

The *Culex pipiens* Complex in Memphis, TennesseeW. L. Jakob,¹ S. A. Dagers,¹ D. B. Francy,¹ J. Mullenix,² and K. Moseley²

ABSTRACT. Extensive resting site collections related to arbovirus investigations in 1977 and 1978 yielded more than 800 male specimens. The DV/D ratios of these specimens were determined. In both years intermediates comprised the greatest proportion of the population on a seasonal basis and *Culex pipiens quinquefasciatus* Say were more numerous than *Cx. p. pipiens* Linnaeus. In this area there appears to be a considerable gene flow between the two subspecies.

INTRODUCTION

Following an outbreak of St. Louis encephalitis (SLE) in Memphis, Tennessee, in 1974 cooperative studies were undertaken to follow the course of the virus in that area. These studies involved the Memphis-Shelby County Health Department and the Center for Disease Control, Vector-Borne Diseases Division, Fort Collins, Colorado. Intensive collections of mosquitoes were made from culverts and similar resting places at sites near human cases and at other suitable sites throughout the metropolitan area. Female specimens were pooled for virus isolation attempts, results of which will be reported separately. Males of *Culex* mosquitoes were determined by the method of Sundararaman (1949), i.e., by the DV/D ratio, the only known reliable means of distinguishing *pipiens pipiens* Linnaeus from *pipiens quinquefasciatus* Say.

Memphis is located at the southern edge of the area bounded by 36°N and 39°N latitudes, the intergradation area outlined by Barr (1957) where both³ the northern form, *pipiens*, and the southern form, *quinquefasciatus*, occur. Intermediates and various mixtures of the three are likely to be found. The tidal nature of the area for SLE and the appearance of insecticide resistance in the vector population dynamics of the complex in the area were needed. The results of species determination of male specimens collected in 1977 and 1978 are herein reported.

¹Vector-Borne Diseases Division, Center for Disease Control, Public Health Service, U.S. Department of Health, Education, and Welfare, P. O. Box 2087, Fort Collins, Colorado 80522.

²Memphis-Shelby County Health Department, Memphis, Tennessee 38104.

³The authors are aware of the reports on the complex by Jupp in South Africa, Miles in Australia, Sirivanakarn in the Orient and the listing of *pipiens* and *quinquefasciatus* as distinct species in the 1978 supplement to A Catalog of the Mosquitoes of the World (Knight and Stone 1977). The authors have opted to follow the generally recognized view that, in North America, the two are subspecies.

METHODS

Collections of mosquitoes by mechanical aspirators from natural resting sites 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 mounted for species determination.

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). The specimens were dissected in clove oil on a slide, and the oil was removed by blotting. A small drop of copal gum was then placed over the specimens which, after gentle positioning, were allowed to "set" at least overnight at room temperature before finishing the mount with balsam and a cover slip. Measurements were then taken immediately by means of an ocular micrometer at 100 diameters magnification. All determinations for specimens collected in 1977 were made by this technique.

The specimens collected in 1978 were similarly prepared; however, measurements were made with the terminalia under a small amount of clove oil. More than 300 terminalia were then further processed through copal gum and balsam (as with 1977 specimens) to provide comparative measurements. The data presented for the 1978 males are those obtained under clove oil except for a few where accurate measurements were obtained only with the permanent mount.

Based on the results obtained by Barr (1957), males with a ratio of 0.20 or less were considered *pipiens* and individuals with values of 0.40 or greater, *quinquefasciatus*. Those with values >0.20 and <0.40 were classed as intermediates.

RESULTS

Comparison of clove oil-permanent readings. The frequency with which *pipiens*, intermediates, and *quinquefasciatus* occurred as demonstrated by the two techniques is shown in the contingency table (Table 1). A significant association was noted between the two techniques; correlation coefficient = 0.95 ($p < 0.01$). No significant difference in the mean DV/D values was obtained between measurements made in clove oil or under permanent mount (Table 2) among the 3 subtypes or the total population. Mean ratio values for the 1977 and the 1978 populations were comparable.

Collection of male specimens. Totals of 318 and 495 males of the *pipiens* complex obtained from approximately 60 sites each year were examined from the 1977 and 1978 collections, respectively. The frequency of finding male specimens in the collections is shown (Table 3). The data show that only one male was found with the greatest frequency and that usually fewer than 10 males were recovered. The greatest number (62 males) from a single collection was obtained on 8-15-78.

