

A STUDY OF THE *CULEX PIFIENS* COMPLEX IN THE OHIO-MISSISSIPPI RIVER BASIN IN RELATION TO ST. LOUIS ENCEPHALITIS^{1, 2}

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ABSTRACT. Following the 1964 outbreak of SLE virus in McLeansboro, Illinois, this virus was isolated from *Culex pipiens* complex mosquitoes each year through 1967. Studies were conducted in St. Louis County, Missouri during an SLE outbreak in 1965 and surveillance was continued over the next 2 years. An intensive effort was made in McLeansboro to determine which members of the complex were present throughout the year by rearing progeny from selected wild captured females. The DV/D ratios were used to distinguish *quinquefasciatus* from *pipiens*; autogeny was the test for *molestus*. Precipitin studies on wild caught females of the complex were processed in all study sites. In 1967 McLeansboro was found to have only

pipiens and the build-up of this population throughout their season is documented. Collections were examined to determine the number engorged, gravid or deplete. A decrease in blood feeding as winter approached is contrasted with the rise in the number of *pipiens* entering hibernation without having taken blood.

In St. Louis County, one area was found to have mixed populations of *quinquefasciatus* and *pipiens*; precipitin studies in this area showed an increase in mammal feeding in contrast to bird feedings. No SLE virus was isolated from females that had progeny reared from them. Suggestions are given as to necessary studies in future epidemics of SLE in the Ohio-Mississippi River Basin.

Because of their role as vectors of disease, mosquitoes of the *Culex pipiens* complex have been of special interest in the Ohio-Mississippi River Basin since the 1930's when they were suspected of being involved in an outbreak of an unknown disease affecting the citizens of Paris, Illinois. Because the central nervous system was affected and because the outbreak occurred late in a hot, dry summer it was concluded it was St. Louis encephalitis. In Lumsden's account of the 1933-1934 St. Louis epidemic of SLE he credited the vast numbers of *Culex* mosquitoes with very probable involvement (Lumsden, 1958). More recent evidence of St. Louis encephalitis (SLE) in the Ohio-Mississippi River Basin occurred at Calvert City, Kentucky, in 1955. In 1964, the virus was isolated from mosqui-

toes associated with an outbreak in McLeansboro, Illinois, as encephalitis in man was reported from Texas to New Jersey.

The Center for Zoonoses Research, University of Illinois initiated arbovirus studies in the Ohio-Mississippi River Basin in 1963, and the center's staff investigated the SLE outbreak in McLeansboro, Illinois in 1964. The results of this central work are reported elsewhere (Kokernot *et al.*, 1967); however, in 1964 a more positive identification of the SLE virus vector was made when virus was isolated from 32 of 56 pools (25 females per pool) of *Culex pipiens* complex mosquitoes. In McLeansboro, SLE virus was isolated from this complex each year from 1964 through 1967; thus it was most apparent that if the flow of virus from mosquitoes to birds and man was to be understood it would be necessary to establish the basic biology of the complex and isolate SLE virus in nonepidemic, as well as epidemic years.

OBJECTIVES. The specific objectives of the study were:

1. To describe the seasonal distribution of the species of the *Culex pipiens* complex in the Ohio-Mississippi River Basin.
2. To describe the blood feeding pat-

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terns in the various study sites of the complex and relate this to the members of the complex in these sites throughout the season.

3. To determine the man biting rates of the complex in McLeansboro, Illinois.

4. To determine by isolation of SLE from wild caught female mosquitoes, and by analysis of blood feeding patterns and population dynamics which species of the complex are involved in SLE transmission.

MATERIALS AND METHODS. The following designations are used referring to the members of the *Culex pipiens* complex: *Culex pipiens pipiens* is called *pipiens*, *Culex pipiens quinquefasciatus* is referred to as *quinquefasciatus*, and *Culex pipiens molestus* is called *molestus*. Hereafter the *Culex pipiens* complex will be referred to simply as the complex.

For all practical purposes, the adult females of the complex are morphologically indistinguishable. Routine processing for arbovirus isolation without first obtaining male progeny from the adult female makes subsequent species determination within the complex impossible.

Wild caught gravid females from select sites in McLeansboro were taken to the laboratory, permitted to deposit eggs and the terminalia of male progeny from these egg rafts were mounted and the DV/D ratio (Sundaraman, 1940)⁴ was measured. Specimens were then assigned to species according to the criteria used by (Barr, 1957): less than 0.2 = *pipiens*; 0.2 — 0.4 = intermediate; 0.4 or greater =

quinquefasciatus. Wild caught males were also measured.

