The Siphonal Index

II. Relative Abundance of *Culex* Species, Subspecies, and Intermediates in Memphis, Tennessee

William G. Brogdon
Division of Parasitic Diseases, Center for Infectious Diseases,
Centers for Disease Control, Public Health Service,
U. S. Department of Health and Human Services, Atlanta, Georgia 30333

ABSTRACT. As part of a larger study of *Culex pipiens* in Memphis (Shelby County), Tennessee, egg rafts were collected from predetermined, widely distributed sites during the 1979 and 1980 breeding seasons. Marked differences in populations of related species and subspecies were observed between the two seasons, with the 1980 pattern more closely resembling that seen in 1975, when Memphis experienced epidemic St. Louis encephalitis (SLE). It is suggested that because of the observed interseasonal variability in numbers of potential vectors, *Culex* species and subspecies populations should be monitored until detailed comparison of epidemic and nonepidemic years becomes possible. The novel methods used herein of monitoring populations by rearing egg-raft collections from specific sites, and measuring siphonal indices of the larvae would be especially cost effective.

INTRODUCTION

Epidemic infection by St. Louis encephalitis (SLE) virus is a recurring problem on the North American continent. Among numerous epidemics in the United States have been those of 1964, 1974, and 1975, in Memphis, Tennessee. In Memphis, as in other heavily populated areas faced with an SLE epidemic, vector control has been the only practical method for human protection (Breeland et al. 1980). Successful vector control programs (those which minimize mosquito production) depend upon continual vector population assessment, viral infection surveillance, and biological understanding of the vector species. Unfortunately, maintaining an effective control program can be difficult. In times of budget cuts between epidemics, the effectiveness of such programs may be progressively reduced through problems in interpreting increasingly less complete surveillance data. For this reason, mosquito and disease control personnel continually seek more efficient means of doing their work.

As part of a larger study of SLE vectors in Memphis, the relative abundance of *Culex* species and subspecies was monitored through the 1979 and 1980 breeding seasons using egg raft collections from selected sites, followed by rearing and identification of the larvae. The immediate objectives of the present work were to describe more accurately the seasonal relative abundance of *Culex pipiens* (including subspecies of intermediates), *Culex restuans* and

Culex salinarius and to evaluate an ongoing method for monitoring *Culex* activity in an area where SLE can be a problem. An ultimate goal is the elaboration of vector surveillance methods that would allow detection of vector population changes significant in SLE infection risk to humans.

MATERIALS AND METHODS

Mosquito surveillance and control efforts are exercised over a 300-square mile area of Memphis/Shelby County. Multiple mosquito surveillance methods (light trap catches, resting station catches, larval survey, egg raft collection) are employed by the Memphis/Shelby County Health Department, and in recent times chemical control (malathion and resmethrin ULV) has been focused on areas where mosquito population indices are high. In addition, virus surveillance activities in Memphis have been conducted for several years by the Vector-Borne Diseases Division of the Centers for Disease Control at Fort Collins, Colorado.

Based on surveillance experience of the local workers, 8 collection sites were chosen that effectively sampled the control area. These sites were found in areas of both higher and lower socioeconomic levels and human population densities and represented waters contaminated with a variety of organic materials, ranging from the wind drift from feed mill separators to feed lot runoff and ice cream plant effluent.

Collections of 5 egg rafts/site were made approximately every 2 weeks from June-October, 1979, and from May-October, 1980, from the chosen sites. Where collections from a specific site were impossible, nearby sites were used. In all cases, the collection address for each raft was recorded.

Rafts were individually packaged on moist paper towelling in plastic vials and sent by air (overnight arrival) to the Centers for Disease Control in Atlanta, Georgia. The rafts were floated in individual 100- x 15-mm petri dishes of deionized water with ample powdered lab feed provided. Egg raft viability was >90%; in most cases, hatched larvae swam off the filter paper as the egg rafts were floated. Following 2 days of development, larvae were transferred to larger 150- x 25-mm petri dishes for rearing. Rearing in petri dishes, which can be stacked while in use, conserves materials and space.

At the late fourth instar stage, larvae were identified as to species, and the siphonal index was measured as previously described (Brogdon 1981) for 25 to 30 individuals from each *Culex pipiens* egg raft. This method involves far less effort and is less subjective than measurements of male genitalia.