Determination of species. The seasonal occurrence of the 3 forms obtained in each year of this study is shown in Table 4. The data show no distinct seasonal pattern of either subspecies and indicate a thorough intermingling of the forms by mid-to-late July. If only the two distinct subspecies are considered, *quinquefasciatus* in each year made up approximately 58 percent of the population.

Culex restuans Theobald males were identified in both years of the study, primarily from collections made before mid-June. Other *Culex* identified from male specimens were *salinarius* Coquillett, *erraticus* (Dyar and Knab), and *peccator* Dyar and Knab.

DISCUSSION

The close correlation of measurements taken under clove oil versus those under permanent mounts indicates that the techniques are comparable. Since this study was conducted as an adjunct to the primary purpose of this laboratory, i.e., isolation of virus from arthropod vectors, it was desirable to find a technique which would eliminate the cumbersome process of mounting genitalia directly in balsam and using broken bits of cover slips to support the cover slip while the preparation was still wet. The copal gum, under local conditions, dried to semihardness overnight, precluding pressure on the specimens from the weight of the cover slip since measurements were made as soon as the balsam and cover slip were added to the preparation. Measurements taken in clove oil further facilitated the technique in that preparation and accumulation of permanent slides was undesired. The differences between the two readings are believed due mainly to refraction in the clove oil and interpretation of points at which measurements should be made by different investigators.

Direct comparison of measurements by either method with those obtained by supporting the cover slip with bits of glass was not attempted. The data from the two methods reasonably agree with the conclusion (Barr 1957) that within the zone of intergradation *pipiens*, *quinquefasciatus*, intermediates, and various mixtures of the three may be found.

Identifications of members of the complex showed no marked temporal trend in the dynamics of either subspecies in either year of the study. In both 1977 and 1978 intermediates constituted the greatest proportion of the population on a seasonal basis and *quinquefasciatus* were more numerous than *pipiens*. If males can be assumed to be representative of the females present, the complex in Memphis is similar to that found in Sacramento County, California (Barr 1957). There appears to be considerable gene flow between the two subspecies which is contrary to the unrecognized isolating mechanism suggested for the complex in other areas within the intergradation zone, such as East St. Louis, Illinois. In California, Iltis (1966) found the cline of the complex to be highly modified by local conditions and provides data which suggest that autogeneous *pipiens* populations are genetically isolated from contiguous *quinquefasciatus* populations but not necessarily from anautogeneous *pipiens* populations.

The small number of specimens collected before June 15 (total of 15 in the 2 years of the study) precludes conclusions regarding the makeup of the complex early in the season. The relative proportion of *pipiens* in the population (40-50%) early in the year, however, is much higher than late in the season (14-23%). Again, assuming that the female population is similar to the male population and that most of such females would hibernate, survival of *pipiens* appears to be markedly higher than for *quinquefasciatus* and intermediates. McMillan (1958) found that only individuals similar to *Cx. p. pipiens* could overwinter successfully in Lawrence, Kansas, an area near the northern limit of the intergradation zone.

The gradual cline in *Cx. p. pipiens* population from south to north is substantiated by the values obtained in this study as compared to a mean of 0.09 (range 0 - 0.26, negative values recorded as 0) for 48 specimens (47 *pipiens*, 1 intermediate) collected from natural resting sites 7-14 and 15-77 in Louisville, Kentucky (Jakob, unpublished data). Ten males reared from larvae collected 6-21-78 in McLeansboro, Illinois showed a mean of 0.06 (range 0 - 0.10, negative values recorded as 0).

Jupp (1978) gives evidence that the 2 taxa are not interbreeding in South Africa and claims that such data and the considerable morphological differences presented are grounds for referring to them as separate species, at least in South Africa. He suggests the presence of an ethological pre-mating isolation mechanism between *pipiens* and *quinquefasciatus* in that region of their distribution. The data obtained in this study clearly demonstrate considerable gene flow between the two subspecies as has also been shown in Sacramento County, California, and to a lesser extent in St. Louis, Missouri (Barr 1957). Introgression of *quinquefasciatus* genes from the south during the latter part of the season is believed responsible for the appearance of intermediate forms in natural populations in Norfolk, Virginia, and Lawrence, Kansas (McMillan 1958). The universal limit of the species concept thus precludes consideration of the nominate subspecies as distinct and separate species.

