

Additional Studies of Male Progeny of Overwintering *Culex*
pipiens Complex Mosquitoes from Memphis, Tennessee

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ABSTRACT. Overwintering females of the *Culex pipiens* complex from Memphis, Tennessee, were acclimated to laboratory conditions and allowed to oviposit. Egg rafts were reared as individual progenies. The DV/D ratios of the male offspring of 23 progenies were determined. The authors compared the results, utilizing the generally accepted interpretation of the DV/D ratio with a suggested more "strict" interpretation, the use of which markedly alters the dynamics of the study population. Further evidence is given that the forms of the complex should be regarded as infraspecific and that a self-sustaining intermediate population exists in the area.

INTRODUCTION

The taxonomic status of members of the *Culex pipiens* complex in North America remains uncertain. *Cx. pipiens* L. and *Cx. quinquefasciatus* Say are listed as distinct species by Knight (1978) on the basis of studies of the complex in Southeast Asia (Sirivanakarn 1976), Australia (Miles 1977), and South Africa (Jupp 1978). Considerable gene flow between members of the complex was found in Memphis, Tennessee in 1977 and 1978 (Jakob et al. 1979) and confirmed in further studies with specimens collected there in 1979 (Jakob et al. 1980a). In addition, studies of male progeny of overwintering *pipiens* complex females from Memphis (collected February and March 1979) continued to indicate the presence of various mixtures of members of the complex (Jakob et al. 1980b). The data in those studies are all based on a single character, i.e., the DV/D ratio of the male genitalia (Sundararaman 1949), which remains the only reliable means of distinguishing adult *pipiens* from *quinquefasciatus* in the U. S.

Further studies on the complex in Memphis, situated near the southern boundary of the intergradation zone (Barr 1957) involved shipment of overwintering females to the Vector-Borne Diseases Division, Center for Disease Control, Fort Collins, Colorado. The mosquitoes were acclimated to laboratory conditions, blood-fed, and allowed to oviposit. Egg rafts were reared as individual progenies. The basis of this report is the DV/D ratios obtained from male offspring produced by these overwintering females.

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MATERIALS AND METHODS

Collections of overwintering females were made from natural resting sites, principally culverts, at two sites on February 4, 1980. Approximately 150 females were shipped in insulated containers with moist towelling and then held in cages at 25° C, 75-80% R.H., for 4 days before being offered an avian host for blood-feeding. Egg rafts were collected over a 3-day period, and each raft was reared separately following standard rearing procedures of this laboratory. The adults of each progeny were killed with cold (-70° C for 45 seconds) and then separated by sex. Female specimens were immediately re-frozen for future reference. Male specimens were stored in appropriately labeled pill boxes at room temperature until they could be examined. Measurements for DV/D ratios in the terminalia were made in clove oil as previously described (Jakob et al. 1979, op. cit.) rather than by preparation of permanent mounts. Several terminalia (usually 10 or 11) were handled on each slide. Following determination of the DV/D ratios in clove oil, permanent mounts of representative samples from each progeny were prepared by the method described (Jakob et al. 1979, op. cit.).

Overwintering females were not given the opportunity to oviposit prior to blood-feeding nor were any specimens examined for the presence of fat bodies. A small number (19) of overwintering females from Memphis (collected March 19, 1980) failed to oviposit prior to blood-feeding 10 days after receipt. Fat bodies were noted in five specimens examined.

Ambient temperatures in the area for the 2-week period preceding the February collection date were equal to or less than normal for 11 of the 14 days.

Mean daily temp. 2° C

Temp. range -8.9° C to 17.8° C

Daily departure from normal (range) -10.6° C to 5.6° C

Mean daily temperatures were below the level at which activity for most mosquito species ceases. The average daily temperature for the month of January 1980 was 6.2° C (a departure from normal of +1.5° C). These climatological data combined with the finding of fat bodies in specimens collected more than a month later indicate that the females collected were probably in a true state of hibernation.

RESULTS

Twenty-three progenies were reared, yielding from 47 to 215 specimens each. The proportion of male specimens in each progeny varied from 38% to 70%. The 3 smallest progenies (47, 52, and 53 adults) had the lowest male counts (40%, 38%, and 40%, respectively), whereas the 3 largest progenies (215, 210, and 203 adults) had male counts of 55%, 49%, and 54%, respectively. Males constituted 53% of the total offspring.

Distribution of DV/D ratios of male terminalia for each progeny are summarized (Table 1). The data show that 10 progenies had specimens which were classed as *pipiens*, intermediates, and *quinquefasciatus* on the basis of generally accepted ratio limits for the 3 forms. In contrast, 12 progenies contained only *pipiens* and intermediates. A single progeny (#13) contained only *quinquefasciatus* and intermediates. *Quinquefasciatus* comprised usually less than 10%, but up to 25% of the progenies which contained this form. None of the progenies contained only 1 form of the 3 possible classifications. Intermediate forms accounted for 75 to 91% of the male offspring in five progenies (#3, 6, 10, 11, and 13). The *pipiens* form accounted for 77 to 93% of the males in nine progenies (#8, 9, 12, 16, 17, 18, 19, 21, and 22).

Progenies #15 and #3 are of particular interest; each had approximately equal numbers of the erstwhile subspecies and at least half were intermediates. Progeny #15 approximates the classical expected Mendelian inheritance from hybrid crosses.

Limitation of DV/D ratios (strict interpretation) to levels originally found for the subspecies (Sundararaman 1949, op. cit.) markedly alters the relative abundance of each form of the complex. With this interpretation, approximately 91% of the population is in the intermediate category. Four of the progenies (#4, 11, 14, and 21) contain only intermediates, 13 of the 23 contain only 1 or 2 specimens of either subspecies, and no progeny contains all three forms. Progenies #3, 6, 13, and 15, however, contain *quinquefasciatus*. Following this interpretation, progenies #3 and #15 are almost entirely intermediates as compared with the more liberal distribution of the forms obtained utilizing the generally accepted interpretation of DV/D ratios.