RESULTS

The seasonal patterns of relative abundance of *Culex pipiens*, *Cx. restuans*, and *Cx. salinarius* were dissimilar for the 1979 and 1980 breeding seasons. In 1979 (Fig. 1), a sharp transition from *Cx. restuans* to *Cx. pipiens* occurred in early July, and the reverse transition to *Cx. restuans* was in early September. The few *Cx. salinarius* egg rafts observed appeared early and late in the 1979 breeding season.

In 1980 (Fig. 2), the transition from *Cx. restuans* to *Cx. pipiens* occurred approximately one month earlier, but this high *Cx. pipiens* relative abundance did not persist, and the mid-season peak of *Cx. pipiens* observed in 1979, was repeated in 1980. However, the reverse transition to *Cx. restuans* occurred much later in 1980 (October) than in 1979. In addition, a pronounced increase in *Cx. salinarius* activity occurred in September, rather than early and late in the season as was the case in 1979.

The relative abundance of *Culex pipiens* subspecies/intermediates was studied by means of the siphonal index. As has been reported (Brogdon 1981), based on this index, 2 forms of *Culex* subspecies intermediates occur in Memphis. The differences in *Culex* species abundance between 1979 and 1980, were reflected in the *Culex pipiens* subspecies/intermediates data for the 2 breeding seasons. In both years (Figs. 3 and 4), the late season egg rafts resembled *Culex pipiens quinquefasciatus* and the early season ones resembled *Culex pipiens pipiens* (except for the earliest collections which will be further discussed). However, the transition from intermediates resembling *Cx. p. pipiens* to those resembling *Cx. p. quinquefasciatus* occurred earlier in 1980, with the result that July populations were predominately the late season intermediate. In addition, a bimodal peak in *Culex pipiens* abundance relative to *Cx. restuans* occurred in 1980, where the early season intermediate population peak is well separated from that of the late season intermediate.

In both seasons, the earliest collections in the spring showed a high number of egg rafts resembling the late season intermediate. These egg rafts may have originated from overwintering females, and the subsequent sharp decline in egg rafts resembling *Cx. p. quinquefasciatus* may have been due to selection by early season conditions. It should be noted that a small number of egg rafts resembling *Cx. p. quinquefasciatus* persisted until the later season when they predominated. Also of interest is the brief late season peak of the intermediate resembling *Culex pipiens pipiens* in both 1979 and 1980. This activity peak closely preceded the transition from *Culex pipiens* to *Culex restuans*. Unlike 1979, however, the late season intermediate maintained its predominance through this period.

DISCUSSION

These data show that in Memphis the relative abundance of the major *Culex* species and subspecies/intermediates differ markedly within a breeding season and from one season to the next. Such differences may be significant with respect to SLE incidence and seasonal occurrence for several reasons. Considerations in assessing the epidemiologic importance of species abundance patterns are species vector ability (toward human or amplification hosts) and factors influencing vector bionomics (especially those modulating vector species abundance through the breeding season).

The most established vector of SLE virus, responsible for virus enzootic circulation and endemic/epidemic virus transmission to man in the East and Southcentral States including Memphis, is *Culex pipiens* and *quinquefasciatus* (Mitchel et al. 1980). Within the same area, however, 2 secondary or accessory vectors, *Cx. restuans* and *Cx. salinarius*, may contribute to enzootic and epidemic virus transmission; the man-biting habits of *Cx. salinarius* implicate this mosquito in transmission to humans (Mitchel et al. 1980).

Factors influencing vector bionomics are frequently those affecting virus transmission or serving as epidemic indicators. These include climatic and socioeconomic factors. Climatic factors, including rainfall, temperature, and humidity, influence prevalence of particular species by affecting, among other things, mating, host seeking, blood feeding, egg maturation, egg-laying, larval growth, and survival of all life stages. An excellent review of these factors as they relate to SLE vectors appears in Mitchel et al. (1980). Socioeconomic factors affecting species prevalence include water quality and degree of man/mosquito contact. These factors, as they relate to SLE epidemiology, have been thoroughly discussed by Monath (1980). Species abundance data from Memphis in 1979 and 1980, may be evaluated in light of the above discussion.

