LOCAL DISTRIBUTION OF *AEDES TRISERIATUS* (DIPTERA:CULICIDAE) AT THE BALTIMORE ZOO[1]

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ABSTRACT. Field studies on the tree hole mosquito, *Aedes triseriatus* were conducted at the Baltimore Zoo. Through larval surveys and ovitrap sampling the oviposition patterns, seasonal dynamics and dispersal of *Ae. triseriatus* were studied in 4 biotopes: forest, field, lawn, and penguin exhibit. Natural sources of this species existed exclusively in the forest and were represented by 164 beech tree holes per hectare. Ovitrap sampling indicated that *Ae. triseriatus* was not strictly confined to the forest where natural development sites exist. Oviposition activity was highest in the beech forest, was less common in the lawn and open field biotopes and was rare at the penguin exhibit.

INTRODUCTION

Aedes triseriatus (Say) is widely distributed east of the Rocky Mountains in the U.S. (Zabortink 1972). It is the most abundant species developing in tree holes in North America and it also develops in tires and other artificial containers in urban habitats (Jenkins and Carpenter 1946). In midwestern U.S. *Ae. triseriatus* is the principal biological vector of La Crosse encephalitis virus (Watts et al. 1972, 1973; Grimstad et al. 1977). This species is also capable of experimentally transmitting eastern equine encephalitis (Chamberlain and Sudia 1961), and is susceptible to dog heartworm *Dirofilaria immitis*) (Intermill 1973) and several species of avian malaria (Huff 1965). Despite its potential as a vector of disease, relatively little is known about the factors affecting the distribution and dispersal of this mosquito.

Monitoring field populations of this species is difficult. Adults are not generally attracted to light traps or bait traps (Loor and DeFoliart 1969). Because tree

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holes are often inaccessible, it is difficult to monitor larval production. However, ovitrap sampling is a highly sensitive, specific and economical method for detecting *Ae. triseriatus* (Loor and DeFoliart 1969, 1970; Furlow and Young 1970). Ovitraps are used to study oviposition site selection (Loor and DeFoliart 1970; Sinsko and Grimstad 1977), mosquito distribution (Gauld et al. 1974), and evaluation of control programs (Garry and DeFoliart 1975).

Field studies of *Ae. triseriatus* were undertaken as part of a larger study to determine natural vectors of avian malaria which affects penguins (*Spheniscus demersus)* at the Baltimore Zoo (Beier 1980; Beier and Stoskopf 1980; Beier and Trpis 1981). This report examines the oviposition patterns, seasonal dynamics and dispersal of *Ae. triseriatus* in 4 biotopes.

MATERIALS AND METHODS

Study Area. A 13 ha tract of land located in the southeast corner of the Baltimore Zoo, Baltimore, Maryland was selected for this study. Five ecologically distinct biotopes, designated as study areas I–V, were surveyed by a registered surveyor to produce a scale map (Fig. 1).

Area I—Forest. Dominant tree species in this 3.48 ha area include beech (*Fagus grandifolia*) and oak (*Quercus* sp.). The forest canopy is contiguous and groundcover is sparse. A road along the north and east boundries separates this area from a larger forest of similar vegetation.

Area II—Field. This is a flat, low-lying area of 1.12 ha positioned adjacent to areas I and III. Twenty mature oak trees grow along the southeast border. The field contains 1 ha of open, uncropped grassy terrain in which there are no trees or saplings.

Area III—Lawn. The lawn is a flat, maintained area of 0.88 ha with shrubbery planted in distinct clusters. Main types of shrubbery include: forsythia (*Forsythia* sp.), pyracantha (*Pyracantha* sp.), viburnum (*Viburnum* sp.), honeysuckle (*Lonicera* sp.), and spiraea (*Spiraea* sp.). There are 20 trees of the following species: oak (*Quercus* sp.), tulip (*Liriodendron* sp.), cherry (*Prunus* sp.), butternut (*Juglans* sp.), ash (*Fraxinus* sp.), cedar (*Juniperus* sp.), and beech (*Fagus* sp.).