ACKNOWLEDGMENTS

The authors thank Mr. Ray Bailey, Vector-Borne Diseases Division, Center for Disease Control, Fort Collins, Colorado for statistical evaluations of the data and the Memphis-Shelby County Health Department (Dr. George Lovejoy, Director) for continued cooperation in these studies.

REFERENCES

- Barr, A. R. 1957. The distribution of *Culex p. pipiens* and *Culex p. quinquefasciatus* in North America. *Am. J. Trop. Med. and Hyg.* 6:153-165.
- Breeland, S. G. 1951. Identification of early larval instars of three common *Anopheles* of southern Georgia, U.S.A. *J. Nat. Malar. Soc.* 10:224-32.
- Iltis, G. W. 1966. Biosystematics of the *Culex pipiens* complex in northern California. Ph.D. Thesis, Univ. Calif., Davis, Calif., 144 p.

- Jupp, P. G. 1978. *Culex (Culex) pipiens pipiens* Linnaeus and *Culex pipiens quinquefasciatus* Say in South Africa: Morphological and reproductive evidence in favour of their status as two species. Mosq. Syst. 10:461-473.
- McMillan, H. L. 1958. Study of a naturally occurring population intermediate between *Culex p. pipiens* and *C. p. quinquefasciatus*. Am. J. Trop. Med. Hyg. 7:505-11.
- Moseley, K., J. Mullenix, and R. T. Taylor. 1977. Organophosphorus resistance in the Memphis, Tennessee *Culex pipiens* complex. Mosq. News 37:271-5.
- Sundararaman, S. 1949. Biometrical studies on intergradation in the genitalia of certain populations of *Culex pipiens* and *Culex quinquefasciatus* in the United States. Am. J. Hyg. 50:307-14.

Table 1. Frequency of *Culex pipiens* complex mosquitoes, Memphis, Tennessee, 1978 by two techniques.

		PERMANENT			
		<i>pipiens</i>	intermediates	<i>quinquefasciatus</i>	Total
OIL	<i>pipiens</i>	53	15	0	68
	intermediates	13	126	17	156
	<i>quinquefasciatus</i>	0	8	71	79
Total		66	149	88	303

Table 2. Mean DV/D ratios of *Cx. pipiens* complex mosquitoes, Memphis, Tennessee, 1978 by two techniques.

	OIL		PERMANENT	
	mean \pm s.e.	% of total	mean \pm s.e.	% of total
<i>pipiens</i>	0.149 \pm .0048	22.4	0.144 \pm .0065	21.8
intermediates	0.295 \pm .0042	51.5	0.292 \pm .0042	49.2
<i>quinquefasciatus</i>	0.569 \pm .0268	26.1	0.569 \pm .0237	29.0
subtotal	0.334 \pm .011		0.340 \pm .012	
total 1977	--		0.337 \pm .011	
total 1978	0.338 \pm .009		--	

Table 3. Number of times specified numbers of males were obtained in collections, Memphis, Tennessee, 1977 and 1978.

<u>Number of Males</u>	<u>1977 (60 sites)</u>	<u>1978 (61 sites)</u>
1	108	91
2	33	33
3	6	10
4	4	5
5	4	9
6 - 10	12	8
11 - 15	0	4
16 - 20	0	2
>20	2	2

Table 4. Temporal occurrence of *Culex pipiens* complex males, Memphis, Tennessee, 1977 and 1978.

Period	<i>Cx. p. pipiens</i>		<i>Cx. p. quinquefasciatus</i>		Intermediates		Totals	
	1977	1978	1977	1978	1977	1978	1977	1978
June	10 (40%) ^a	7 (28%)	8 (32%)	8 (32%)	7 (28%)	10 (40%)	25	25
July	11 (27%)	18 (19%)	11 (27%)	35 (38%)	19 (46%)	40 (43%)	41	93
August	28 (29%)	48 (21%)	29 (30%)	54 (24%)	40 (41%)	122 (54%)	97	224
September	16 (17%)	23 (22%)	42 (45%)	27 (26%)	36 (38%)	53 (51%)	94	103
October	14 (23%)	7 (14%)	25 (41%)	18 (36%)	22 (36%)	25 (50%)	61	50
TOTAL	79 (25%)	103 (21%)	115 (36%)	142 (29%)	124 (39%)	250 (50%)	318	495

^aPercent of total specimens for the month.