Epidemics of SLE generally peak in the late season months, August-October (Monath 1980). In Memphis in 1979, this period was characterized by a sharp decline in *pipiens* abundance. Relative to *Cx. restuans*, *Cx. salinarius* was virtually absent throughout this period. It seems a good assumption, based on what is known about SLE virus transmission, that decreased risk of SLE transmission to humans could have been detected in 1979, concurrent with and based upon the egg raft survey. A concomitant savings in *Culex* control activities would also have been possible, since *Cx. restuans* is also not a nuisance mosquito. Also of great importance in comparisons of 1979 and 1980, is the fact that *Culex pipiens quinquefasciatus* is more of a human feeder than is *Culex pipiens pipiens*, which is a canopy feeder on birds. Whether the intermediates resembling the 2 subspecies also show this different biting behavior remains to be learned. If they do, the transition between these forms could be a very important factor in SLE transmission.

In 1980, high relative abundances of both *Cx. pipiens* and *Cx. salinarius* during the same late season period were similar to those observed (based on mosquitoes collected for viral assay) in the Memphis SLE epidemic years 1974 and 1975. In these years, early (May) replacement of *Cx. restuans* by *Cx. pipiens* occurred, and *Cx. pipiens* and *Cx. salinarius* relative abundances were high late in the season. The 1980 season is the first since the epidemic years 1974 and 1975 when *Cx. salinarius* showed such a peak of activity.

Since factors other than high vector populations also influence human SLE occurrence, it follows that egg raft surveillance would be most useful in detection of seasonally low *Cx. pipiens* and *Cx. salinarius* populations, thus allowing savings in routine control efforts. Conversely, demonstration of high *Cx. pipiens* and *Cx. salinarius* relative abundances at a time when viral surveillance shows high SLE activity could allow assessment and localization of risk to humans.

The advantages of egg raft surveillance lie in the minimal resources and training of personnel required on the part of local control agencies. *Culex pipiens*, *Cx. restuans*, and *Cx. salinarius* are easily distinguished as larvae and, using petri dish rearing, hundreds of egg rafts can be processed for evaluation in a limited space. Should differentiation of *Culex pipiens* subspecies prove to be important in assessing the disease threat, the siphonal index would provide a relatively simple procedure for these determinations.

In Memphis, as in other areas where good surveillance and control are exercised, much time is spent in accurate mapping of breeding sites within the control area. Knowledge of these sites would allow egg raft surveillance to be especially effective, as in SLE control areas, where foci of human infection are highly localized and the areas of potential risk must be closely monitored.

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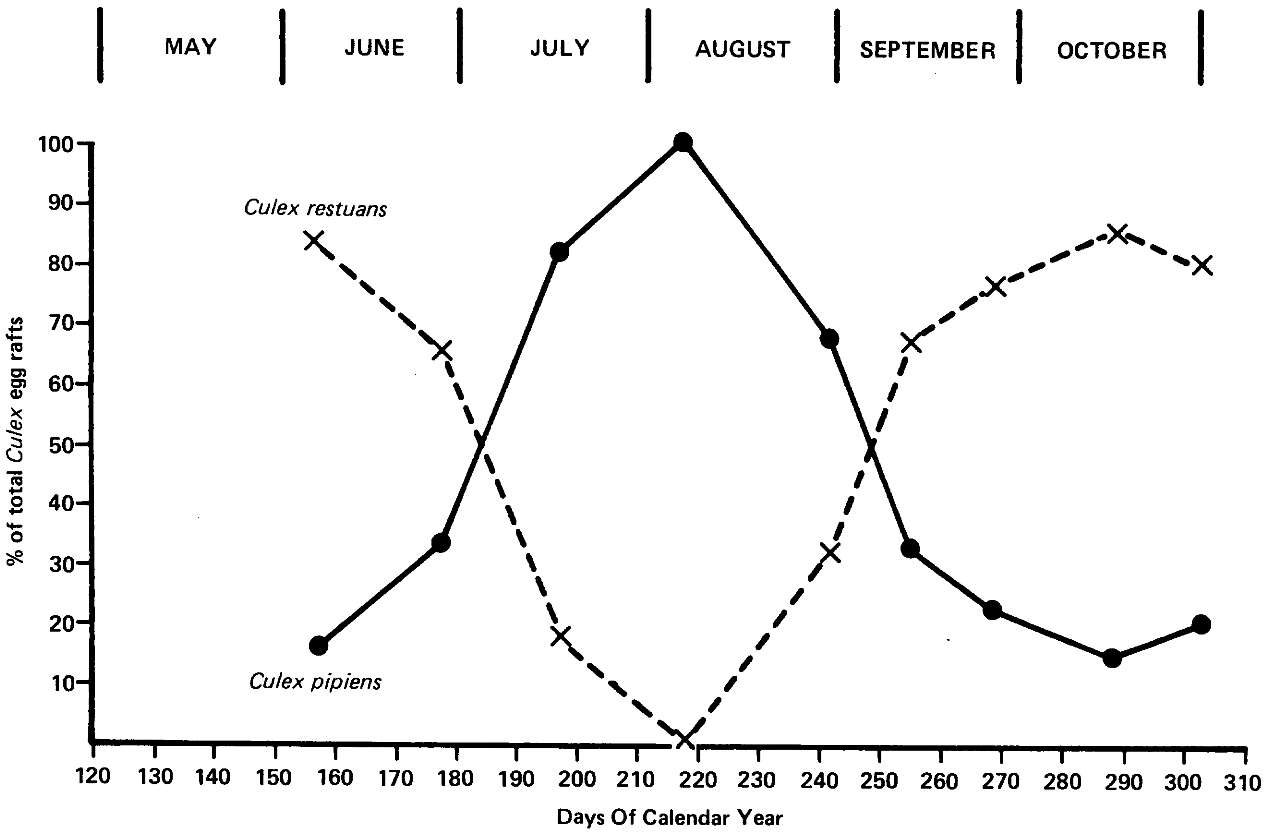