Area IV—Penguin Exhibit. The exhibit, housing a colony of 50 penguins, consists of an oval-shaped rock structure (31 × 12 × 9 m) surrounded by a fresh water moat (4.5 m wide and up to 1.3 m deep). This exhibit is constructed of concrete, stone, and steel. It encompasses an area of 0.16 ha and is located within area III.

Area V—Construction Zone. Construction in area V made it impossible to do field work here. At the time of this study the area consisted of open, grassy parkland with a few beech trees.

LARVAL SAMPLING. Larval surveys were made in the study areas to locate and monitor natural breeding sites of *Ae. triseriatus*. Surveys were conducted twice per month from April through October in 1978 and 1979, and single examinations were made in December 1978 and February 1979. During each survey, water from at least 15 tree holes in the forest was examined for mosquito larvae. Samples of larvae were collected and identified. No attempt was made to identify *Ae. hendersoni* (Cockerell), a sibling species which is similar to *Ae. triseriatus* and apparently occurs throughout the geographic range of *Ae. triseriatus* (Zavortink 1972). Therefore the possibility exists that some of the specimens considered to be *Ae. triseriatus* were actually *Ae. hendersoni*.

ASSESSMENT OF LARVAL SOURCES. Larval surveys indicating abundant *Ae. triseriatus* larvae in basal tree holes of beech trees in the forest (Area I) made it desirable to quantitate the habitat available for larval development. Trees in five 0.1 ha plots in this area were examined to determine: 1) the number of beech and oak trees over 40 cm in circumference, 2) the number with basal tree holes, 3) the mean number