Female progeny reared from rafts were maintained on a carbohydrate diet and observed for evidence of autogeny, then frozen for future dissection to confirm that there was no ovarian development. For a future attempt to isolate virus, the female parent from which progeny were obtained was frozen. To measure the buildup of the resting population, collections of resting mosquitoes were started with one man collecting for 30 minutes in each of three sites; however, numbers in excess of those needed were obtained, and the collecting time was reduced to 15 minutes per site from August until November. All female mosquitoes from resting collections were classified as to being engorged, gravid or deplete.

To determine the time and frequency of biting by the mosquitoes upon man, overnight biting collections were initiated in June 1967 in McLeansboro at Site 41. Females had not been collected in day-biting captures in previous years so collections were made for 12-hour periods from at least a half hour before sunset to a half hour after sunrise. Site 41 which was at the edge of a railroad was covered by honeysuckle, honey locust and willow trees, bordering upon an abandoned garden spot. The nearest houses were 100 yards away. Collecting was done in 2-hour shifts with two men present throughout the summer and fall. Collections of bird-attracted mosquitoes were made in 3' x 3' x 2' cages modified to capture mosquitoes attracted to chickens. These specimens were processed for arbovirus isolation. Routine resting captures of the complex were made by the St. Louis County Health Department in a surveillance program done throughout 1966-1967. This program was a continuance of the work in 1965 by the Center for Zoonoses during an outbreak of SLE (Kokernot and Brandley, 1969). Some females captured in St. Louis County were processed for virus isolation

⁴ Determination of the DV/D ratio

Culex pipiens quinquefasciatus is best distinguished from *Culex pipiens pipiens* by the basic structure of the aedeagus. Sundaraman (1949) developed the DV/D ratio which compares the extension of the distal ends of the ventral arms of the phallosome laterally beyond the outer point of the intersection of the dorsal arms with the ventral arms of the phallosome (DV) to the distance between the outer point of the intersection of the dorsal arms with the ventral arms (D). Individuals having a DV/D ratio of 0.20 may be considered as *pipiens*; *quinquefasciatus* is designated by a ratio of 0.40 or greater.

and suitable engorged specimens were saved for precipitin study. Males were mounted and DV/D ratios determined. Collections of resting adults were also made in Eldorado, Illinois, a community of similar size and only 20 miles south of McLeansboro, to compare with the McLeansboro collections. Wild caught males were examined and females of the complex were processed for virus isolation.

LOCATION OF STUDY SITES. The map (Fig. 1) indicates the location of the various collecting sites within the Ohio-Mississippi River Basin. The principal study site was McLeansboro, location of the 1964 outbreak of SLE. In 1965 an outbreak of SLE was detected in St. Louis County. Collections were made at four major sites in the municipalities of Ladue, University City, LeMay and a series of storm drains along the River Des Peres, all of which were within 10 miles of each other.

RESULTS. McLeansboro-Eldorado Area. *Pipiens* was found in McLeansboro in the fall of 1966 (DV/D mean = 0.43 ± 0.01 , $N = 398$). No *quinquefasciatus* was found by examination and measurement of 4,270 progeny of 480 females reared from June 1967 to July 1968 and of 444 wild captured males collected from June through October 1967 in McLeansboro. The sampling from the nearby community of Eldorado was also negative for *quinquefasciatus*; all wild caught males, numbering 289, were *pipiens* and were captured from June to October 1967.

The average measurements of 10-15 male progeny per female taken in resting captures from June to October 1967 at McLeansboro were (mean DV/D ratio \pm standard error of mean): June, 0.46 ± 0.04 ; July, 0.31 ± 0.04 ; August, 0.41 ± 0.04 ; September, 0.39 ± 0.033 ; October, 0.34 ± 0.04 . No evidence of autogeny was ever demonstrated, thus I feel confident in saying only *pipiens* was present in McLeansboro in 1967 and all observations of the complex during this time can be attributed solely to *pipiens*.

Male progeny from 20 females (bird

attracted) were successfully reared and all were typical *pipiens*. Since vandals released the chickens and destroyed the cages, this aspect of the study was not further pursued. Only 1 egg raft was obtained from a man-attracted female; male offspring were typical *pipiens*. SLE virus was isolated each year in McLeansboro from 1964 through 1967 in wild captured complex females (Kokernot 1969).

Since the 1964 outbreak of SLE, resting captures from storm sewers have yielded nearly all of the specimens collected for attempted virus isolation and as in previous years, the population build-up in storm sewers started in June and continued until cold weather set in. In addition to the complex, *Culex restuans* was the only other mosquito present in resting captures in significant numbers, constituting 25 percent of the collection in June (46 of 184). This percentage, in general, decreased as the season progressed and by late September this species was absent. *Culex salinarius*, *Culex (Melanoconion)* species, *Culex territans*, and *Anopheles quadrimaculatus* were never present in proportions greater than 3 percent. Females of the complex increased steadily in numbers collected as the season progressed. The number collected per man-minute of collecting was 4.1 on June 19; 6.9 on July 15; 9.9 on August 14; 11.3 on September 5; 14.2 on September 20 and 18.7 by October 5 (Table 1).

