The Egg Raft Seam as an Indicator of Species in *Culex pipiens* and *Culex restuans*[1]

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ABSTRACT. Studies of egg rafts produced by *Culex pipiens* and *Cx. restuans* indicate presence of a longitudinal seam to be more typical of rafts produced by *Cx. pipiens* than by *Cx. restuans*. In 1982, rafts produced by *Cx. pipiens* were 56.8% seamed and *Cx. restuans* rafts were 11.7% seamed. Tested by chi-squared, the differences between species, and the seamed/unseamed raft ratio of each species had a *p*-value of 0.0001.

INTRODUCTION

Adults of *Culex pipiens (sensu latu)*, *Cx. restuans* Theobald and *Cx. salinarius* Coquillett cannot be reliably distinguished from one another after they have been captured in light traps which use fans, because scale patterns necessary for species separation are damaged. Further, even untrapped, the subspecies and intermediates of *Cx. pipiens (s. l.)* cannot be separated in the adult stage. This problem led Brogdon (1981) to refine an index, based on siphonal length-to-width ratios of fourth instar larvae (McMillan 1958), to permit larval separation of *Cx. pipiens pipiens* L., *Cx. p. quinquefasciatus* Say, and field-collected intermediates. Thus, egg rafts produced by the *Cx. pipiens* subspecies may be distinguished, retrospectively, using the siphonal indexes of larvae reared from them (Brogdon 1984a).

During a field study of *Culex pipiens (s. l.)* and *Culex restuans* oviposition behavior, a number of egg rafts possessing a longitudinal seam, or furrow, were found (Figure 1, A). Most seams ran the entire length of the raft, a few ran only part way, but approximately half of the rafts observed had a seam. Earlier workers also noticed this phenomenon (Christophers 1945, Beament and Corbet 1981). In one sample of *Cx. p. pipiens* eggs examined by Beament and Corbet, 23 of 30 rafts (76.7%) were seamed.

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Diagnostic characters which allow identification of eggs to species are known for various mosquito genera. Eggs of many anophelines are sufficiently distinct that they can be identified by workers familiar with a local fauna (Theodor 1924, Bates 1941, Saliternik 1942, D'Abrera 1944, Causey et al. 1944). Hinton (1968) presents a general review of the taxonomy and biology of *Anopheles* eggs. Craig (1956) described and illustrated eggs of 35 nearctic *Aedes* species, finding specific egg characters for most of the species examined. Egg keys are available for *Aedes* species of California and Nevada (Myers 1967), and Illinois (Ross and Horsfall 1965).

In contrast, *Culex* eggs and egg rafts have received little study for taxonomic characters which might be used to distinguish species. Roubaud (1935) described eggs of three races of *Cx. pipiens: Cx. p. pipiens, Cx. p. berbericus,* and *Cx. p. autogenicus*. Cervone (1957) examined the reticulum of the micropile region of *Cx. autogenicus* and compared it to the other races studied by Roubaud. Mattingly (1976, 1975, 1970) has provided descriptions of eggs produced by species from various subgenera of *Culex,* and gives general egg raft configurations for a number of *Culex* species. Christophers (1945) gave general egg raft descriptions for *Cx. fatigans, Cx. pipiens,* and *Cx. molestus*. Those descriptions suggest that rafts of *Cx. fatigans* and *Cx. pipiens* regularly are seamed, while those of *Cx. molestus* are rarely so. *Culex fatigans* is a synonym of *Cx. quinquefasciatus,* and *Cx. molestus* is very likely an autogenous strain of *Cx. pipiens* (Barr 1960). No keys have been published for determination of *Culex* species by use of egg, or egg raft, characteristics.

It appeared possible that the seam found in some *Culex* egg rafts might be a character useful in separating rafts of one *Culex* species from another. This study was undertaken to determine whether rafts produced by *Cx. pipiens (s. l.)* and *Cx. restuans* could be reliably differentiated by presence or absence of a seam. Identification of *Cx. pipiens* subspecies was not attempted, but work done by Barr (1957) suggests that this local population of *Culex pipiens* should belong to *Cx. p. pipiens* L., which is the more northerly subspecies, occurring north of 39° N. Newark, Delaware, where the present study was conducted, is located at 39° 41' N. (USGPO 1984).

Beament and Corbet (1981) examined the behavior of *Cx. p. pipiens* females during production of the egg raft, and explained the manner in which a seam, of "parting," is produced during raft formation. That behavior was also observed during this study, and is briefly as follows. Eggs of *Cx. pipiens* and *Cx. restuans* are slightly curved along their length. The female places eggs against the forward (blunt) end of the raft as they are laid, rotating them about their long axes so they lock into position in the angle between two previously layed eggs. Eggs are held in place, and the raft is held together, by microscopic pegs on the exochorion which have been described and illustrated by Beament and Corbet (1981). A seam is caused in a raft when two adjacent longitudinal rows of eggs have their outer, curved surfaces arranged toward each other creating a greater space between the egg tips of those rows than between tips in other rows with the curved surfaces nested (Figure 1, B).

MATERIALS AND METHODS

During the summers of 1981 and 1982, completed *Culex* egg rafts were collected from an oviposition trap consisting of a plastic tub, 34.3 cm in diameter, filled with 3.8 l of tap water and 113 g of wheat straw. Simple infusions of rotting wheat straw are quite attractive to gravid females of several *Culex* species (Madder et al. 1980; Murphey and Burbutis 1967; R. M. Weber, unpublished data). The trap was placed near an outbuilding used as a mosquito research facility on the University of Delaware Research Farm at Newark, Delaware. The water/wheat straw mixture was replaced a week before collections were to be made. Wild mosquitoes from the local population oviposited freely in the trap. Collections were made monthly in June, 1981, and May through September, 1982.