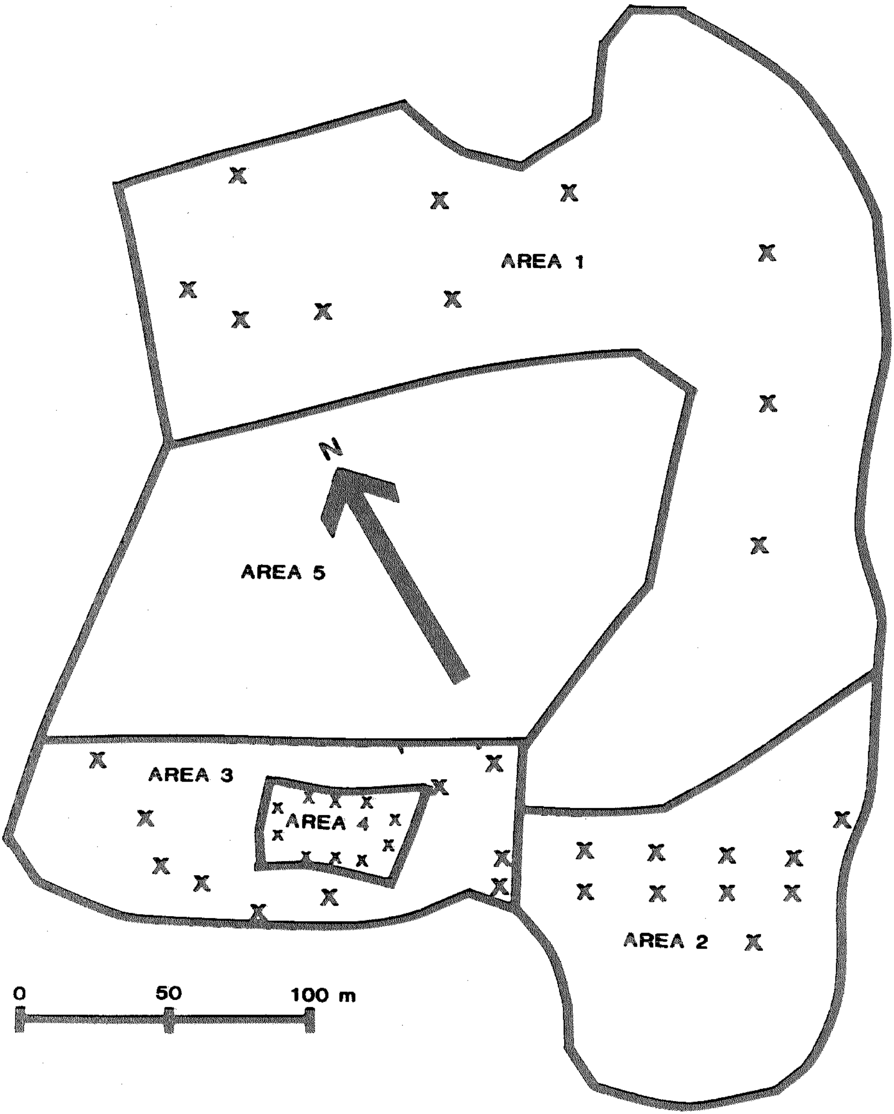

Fig. 1. Scale map of biotopes sampled for *Aedes triseriatus*. Area I—Forest, Area II—Field, Area III—Lawn, Area IV—Penguin exhibit, Area V—Construction zone. Ovitrap placement is marked by "X".

of tree holes per positive tree, and 4) the relationship between beech tree circumference and number of basal tree holes per beech tree.

OVITRAP SAMPLING. Ovitraps (Loor and DeFoliart 1969) were used to monitor *Ae. triseriatus* in study areas I—IV. Traps consisted of 12 oz (355 ml) aluminum cans covered with masking tape. Black cloth sleeves (8× 12 cm) of coarse texture lined the inside and extended over the top where they were fastened by 2 paper clips. In the field, each trap was partially filled with tapwater. In areas I–III, traps were fastened to pine stakes (50 × 1 cm) driven into the ground so that the top of each trap was 30 cm above ground level. In area IV, traps were secured in rock crevices on the outside of the penguin exhibit and trap height ranged from 1 to 3 m above ground level.

Ovitrap placement is shown in Figure 1. In the forest (Area I) all ovitraps were placed within 1 m of mature beech trees. Five traps were placed next to trees containing basal tree holes while 5 traps were placed next to trees with no tree holes. In the field (Area II) a double line transect with 8 traps was positioned as shown in Figure 1. Single traps were also positioned near a storm sewer opening and by a drain with running water. On the lawn (Area III), traps were placed in various types of shrubbery.

Ten ovitraps were maintained in areas I–IV for 11 weeks (18 June to 27 August 1979). Traps were monitored weekly and used liners were returned to the laboratory in marked containers. Partially dried liners were examined for mosquito eggs under a dissecting microscope (70 X). Liners containing eggs were pooled and stored in plastic bags. Samples of eggs were incubated in an insectary for at least 14 days at 21°C, then hatched and identified.

RESULTS

Aedes triseriatus larvae were found in beech tree holes in the forest in 22/26 (85%) larval surveys from April through October in 1978 and 1979. Most beech tree holes which contained water also contained *Ae. triseriatus* larvae from April through the end of August. In September and October, few larvae were found. Pupae were present in tree holes from May through the end of August. Collections in December and February yielded many *Ae. triseriatus* eggs and a few 3rd and 4th stage larvae. Four *Toxorhynchites rutilus* (Coquillett) larvae were found in beech tree holes containing *Ae. triseriatus* larvae. *Culex restuans* (Theobald) was the only other mosquito species collected in tree holes, and these were found on only one occasion in one tree hole. No natural or artificial sources of this species were found in areas II, III, or IV. During larval surveys in 1978 no sources of *Ae. triseriatus* were found in area V but construction during 1979 made it impossible to examine this area.