DISCUSSION

Ready hybridization of *pipiens* and *quinquefasciatus* was shown by Sundararaman (op. cit.) and Farid (1949) with laboratory models. Hybrid field populations were found in several areas of the United States (Barr, op. cit.) and in various parts of California (Iltis 1957). Intermediates were found to constitute 50 to 52% of the male population of the complex in 1978 and 1979 (Jakob et al. 1979 and 1980a). Similarly, approximately 47 to 53% of the male offspring of overwintering females collected in 1979 were determined to be intermediate forms on the basis of the generally accepted interpretation of DV/D values. In this study, intermediates accounted for 43% of the male offspring of the 23 progeny from females overwintering in 1980.

The ratios for "pure" *pipiens* from areas known not to contain *quinquefasciatus* have been shown to be 0.10 or less, while the ratios for "pure" *quinquefasciatus* are 0.60 or greater. Barr summarized, "It was found that the average ratio in a collection of *pipiens* is usually 0.1 or less and in *quinquefasciatus* 0.6 or more. In individual specimens, the ratio is usually less than 0.2 in the former and more than 0.4 in the latter." This commonly accepted interpretation was devised at a time hybridization of the subspecies was initially investigated in field populations. If the more rigid parameters for

the "pure" subspecies had been applied, the results, particularly within or near the borders of the intergradation zone, would have been markedly different. In this study, application of the "strict" interpretation of the DV/D ratios significantly increases the percentage of intermediates to >90% of the offspring. That such an interpretation may be valid is suggested by the lack of mean values fitting the criterion of Barr (only progeny #8 - mean 0.105 - is close to this standard). Indeed, almost half of the progenies had mean values of 0.20 or greater. The relative reduction in number of *pipiens* utilizing this "strict" interpretation is markedly less than for *quinquefasciatus*, which would support the belief that *quinquefasciatus* do not hibernate. The few *quinquefasciatus*-like individuals found probably result from inheritance factors, thought to be multifactorial, of the parent cross.

Regardless of the DV/D interpretation followed, the data support the findings of considerable gene flow in the *pipiens* complex in Memphis and strongly suggest the presence of a continuing self-maintaining intermediate population. Similar situations are likely to be found following intensive study within and below the intergradation zone and provide evidence that the forms of the complex should be regarded as infraspecific. Permanent slides of terminalia representative of each progeny will be deposited with the Medical Entomology Project of the Smithsonian Institution, U. S. National Museum, Washington, D. C. The slides constitute approximately 58% of the terminalia examined, varying from 44% of the males in a progeny to all of the males in two of the smaller progenies.

We are aware that the data presented are based entirely on a single biometric evaluation; nevertheless, the DV/D ratio of the male terminalia remains the most reliable means of distinguishing *pipiens* from *quinquefasciatus*. The data also suggest cautious interpretation of DV/D values of specimens from areas where hybridization might occur regularly or at specific periods of time. Late season changes in DV/D ratios have previously been found for populations in Lawrence, Kansas (McMillan 1958) and Salt Lake City, Utah (Rosay and Nielsen 1973).

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Table 1. Male Offspring of Overwintering *Culex pipiens* complex Females Collected in Memphis, Tennessee, February 4, 1980

Progeny No.	DV/D ratio		No of. <i>pipiens</i> — intermediates — <i>quinquefasciatus</i> based on DV/D interpretation	
	range	mean ± s.e.	Standard*	Strict**
1	0.05 — .28	0.191 ± .005	52-23-0	1-74-0
2	0.10 — .36	0.228 ± .006	35-60-0	1-94-0
3	0.14 — .85	0.289 ± .009	7-80-5	0-91-1
4	0.11 — .31	0.212 ± .007	25-25-0	0-50-0
5	0.08 — .33	0.185 ± .005	78-36-0	2-112-0
6	0.16 — .71	0.295 ± .009	2-68-5	0-74-1
7	0.09 — .39	0.234 ± .011	15-25-0	1-39-0
8	0.01 — .28	0.105 ± .007	74-6-0	45-35-0
9	0.08 — .26	0.154 ± .006	34-4-0	2-36-0
10	0.07 — .49	0.259 ± .011	7-35-1	1-42-0
11	0.13 — .53	0.307 ± .012	3-40-9	0-52-0
12	0.06 — .28	0.143 ± .014	16-3-0	8-11-0
13	0.23 — .60	0.345 ± .027	0-12-4	0-15-1
14	0.14 — .49	0.260 ± .011	18-36-4	0-58-0
15	0.12 — .62	0.301 ± .012	20-56-23	0-98-1
16	0.02 — .48	0.165 ± .004	55-15-1	10-61-0
17	0.03 — .24	0.162 ± .006	44-8-0	4-48-0
18	0.04 — .30	0.150 ± .006	77-10-0	17-70-0
19	0.00 — .42	0.146 ± .007	92-21-1	32-82-0
20	0.10 — .33	0.192 ± .006	34-31-0	1-64-0
21	0.12 — .42	0.175 ± .011	26-5-1	0-32-0
22	0.09 — .24	0.166 ± .009	16-2-0	1-17-0
23	0.04 — .58	0.220 ± .010	32-28-3	2-61-0
total population	0.00 — .85	0.21 ± .002	762-629-57	128-1316-4

* *pipiens* ≤0.20, intermediates = 0.21-.39, *quinquefasciatus* ≥0.40

** *pipiens* ≤0.10, intermediates = 0.11-.59, *quinquefasciatus* ≥0.60