Existing egg rafts were removed from the trap on the afternoon preceeding a day when rafts were to be collected. On collection days, all rafts present were evaluated on the water surface, using a hand magnifying lens, for presence of seams. They were then removed with a spoon-type section lifter. By using such a dished instrument it was possible to avoid stressing any particular row of eggs in a raft, which might have caused an artifact seam. Rafts were examined with a stereoscopic microscope at magnifications up to 15 X, using oblique illumination which enhances visibility of seams.

Some raft seams could be artifacts of egg tanning, that is, some seams might appear after the female leaves the raft rather than being created only as she produces it. To investigate this possibility, 73 untanned rafts were examined a second time approximately 5 hours after the initial observation, and a third time before hatching. Forty-eight of the 73 untanned rafts were examined a fourth time between initial observation and hatching. Examinations were made with rafts afloat in their culture dishes.

After collection each raft was put into its own separate culture dish, where resulting larvae were reared to 3rd or 4th instar for identification. Representative larvae from each egg raft were killed and preserved in AGA (Martin 1978) for storage and later identification. Identified voucher specimens have been placed in the insect collection of the Department of Entomology and Applied Ecology, University of Delaware (accession numbers 2142-2147, Alcohol Collection).

RESULTS AND DISCUSSION

Of the 105 rafts collected, 51 had no seam, 41 had complete seams, 5 had half-seams, and 8 changed in seam manifestation after collection. Data are presented in Table 1. Half-seams occurred 3 times in *Cx. pipiens* rafts; twice in rafts of *Cx. restuans*. Rafts found with half-seams were included with the complete-seamed rafts for statistical analysis. At time of collection, 73 rafts were 1-2 hours old, as indicated by their pale yellow, or light gray color. The remaining 32 rafts were fully tanned, and black.

Between the initial and second examinations 4 rafts developed half-seams, 1 developed a complete seam, and the seam disappeared from 1 raft. Between second and third examinations 2 additional rafts developed half seams. Both species showed similar percentages of rafts with changes in seam character. Because untanned rafts which changed in seam manifestation were found only in 1982 collections they were not included in percentage calculations, but appear separately in Table 1.

As identified from reared larvae, 39 rafts were produced by *Cx. restuans;* 66 by *Cx. pipiens* (Table 1). In both years percentages of seamed and unseamed rafts for a species appear to be similar, but 1981 sample sizes are too small for valid statistical comparison of data from both years. However, comparison of 1982 data shows that the apparent differences between species, and seamed/unseamed raft values for each species, are significant at the 5% level. In this case, the chi-squared p-value was 0.0001. Data from 1982 do not contradict apparent ratios in the 1981 data, but cannot confirm their accuracy.

In the 1982 collections, a seamed raft was most likely to have been produced by *Cx. pipiens.* Data reported here may characterize the local Newark, Delaware, populations, or only the subspecies *Cx. p. pipiens,* but might be of broader importance. Larger samples, collected monthly over several seasons, preferably at separate localities, will be required to demonstrate consistency of these data. It is also necessary to determine whether any seasonal effect on seamed/unseamed ratios of *Culex* spp. exists. Various species of *Anopheles* are polymorphic with respect to overall egg architecture, and the egg chorion is known to vary in structure according to time of year in which eggs are laid (Hinton 1981).

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Table 1. Seam distribution observed in field-collected *Culex* egg rafts.

		Cx. pipiens		*Cx. restuans*	
1981	With seam	12	70.6%	1	33.3%
	Without seam	5	29.4%	2	66.7%
	Changed	0	0.0%	0	0.0%
1982	With seam	29*	56.8%	4*	11.7%
	Without seam	17*	33.3%	27*	79.4%
	Changed	5	9.8%	3	8.8%
Total	With seam	41	60.3%	5	13.5%
	Without seam	22	32.3%	29	78.4%
	Changed	5	7.3%	3	8.1%

*Difference between species, and ratios of seamed/unseamed rafts tested by chi-square, p = 0.0001.

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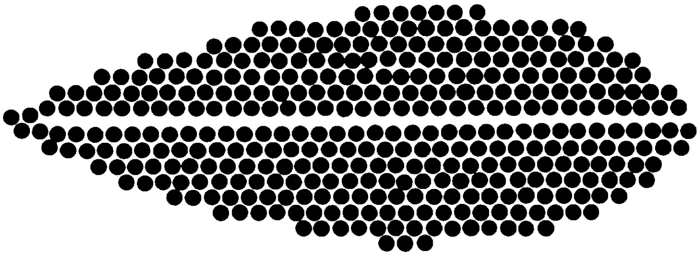
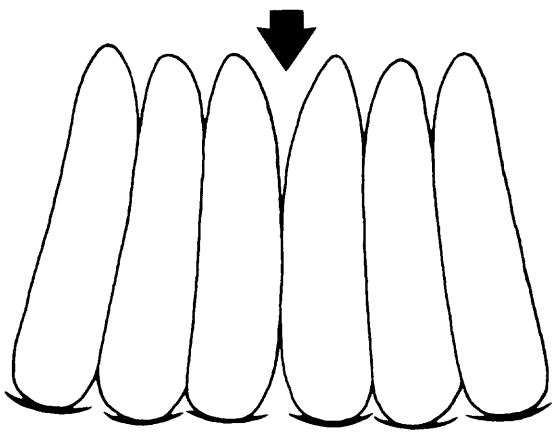
Fig. 1



A



B

Figure 1. (A) Diagrammatic top view of a *Culex* egg raft showing position of seam. (B) Side view of eggs adjacent to the longitudinal midline of a seamed raft, showing how egg orientation during raft construction produces the seam. Arrow indicates gap between egg tips, which gives seamed appearance to top of raft.