Fig. 1. Relative abundance of *Culex* species in Memphis from June-October, 1979. Data based on collections of 362 egg rafts.

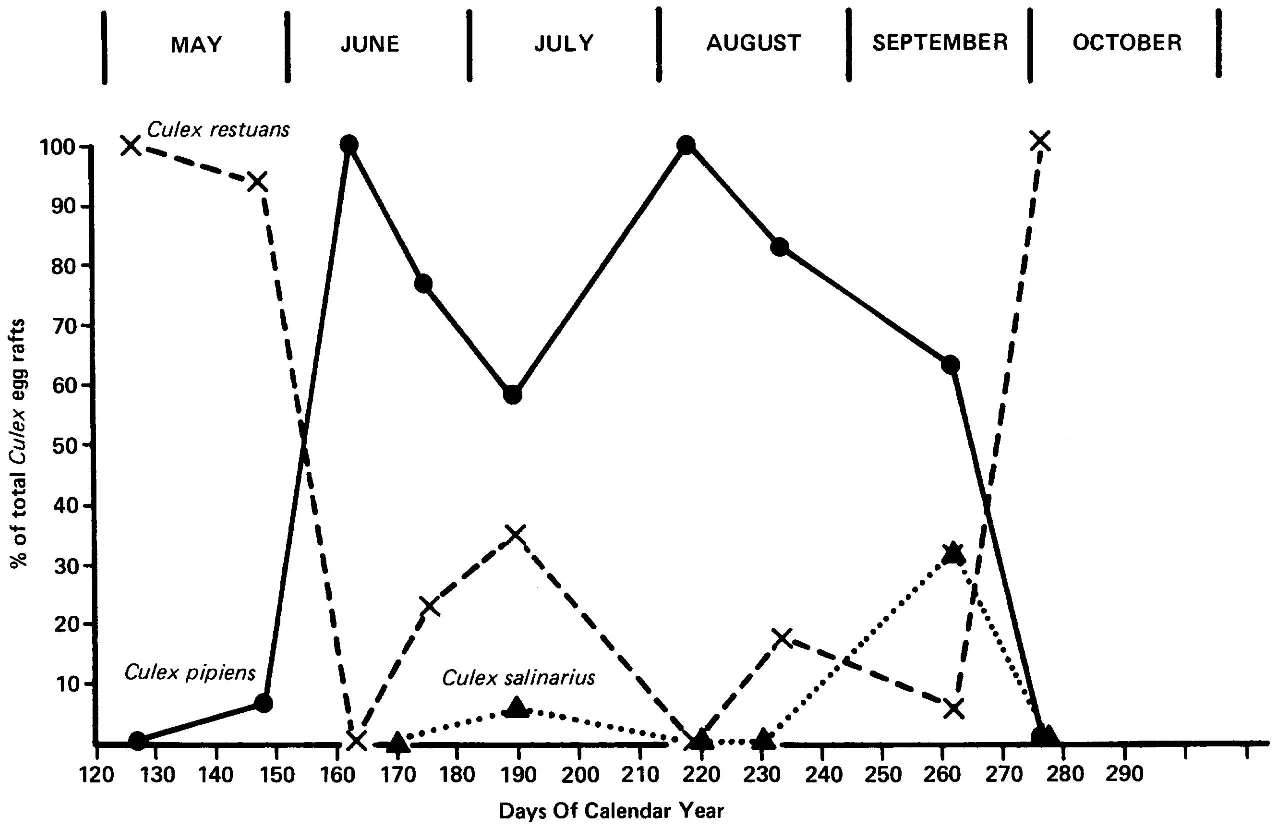

Fig. 2. Relative abundance of *Culex* species in Memphis from