In Table 2, the numbers of gravid complex females collected for rearing reflected an increase similar to what one would expect with the coinciding increase in numbers of engorged females; however, as cold weather approached, females which had fed on other substances developed large fat bodies and might be classified as being gravid by the unwary. The increased feeding on other substances (presumably carbohydrates) as winter approached was well illustrated by the fact that from June to the end of August there were no "carbohydrate-fed" females, at the beginning of September there were 33 percent, two weeks later, over 50 per-



FIG. 1.—Map showing location of study sites, Courtesy Medical Communications, Univ. of Texas.

TABLE 1.—Numbers of mosquitoes taken in resting captures from sites 39, 47 and 49 McLeansboro, Illinois 1967.

Date	June 19	July 18	Aug. 14	Sept. 5	Sept. 20	Oct. 5
	1 man 30 min.	1 man 30 min.	1 man 15 min.	1 man 15 min.	1 man 15 min.	1 man 15 min.
<i>Culex pipiens</i> comp.	136	209	149	179	214	281
<i>Culex restuans</i>	46	34	45	16	7	0
<i>Culex salinarius</i>	2	5	7	2	0	1
<i>Culex territans</i>	0	3	4	2	3	4
<i>Culex (Melanoconion) species</i>	0	1	2	1	4	3
<i>Anopheles quadrimaculatus</i>	0	2	5	2	4	1
Total	184	254	212	202	232	290

cent and by October 31, 99 of 101 females collected were "carbohydrate fed" (Fig. 2.) In other words, I selected gravid females with 100 percent accuracy from June until the end of August. From the end of August an increasing percentage of apparently gravid females were unable to lay eggs and required blood meals to oviposit.

Figure 2 demonstrates the seasonal reduction of blood feeding and contrasts the increase of "false positive" gravids against the number of truly gravid females.

St. Louis County, Missouri Area, 1967.

Along the River Des Peres and in Lemay, St. Louis County, during 1967, a mixed population of *pipiens* and *quinquefasciatus* was found. In contrast to the usual dominant bird feedings by the complex in Ladue and University City,

Missouri and in McLeansboro and Eldorado, Illinois, where the population was practically pure *pipiens*, female mosquitoes from this area exhibited a greater preference for mammal blood. From University City and Ladue, 428 *pipiens* males and no *quinquefasciatus* were found in August; in September 234 *pipiens* and 3 *quinquefasciatus* were present; by October only 35 *pipiens* and no *quinquefasciatus* were collected. The River Des Peres—Lemay area had 95 *pipiens* and 64 *quinquefasciatus* in August. By September the ratio changed to 21 *pipiens* to 30 *quinquefasciatus*; in October the ratio was 7 *pipiens* to 8 *quinquefasciatus*.

Although 1,381 males were examined in the mixed population breeding side by side, only 2 specimens that could be regarded as hybrids were found (Table 3).

Blood feeding in the River Des Peres—Lemay area was as follows: 4 mammals versus 32 birds in July, 20 mammals

TABLE 2.—Physiological reproductive status of *Culex pipiens* complex females in McLeansboro, Illinois 1967.

Month	No. & % engorged	No. & % with fat body	No. & % deplete	Total
June	157 (22%)	241 (32%)	345 (46%)	743
July	223 (44%)	155 (31%)	125 (25%)	503
August	676 (27%)	550 (22%)	1255 (51%)	2841
September	74 (3%)	1275 (46%)	1385 (51%)	2734
October	30 (1%)	2426 (83%)	455 (16%)	2911
November	1 (0.5%)	586 (99.5%)	—0—	587
				9959

TABLE 3.—Ratio of *Culex pipiens pipiens* to *Culex pipiens quinquefasciatus* from selected communities in St. Louis County, Missouri, 1967 as determined by male terminalia.

