

Further Studies on the *Culex pipiens* Complex
in Memphis, Tennessee

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ABSTRACT. Extensive resting site collections related to arbovirus investigations in 1979 yielded more than 1,000 male specimens. The DV/D ratios of these specimens were determined. *Culex pipiens pipiens*-like individuals were more numerous than *Cx. p. quinquefasciatus*-like individuals but intermediates constituted more than 50% of the population. The increase in *pipiens*-like individuals is attributed to a severe winter and excessive rainfall, particularly early in the mosquito season. The considerable gene flow between the two subspecies found in this area in the previous 2 years is substantiated.

Intensive resting site collections relating to arbovirus investigations were continued in 1979 as part of collaborative studies between the Memphis-Shelby County Health Department and the Center for Disease Control, Vector-Borne Diseases Division, Fort Collins, Colorado. Mosquitoes were collected from established natural sites, principally culverts, at locations near dwellings of human cases in the 1974 St. Louis encephalitis outbreak and at other suitable sites throughout the metropolitan area. Female specimens were pooled for virus isolation attempts, while males of the *Culex pipiens* complex were examined by the method of Sundararaman (1949), i.e., for DV/D ratio, the only known reliable means of distinguishing the adult males of *pipiens pipiens* Linnaeus from those of *pipiens quinquefasciatus* Say.

Considerable gene flow between members of the complex was shown (Jakob et al., 1979) in a similar study of male specimens obtained from collections in Memphis in 1977 and 1978. Memphis is located at the southern edge of the intergradation zone outlined by Barr (1957) where *pipiens*, *quinquefasciatus*, intermediates and various mixtures of the three are likely to be found. The collections in 1979 were begun earlier in the year than in the previous years

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in attempts to isolate virus from members of the complex and/or *Cx. restuans* Theobald, a species known to produce peak populations in spring, and to investigate the dynamics of the population as early in the mosquito season as possible. The results of species determination of male specimens from these collections are reported here.

METHODS

Collections of mosquitoes (May through November) from natural resting sites by use of mechanical aspirators were made weekly or biweekly at specific sites and at less frequent intervals at other established sites. The mosquitoes were frozen and shipped to Fort Collins where females were pooled for virus isolation attempts. Male specimens were removed and stored at room temperature, with collection sites and dates noted, until terminalia could be examined.

Terminalia were cleared for 7 to 10 minutes in hot 10 percent KOH, washed in water, dehydrated with glacial acetic acid, and softened in glacial acetic acid: oil of cloves (1:1) for 10 to 15 minutes, and then soaked in glacial acetic acid: oil of cloves (1:2) for at least 30 minutes (Breeland, 1951). All measurements were made with the terminalia in a small amount of clove oil; no permanent mounts were made.

On the basis of the results obtained by Barr (1957), males with a DV/D ratio of 0.20 or less were considered *pipiens*-like and individuals with values of 0.40 or greater, *quinquefasciatus*-like. Those with values >0.20 but <0.40 were classed as intermediates.

RESULTS

More than 1090 male specimens were examined from the 97 sites from which such specimens were recovered (Table 1). The data show most frequently that only one or two males were obtained from a given collection site, thus reducing the problem of bias associated with collection sites. Male specimens were recovered only once from 42 of the 97 sites and more than 50 specimens were obtained from only 5 sites. Seventy-five of the sites yielded 10 or fewer males during the collection period.

The seasonal occurrence of the three forms is shown in Table 2. The data show no distinct seasonal pattern of either subspecies and indicate that intermediates were more numerous than either of the parental-like forms throughout the collection period except for the October collections. If only the two subspecies are considered, *pipiens*-like males in 1979 made up approximately 59% of the complex as compared to approximately 42% in 1977 and 1978 (Jakob, et al., op. cit.). Intermediate forms constituted 52% of the population in 1979 as compared with 50% in 1978.

The distribution of DV/D ratios for the 1979 study is shown in Figure 1. Negative values were recorded as 0; thus the range of values obtained was 0 to 1.65. Many of the highest values were obtained in October, when a significant increase in the occurrence of *quinquefasciatus*-like males was noted. The histogram shows that most DV/D values fell in the range of >0.10 to <0.40.