May-October, 1980. Data based on collections of 357 egg rafts.

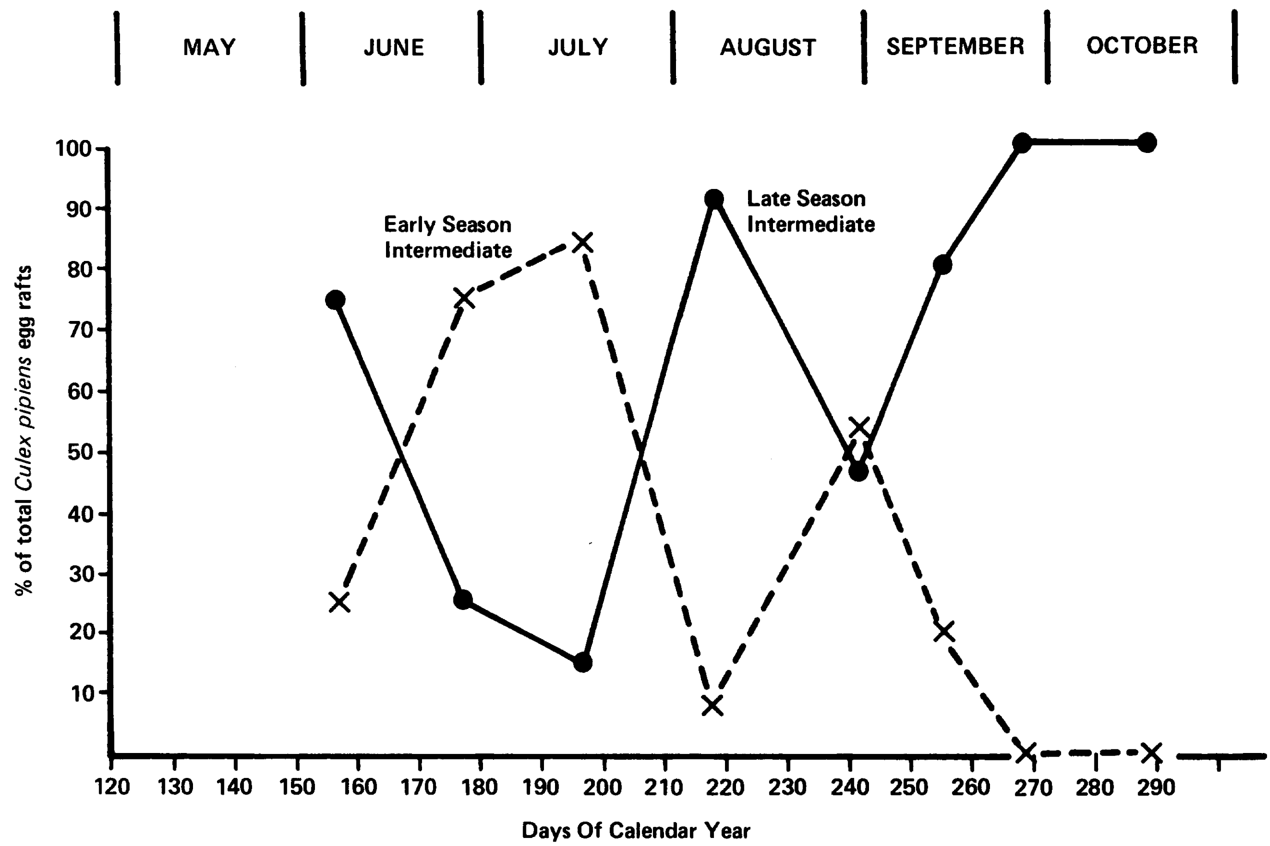

Fig. 3. Relative abundance of *Culex pipiens* intermediates in Memphis from June-October, 1979. Data based on 159 egg rafts.

