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## THE SALIVARY GLAND CHROMOSOMES OF *CULEX PIPPIENS* L.<sup>1, 2</sup>

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**ABSTRACT.** The salivary gland chromosomes of *Culex pipiens* L. are described and compared with previous descriptions of chromosomes of the *C. pipiens* complex. Differences which have been

described among the various forms may relate to techniques of preparation rather than to real differences among the forms.

### INTRODUCTION

Sutton (1942) described the salivary gland chromosomes of *Culex pipiens* L. as being three pairs, synapsed along most of their lengths. The three pairs were completely separated from each other and were without obvious chromocenters. No difference was detected between the chromosomes of females and males. A detailed

study of the banding pattern was not attempted. Kitzmiller and Clark (1952) and Kitzmiller and Keppler (1961) reported the preparation of a map of the salivary gland chromosomes for the subspecies *pipiens*. Dennhöfer (1968) described and mapped the salivary gland chromosomes of an autogenous strain of this subspecies (sometimes called subspecies *molestus*). Sharma *et al.* (1969) and Kanda (1970) described the salivary gland chromosomes of the subspecies *quinquefasciatus* (= *fatigans*). Although there are differences in the salivary gland maps which have been published to date, there

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are no differences which have been related to subspecific status. Hybridization studies suggest that there are no major differences in the chromosomes of the various taxonomic entities of the *Culex pipiens* complex. The purpose of the present study was to try to reconcile the various chromosomal banding maps which have been published.

## MATERIALS AND METHODS

An autogenous strain of *Culex pipiens* from Kawasaki, Japan supplied by Dr. Takeo Tadano was used for this study. Larvae were reared one egg raft to a pan about 29 x 17 x 4 cm. The best smears were of salivary glands from large, 4th-instar larvae which were just ready to pupate. The glands were dissected in 1% sodium citrate and then fixed for no longer than a minute in a 1:1:1 solution of absolute ethyl alcohol, glacial acetic acid, and chloroform. The glands were stained for 5 minutes in lacto-aceto-orcein which was made by dissolving 2 gm of orcein in 100 cc of a 1:1:1 solution of distilled water, glacial acetic acid, and lactic acid. Afterward, the glands were lightly squashed. Only temporary preparations sealed with clear nail polish were used in this study. Good preparations with the 3 chromosomes completely separated are difficult to obtain because of the absence of a chromocenter and the length of the 2nd and the 3rd chromosomes.

## RESULTS

The salivary gland nuclei of *C. pipiens* contain 3 long polytene chromosomes with various degrees of asynapsis which may involve the centromeres. Each chromosome has free ends and a characteristic enlarged centromere which has a characteristic banding pattern (Fig. 1). The chromosomes of both sexes are alike. As in the study of Dönhöfer (1968), the total chromosome length was divided into 70 zones and each zone into one or more regions (Fig. 2). The ratio of the lengths of the chromosomes is 19:39:42. Because

of the length of the 2nd and 3rd chromosomes, they are frequently intertwined. The length of the asynaptic regions depends upon the degree of squashing and the size of the nucleus.

**CHROMOSOME I. RIGHT ARM (1R).** The free end of this arm forms a small puff in region 1A and 1B which has a dotted band in 1B. It is followed in 1C by a characteristic puff with a double band in the middle. Zone 2 contains a series of light bands, and 3 relatively strong bands of light bands, and 3 relatively strong bands in regions 2A, 2B, and 2C. Zone 3 contains a series of dark bands. There is a characteristic elongated puff with 2 dark heavy bands in 4C, and a double band in 5A. In 5C there is a small puff with 2 double bands, and in 6C a large puff with 5 dark bands. There is a large puff in regions 7C and 8A with 2 strong dark bands in 7C and 2 dark bands in 8A. A series of 5 dark bands in regions 7A and 7B are followed by 2 light bands in 7C. Region 8B contains 5 light bands before it connects with the right side of the centromere.

**CENTROMERE (C1).** This centromere consists of a characteristic, large, spherical puff, zone 9, which has 2 strong, dark bands in region 9B which divide the centromere into 2 parts and forms its waist. Its right side, region 9A, contains 3 relatively light bands followed by a dark one, and its left side, region 9C, contains three light bands.

**LEFT ARM (1L).** The free end of this arm is marked by a small puff with 2 relatively dark bands in region 16C followed by 3 light bands in 16B. 15C is characterized by a small puff with a heavy dark band in the middle. Region 14B contains a relatively large puff with 3 heavy dark bands. Regions 13B, 13A, and 12C contain dotted bands in small puffs. Region 11B contains a large puff with 2 characteristic, heavy, dark bands. There is a characteristic double band in 10C, a dotted band in 10B, and 3 light bands in 10A.