By sampling trees in five 0.1 ha plots in the forest (Area I), it was possible to determine the extent of natural larval development sites of *Ae. triseriatus*. For each one-tenth hectare of forest, there were 12.6 (S.D. = 2.7) beech trees and 3.8 (S.D. = 2.7) oak trees. At the time of sampling 52% of all beech trees contained tree holes holding water; no water-filled tree holes were found on oak trees. Beech trees with tree holes contained a mean of 2.48 holes (range: 1 to 6). Approximately 16.4 (S.D. = 5.8) beech tree holes were located in each one-tenth hactare of forest. Therefore, within the 3.48 ha forest study area there were approximately 439 beech trees which contained 566 basal tree holes.

A sample of 60 beech trees in area I was examined to determine the relationship between tree circumference at 1 meter and number of basal tree holes per beech tree. The linear regression of beech tree circumference on the number of basal tree holes per beech tree indicated that larger beech trees contained the most basal tree holes (ANOVA, $p < 0.05$) (Fig. 2).

During the 11 week sampling period, *Ae. triseriatus* eggs were collected weekly in

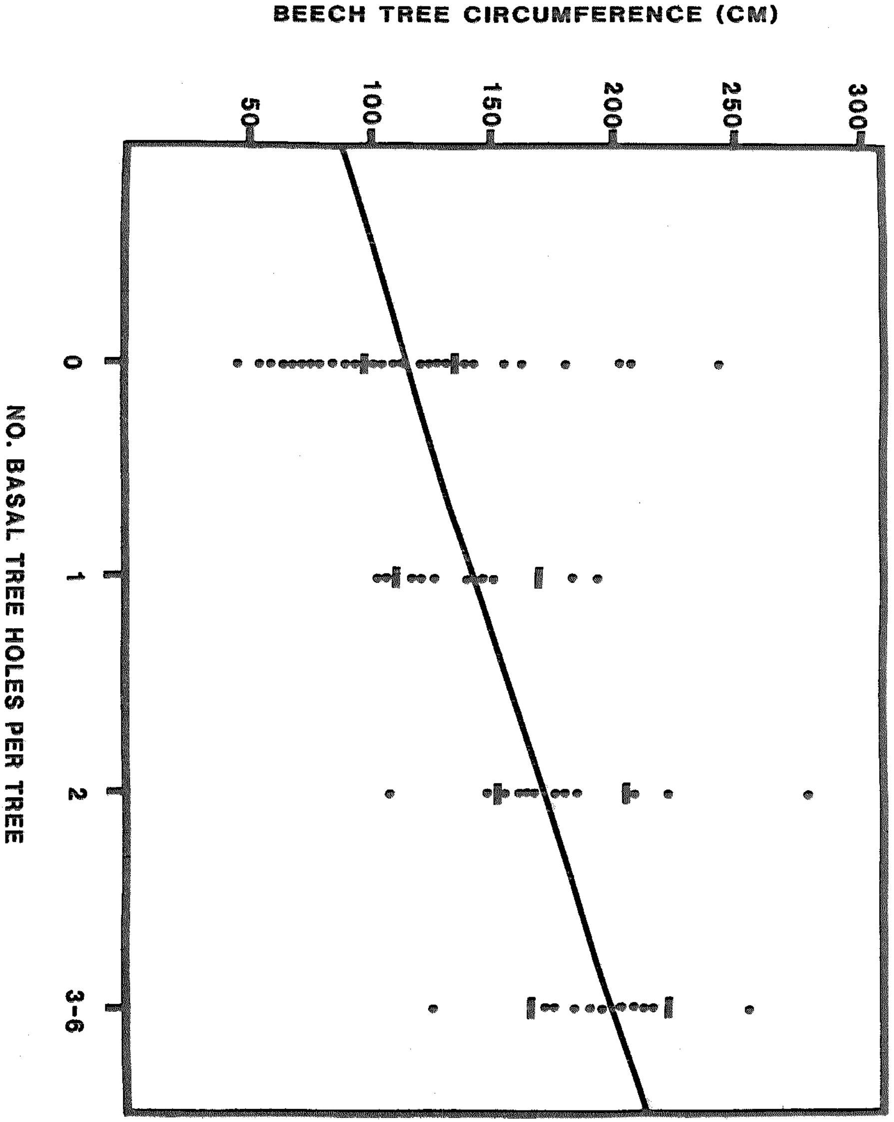

Fig. 2. Linear regression of beech tree circumference on the number of basal tree holes per beech tree. The 95% confidence limits of the mean are identified by "-" signs.

ovitraps from the 4 study areas (Table 1). During June and July, the weekly number of eggs from 40 traps ranged from 115 to 603. The weekly number of eggs collected in August ranged from 6 to 58. Table 2 summarizes results of all ovitrap collections.

Table 1. Seasonal distribution of oviposition of *Aedes triseriatus*.

	Date of Collection										
Area	25 June	2 July	9 July	16 July	23 July	30 July	6 Aug.	13 Aug.	20 Aug.	27 Aug.	Total no. eggs
I—Forest	119	450	144	294	60	237	29	13	22	6	1,374
II—Field	0	2	97	5	7	70	29	0	0	0	210
III—Lawn	12	151	2	62	39	2	0	32	3	0	303
IV—Exhibit	0	0	0	2	9	0	0	0	0	0	11
Total eggs	131	603	243	363	115	309	58	45	25	6	1,898

Table 2. Oviposition of *Aedes triseriatus* in four biotopes.

Area	Number trap Collections	Number positive collections	% positive	Total number eggs	Mean no. eggs/positive collection	% of total eggs collected
