.

Berry, R. L. 1983. *Aedes triseriatus*, man-made containers and La Crosse encephalitis in Ohio. Proc. Ohio Mosq. Control Assoc. 18:13–17.

Deese, P. L., J. F. Hudson, R. C. Innes and D. Funkhouser. 1981. Options for resource recovery and disposal of scrap tires. EPA Mimeograph Publ. 144 pp.

Grimstad, P. R. and L. D. Haramis. 1983. Evaluation of immunofluorescence and cell culture techniques for detecting La Crosse virus infection in individual *Aedes triseriatus* (Diptera: Culicidae). J. Med. Entomol. 20:458–460.

Grimstad, P. R. and L. D. Haramis. 1984. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus. III. Enhanced oral transmission by nutrition-deprived mosquitoes. J. Med. Entomol. 21:249–256.

Grimstad, P. R., G. B. Craig, Jr., Q. E. Ross and T. M. Yuill. 1977. *Aedes triseriatus* and La Crosse virus: geographic variation in vector susceptibility and ability to transmit. Am. J. Trop. Med. Hyg. 26:990–996.

Grimstad, P. R., S. L. Paulson and G. B. Craig, Jr. 1985. Vector competence of *Aedes hendersoni* Cockerell (Diptera: Culicidae) for La Crosse virus and evidence for a salivary gland escape barrier. J. Med. Entomol. 22:447–453.

Grimstad, P. R. and E. D. Walker. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus. IV. Nutritional deprivation of larvae affects the adult barriers to infection and transmission. J. Med. Entomol. (In press).

Haramis, L. D. 1983. Increased adult size correlated with parity in *Aedes triseriatus*. Mosq. News 43:77–79.

Haramis, L. D. 1984. *Aedes triseriatus*: a comparison of density in tree hole vs. discarded tires. Mosq. News 44:485–489.

Hedberg, C. W., J. W. Washburn and R. D. Sjogren. 1985. The association of artificial containers and La

Crosse encephalitis cases in Minnesota, 1979. J. Am. Mosq. Control Assoc. 1:89–90.

Jenkins, D. W. and S. J. Carpenter. 1946. Ecology of the tree hole breeding mosquitoes of Nearctic North America. Ecol. Monogr. 16:32–47.

Matthews, T. C. 1983. Population genetics of the treehole mosquito *Aedes triseriatus*: no correlation between est-6 and larval habitat. Heredity 52:133–139.

Matthews, T. C. and G. B. Craig, Jr. 1980. Genetic heterozygosity in natural populations of the treehole mosquito *Aedes triseriatus*. Ann. Entomol. Soc. Am. 73:739–743.

Nasci, R. S. 1981. A lightweight, portable aspirator for collecting resting mosquitoes in the field. Mosq. News 41:808–811.

Nasci, R. S. 1988. Biology of *Aedes triseriatus* (Diptera: Culicidae) developing in tires in Louisiana. J. Med. Entomol. 25:402–405.

Novak, R. J. and D. A. Shroyer. 1978. Eggs of *Aedes triseriatus* and *Aedes hendersoni*: a method to stimulate optimal hatch. Mosq. News 38:515–521.

Parry, J. E. 1983. Control of *Aedes triseriatus* in La Crosse, Wisconsin. pp. 355–363. *In:* C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. Alan R. Liss, Inc., New York.

Patrican, L. A. and G. R. DeFoliart. 1985. Lack of adverse effect of transovarially acquired La Crosse virus infection on the reproductive capacity of *Aedes triseriatus* (Diptera: Culicidae). J. Med. Entomol. 22:604–611.

Paulson, S. L., P. R. Grimstad and G. B. Craig, Jr. 1989. Midgut and salivary gland barriers to La Crosse virus dissemination in mosquitoes of the *Aedes triseriatus* group. Med. Vet. Entomol. 3:113–123.

Paulson, S. L. and P. R. Grimstad. 1989. Replication and dissemination of La Crosse virus in the competent vector *Aedes triseriatus* and the incompetent vector *Aedes hendersoni* and evidence for transovarial transmission by *Aedes hendersoni* (Diptera: Culicidae). J. Med. Entomol. 26:602–609.

Pinger, R. R., P. R. Grimstad, M. J. Sinsko and C. H. Calisher. 1983. Isolation of La Crosse and other arboviruses from Indiana mosquitoes, 1961–1982. Mosq. News 43:459–463.

Rutledge, L. C., R. A. Ward and D. J. Gould. 1964. Studies on the feeding response of mosquitoes to nutritive solutions in a new membrane feeder. Mosq. News 24:407–418.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. The principles and practice of statistics in biological research. W. H. Freeman and Co., San Francisco.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill Book Company, Inc., New York.

Thompson, W. H. and C. B. Gunderson. 1983. Occurrence of disease and control in a suburban area, pp. 225–236. *In:* C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. Alan R. Liss, Inc., New York.

Walker, E. D., R. S. Copeland, S. L. Paulson and L. E. Munstermann. 1987. Adult survivorship, population density, and body size in sympatric populations of *Aedes triseriatus* and *Aedes hendersoni* (Diptera: Culicidae). J. Med. Entomol. 24:485–493.