	August		September		October	
	<i>Culex pipiens</i>	<i>Culex quinquefasciatus</i>	<i>Culex pipiens</i>	<i>Culex quinquefasciatus</i>	<i>Culex pipiens</i>	<i>Culex quinquefasciatus</i>
River Des Peres	87	9	9	16	4	5
	1	Intermediate	1	Intermediate		
Lemay	8	55	12	14	3	3
University City	388	0	21	3	21	0
			1	Intermediate		
Ladue	40	0	213	0	14	0

Physiological Status of *Culex pipiens* Complex
Females in McLeansboro, Illinois
June through November 1967

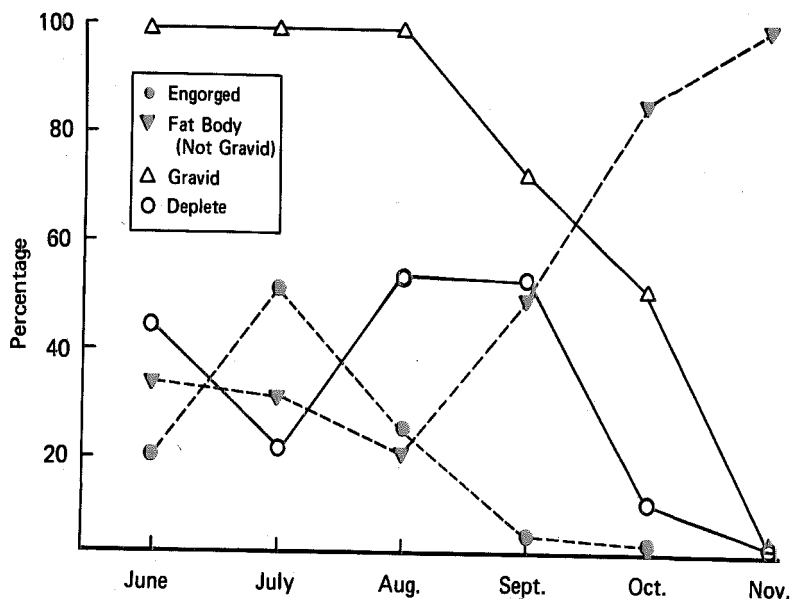


FIG. 2.—Courtesy Medical Communications, Univ. of Texas.

versus 51 birds in August, and 11 mammals versus 9 birds in September. The only blood meal obtained in October was mammalian. In contrast to the above area, the Ladue—University City area had only 6 mammal feedings recorded for the entire season: 1 in July; 3 in August; and 2 in September. There were 63 bird feedings in July, 64 in August, 51 in September and 3 in October.

DISCUSSION. The phenomenon of carbohydrate feeding and reduced blood feeding as autumn approaches appears to have a significant role in the reduction of virus present in *pipiens* in epidemic situations. During the 1964 McLeansboro SLE epidemic, nearly every other pool of 25 complex mosquitoes was positive for SLE virus. (Kokernot *et al.*, 1967). One month later, 856 complex mosquitoes were collected and only 2 of 18 pools were SLE positive. In November, 1964, 1,150 females were processed in 23 pools and only one pool was positive. In December 1964, 625 complex females were processed in 13 pools and none was positive.

To further illustrate the probable involvement of reduced blood feeding in reduced virus transmission are data from two sites in St. Louis County in 1966. From September 2 to September 5, 1,147 complex females processed in 47 pools yielded 10 positive pools, yet two weeks later 922 complex females processed in 36 pools, yielded no virus. (Kokernot *et al.*, 1969.) This is the same time of year that reduced blood feeding, and increased consumption of other substances (presumably carbohydrates) was documented in McLeansboro, Illinois in 1967. Host immunization and many other factors must be considered. However, the phenomenon of feeding on substances other than blood warrants considerable detailed study. The physiological requirements for both sugar and blood throughout the season present interesting problems.

The presence of *quinquefasciatus* in the River Des Peres—Lemay area of St. Louis County was quite demonstrable using measurements of male terminalia.

A monthly mean increase in the DV/D ratio from .283 in August to .527 in September coupled with the increased rate of mammal feedings from 28 percent in August to 55 percent in September, clearly demonstrates the potential of *quinquefasciatus* to introduce virus into the mammal populations. It is my opinion that *quinquefasciatus* would be most conducive to the introduction of SLE virus into man. There is still a need for detailed study of the complex in epidemic situations in the Ohio-Mississippi River Basin. Future investigations must not be content to process complex mosquitoes without rearing progeny to determine specifically which members of the complex carry the virus.

In other parts of the world *quinquefasciatus* feeds readily upon man; de Meillon and Sebastian (1967) found 89 percent of this species engorged on human blood. Precipitin studies from McLeansboro (1967) which had a *pipiens* population showed that population to be ornithophilic. Other studies of *quinquefasciatus* vary: Hammon *et al.* (1945) in a California study showed an 87.6 percent feeding on birds. My precipitin studies in Houston also substantiate the fact that *quinquefasciatus* feeds readily on man. Future studies should be made to determine the sequence of the movement of *quinquefasciatus* into the Ohio-Mississippi River Basin. The movement of *quinquefasciatus* up rivers has been documented in California by Iltis (1957).

In future outbreaks of SLE it is hoped that the interaction of man and vector will be closely examined. Only by rearing out progeny from man attracted complex females will it be learned which members of this complex are responsible for human cases of SLE.

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