The temporal frequency of the ratios is shown in Table 3. No trend is evident. Most of the values for each month are in the >0.10 to <0.40 range, and the increase of *quinquefasciatus*-like individuals in October is marked.

DISCUSSION

The relative abundance of *pipiens*-like individuals in Memphis in 1979 was unexpected in view of the preponderance of *quinquefasciatus*-like individuals during the previous 2 years. The very cold winter of 1978-79, combined with an unusually cool spring and excessive rainfall throughout the mosquito season, particularly April and May (>9 inches in excess of normal), may well have contributed to the abundance of the *pipiens*-like form. During January and February, 1979 the mean temperatures were 4.9 and 2.5° C below normal.

The mean DV/D ratio of the population was reduced to $0.307 \pm \text{s.e. } .006$ as compared to $0.337 \pm .011$ and $0.338 \pm .009$ in 1977 and 1978, respectively. Thus a significant change in the Memphis population occurred in 1979 [p < .01, t-test (1978)]. Similarly, in California, Iltis (1966) found the cline of the complex to be highly modified by local conditions.

We can only speculate that the increase in *quinquefasciatus*-like specimens in October was the result of the oft-noted northern extension of this form during the latter part of the season. No severe weather conditions which might favor incursion were noted in climatological records, but wind direction in late September and early October was generally from the S-SW and might have facilitated the northward movement of this subspecies. Such late-season northern movement by *quinquefasciatus*-like individuals have been observed in Kansas (McMillan, 1958) and Utah (Rosay and Nielsen, 1973). That an influx occurred is suggested by the markedly higher mean DV/D ratio for this form (0.71) in comparison to values obtained for earlier collection periods (0.47 to 0.57). In addition, the comparable mean of 74 males collected in mid-October in several areas to the south, in Mississippi, was 0.65.

These data for 1979 confirm the considerable gene flow in the *pipiens* complex population in the Memphis, Tennessee area where the ranges of *p. pipiens* and *p. quinquefasciatus* overlap. In this area, and also probably in other sections of the intergradation zone not heretofore adequately studied, the *pipiens* complex continues to be something of an enigma, defying efforts to determine the role of each subspecies and the intermediates in relation to parameters such as vector efficiency, morphology, and hibernation capability.

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Table 1. Yield of *pipiens* complex males from natural resting site collections, Memphis, Tennessee, 1979.

<u>No. Males</u>	<u>No. Times</u>
1 or 2	233
3 - 5	81
6 - 10	25
11 - 20	14
>20	3

n = 97 sites

Table 2. Temporal occurrence of *Culex pipiens* complex males, Memphis, Tennessee, 1979.

<u>Period</u>	<u><i>p. pipiens</i></u>		<u><i>p. quinquefasciatus</i></u>		<u>intermediates</u>		<u>Total</u>
May	8	(31%) ^a	5	(19%)	13	(50%)	26
June	78	(34%)	36	(15%)	118	(51%)	232
July	90	(27%)	64	(19%)	179	(54%)	333
August	49	(26%)	18	(10%)	118	(64%)	185
September	40	(36%)	21	(19%)	50	(45%)	111
October	25	(17%)	63	(42%)	61	(41%)	149
November	16	(29%)	6	(11%)	33	(60%)	55
Total	306	(28%)	213	(20%)	572	(52%)	1091

^aPercent of total specimens for the month.

Table 3. Temporal Frequency of DV/D Ratios of *Culex pipiens* complex males, Memphis, Tennessee, 1979.

<u>DV/D Ratio</u>	<u>May</u>	<u>June</u>	<u>July</u>	<u>August</u>	<u>September</u>	<u>October</u>	<u>November</u>
0-0.10	0	10	16	6	3	6	2
0.11-0.20	8	68	74	43	37	19	14
0.21-0.30	6	79	121	84	40	37	19
0.31-0.39	7	39	58	34	10	24	14
0.40-0.49	3	20	35	14	14	19	1
0.50-0.59	0	8	14	3	2	10	1
≥ 0.60	2	8	15	1	5	34	4

FIG. 1 HISTOGRAM SHOWING DISTRIBUTION OF DV/D RATIOS OF CULEX PIPIENS COMPLEX MALES, MEMPHIS, TENN., 1979.