I—Forest	94	44	47	1374	31	72
II—Field	100	11	11	210	19	11
III—Lawn	100	14	14	303	22	16
IV—Exhibit	100	2	2	11	6	1
Total	394	71	18	1898	27	100

Eggs of *Ae. triseriatus* were the only mosquito eggs collected in ovitraps. In the forest (Area I) significantly more eggs were collected from traps positioned near beech trees with no holes than from traps by trees with tree holes (Wilcoxon-Mann Whitney Non-Parametric test, $p < 0.05$). Traps placed next to beech trees with no holes were positive for eggs in 55.6% (24/45) of the collections while traps next to trees with tree holes were positive in 38.8% (19/49) collections. These differences were not significantly different (Chi-Square Test, $p > 0.05$).

In the field (Area II) 78% (163/210) of the eggs were collected from 2 traps located approximately 10 m from mature oak trees. These 163 eggs came from 3 positive collections. The remaining 22% (47/210) of the eggs were collected from 3 traps located in completely exposed areas. These 3 traps were located 50 m from the forest edge and at least 60 m from oak trees near the southeastern border of this area. The 47 eggs represented 8 positive collections. No eggs were collected from single traps located near the shaded tile drain or the exposed sewer opening.

On the lawn (Area III), 65% (196/303) of the eggs were collected from 2 traps located within viburnum shrubbery. Two traps located in clusters of pyracantha accounted for 19% (57/303) of the eggs collected. Few eggs were collected from the remaining 6 traps secured in honeysuckle, forsythia, and spiraea shrubbery.

DISCUSSION

Aedes triseriatus is capable of developing in tree holes of many species of deciduous trees (Jenkins and Carpenter 1946). In this study, basal cavities of mature beech trees comprised the dominant natural sources of *Ae. triseriatus*. These tree holes are classified as "pans" (Kitching 1971) and are formed by natural growth processes of the beech tree. The finding of up to 164 beech tree holes per hectare emphasizes the magnitude of potential

Ae. triseriatus sources in this particular forest.