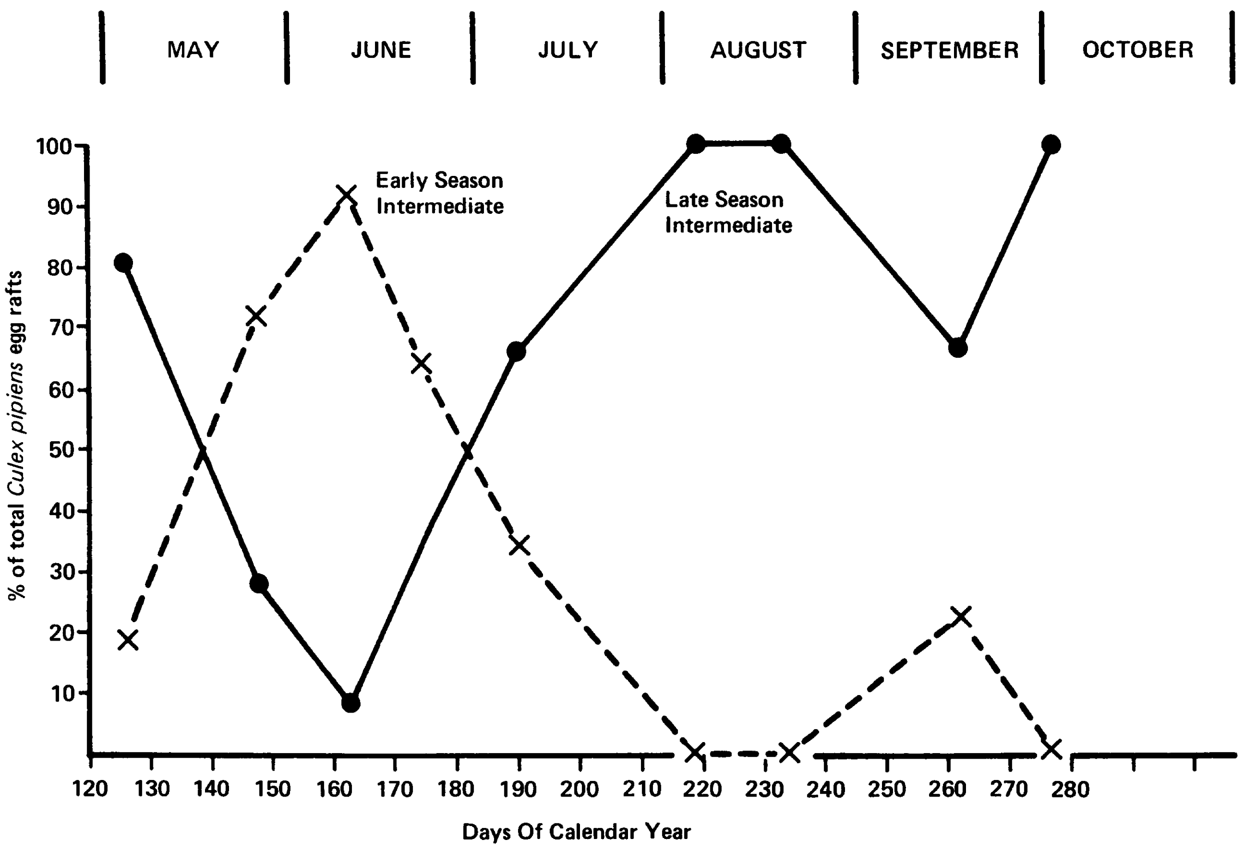

Fig. 4. Relative abundance of *Culex pipiens* intermediates in Memphis from May-October, 1980. Data based on 193 egg rafts.