Larval surveys indicated that the larval habitat of *Ae. triseriatus* existed exclusively in the forest. By monitoring pupae in tree holes, it was possible to determine that adults emerged from May through the end of August. The scarcity of 1st instar larvae in tree holes during September suggests that the onset of diapause for eggs of *Ae. triseriatus* began in August. These findings cannot be attributed to decreased precipitation. Egg diapause in this mosquito is controlled by photoperiod (Shroyer and Craig 1980). The temporal onset of diapause for *Ae. triseriatus* is not well documented throughout the range of natural populations but in Indiana it begins the first week in August (G. B. Craig, Jr., personal communication), and in Wisconsin it begins the end of July (Scholl and DeFoliart 1977).

Most eggs of *Ae. triseriatus* were collected from ovitraps located in the forest. Other ovitrap studies have sampled almost exclusively in the forest (Loor and DeFoliart 1969, 1970; Garry and DeFoliart 1975). Adult *Ae. triseriatus* are generally believed to be restricted in range to the vicinity of their larval habitat. If sources occur in the forest, then the mosquito is confined to the forest (Sinsko and Craig 1978). If the source is a domestic one, such as artificial containers, then the mosquito remains around dwellings, as is common in southern U.S. (Horsfall 1955). When both tree holes and artificial containers are located within the same area, Means et al. (1977) suggest that two behaviorally distinct populations of *Ae. triseriatus* may exist.

In view of this it is significant that eggs of *Ae. triseriatus* were collected so frequently from ovitraps located in the field and lawn areas. These findings provide additional evidence that *Ae. triseriatus* females disperse from the breeding habitat (Garry and DeFoliart 1975; Scholl et al. 1979). The extent of dispersal may depend upon the size and structure of the woodland habitat as well as ecological conditions of peripheral areas. Even though conditions in the field and lawn clearly distinguished these areas from the forest biotope, vegetational conditions did not permit adequate isolation necessary to confine this mosquito to the wooded area where breeding sites exist.

The distribution of eggs from ovitraps in the forest, field, and lawn was not uniform within areas. In the forest, significantly fewer eggs were collected in traps located next to beech trees containing natural holes than from traps near beech trees with no holes. This suggests that, given the choice between tree holes and ovitraps, *Ae. triseriatus* females prefer to oviposit in tree holes. This factor is important in the interpretation of ovitrap sampling data. Differences in the number of eggs per ovitrap probably do not reflect differences in density of *Ae. triseriatus* females. Investigators working in mature beech forests and other areas may encounter this competition between ovitraps and tree holes because it is the older, larger beech trees that have the most tree holes suitable for *Ae. triseriatus* development (Fig. 2).

In the field, far more eggs were collected from traps in shaded areas than in exposed areas. This suggests that isolated trees or clusters of trees may serve as peripheral habitat for this mosquito even though larval development sites may be lacking.

The distribution of eggs from traps on the lawn was influenced by the type of shrubbery sampled. Most eggs were collected from traps positioned in viburnum and pyracantha shrubbery. These shrubs are not dense near ground level. Perhaps the few eggs collected in other types of shrubbery reflect an inhibition of trap use due to vegetation density.

As measured by oviposition, *Ae. triseriatus* is infrequently present at the penguin exhibit. Although ovitraps positioned in rock crevices represent an unnatural situation for this study site, this species is known to breed in water-filled crevices (Hanson and Hanson 1970). Since subsequent laboratory studies indicated that *Ae. triseriatus* is refractory to *Plasmodium elongatum*, the malarial species which affects penguins at the Baltimore Zoo, it is doubtful that this mosquito serves as a natural vector (Beier and Trpis 1981).

This study indicates that *Ae. triseriatus* utilizes habitats adjacent to the forest where larval development sites exist. This factor would enhance the potential for mosquito contact with humans, especially in suburban residential areas located near sylvan environments. For further quantification of La Crosse virus epidemiology, additional studies are needed to evaluate environmental conditions which permit *Ae. triseriatus* mosquitoes to disperse from the confines of the woodland habitat.

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