**CHROMOSOME II. RIGHT ARM (2R).** The free end of this arm is an elongated puff

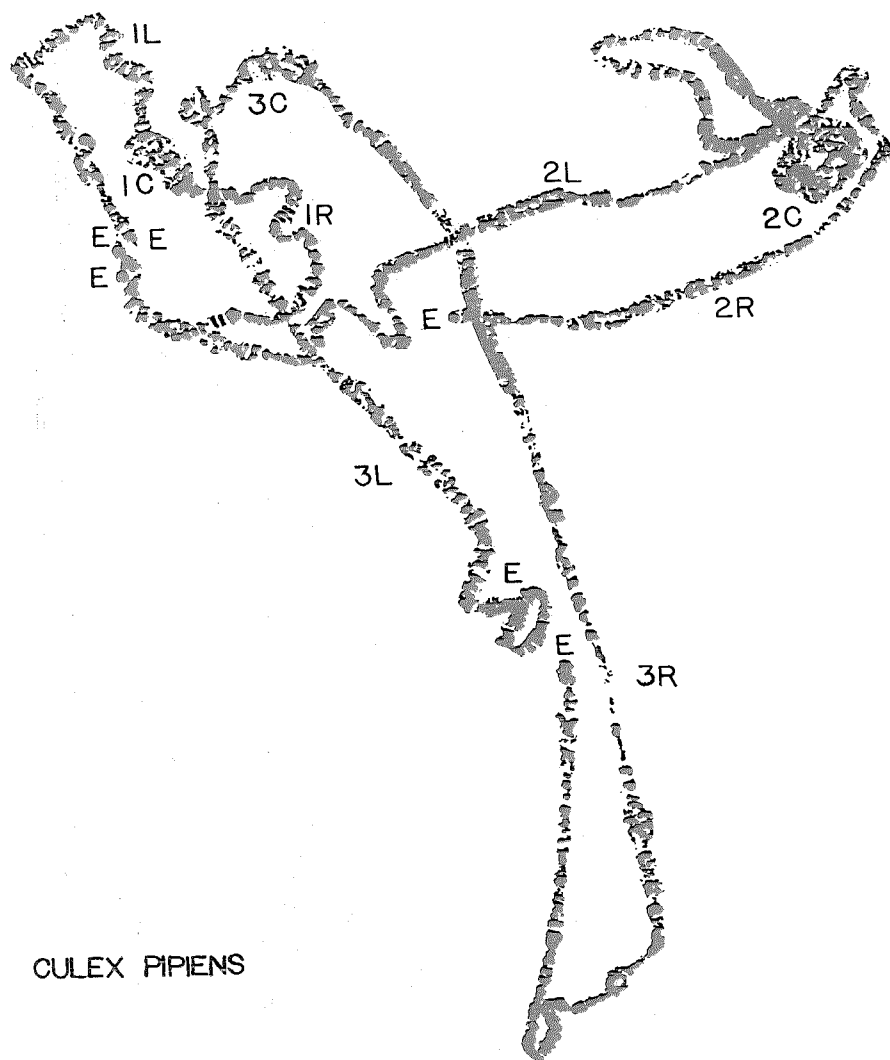


FIG. 1.—Photomicrograph of the nucleus of a salivary-gland cell of *Culex pipiens* after complete squashing. The chromosomes are completely separated and take different shapes according to the locations of the strong bands.



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with a characteristic, small, heavy, dark band in region 17C. A series of dark and thin bands are in zones 18 and 19. In zone 20, there are 2 puffs, a large one with a heavy, dark band in region 20A, and a small one with 2 dark bands in region 20C. Zone 21 contains a series of dark and thin bands. Zone 22 contains a large puff with relatively strong bands in regions 22B, 22C, and 22D. A light puff in zone 23 contains a strong band in region C. Zone 27 contains a dark elongated puff with strong bands in regions 27A and 27B. Zone 28 is characterized by a dotted band in region D.

**CENTROMERE (C<sub>2</sub>).** This centromere is a large, spherical puff divided by a narrow region, 30B, characterized by 3 heavy, dark bands. Region 30A contains 3 relatively dark bands, and 30C contains 2 thin bands followed by 2 dark bands in region 30D.

**LEFT ARM (2L).** The free end expanded into a long puff contains, in region 41C, 3 small, dark bands, followed in region 41B by 3 light bands and 3 dark bands. Region 41A contains 2 heavy bands. Zone 40 contains a small characteristic puff in region C, with a heavy, dark band in the middle. A characteristic small puff in 39A contains a strong band. Zone 38 is a dark, long puff with a series of thin and heavy bands. Zone 37 contains a small puff with 2 relatively strong bands in region C and an elongated puff in regions B and A. A series of heavy bands in regions 36D and 36C is followed by a characteristic small puff with a dark band in region 36A. Zone 35 in regions D, C, and B contains 3 small puffs and a series of dark bands. Two characteristic large puffs in region 32B contain relatively strong, dark bands. Zone 31 is stretched, and contains an elongated puff with a dotted band in region B and a characteristic dark band in region A.

**CHROMOSOME III. RIGHT ARM (3R).** The free end of this arm is a small spherical puff with 3 light bands followed by 2 heavy bands in region 42A. Region 42B contains another small puff, and region 42C contains a strong band. Region 43A contains a small puff with a strong, dark

band in the middle. Region 43C has a double band. Zones 44 and 45 are generally light areas. Region 44B contains a heavy, strong band, and region 45D contains 2 strong, dark bands. Zones 46 and 47 are dark with a series of dark bands. Zone 48 contains 2 small puffs, and in regions A and C, a dotted band and a heavy, strong, dark band. Zone 49 contains 3 puffs, one with 3 strong bands in region A, one with 1 band in region B, and one with light bands in C. Zone 50 contains 2 puffs, each with a dark band, in region B. Zone 51 contains in region A 3 strong dark bands. Zone 52 contains a dotted band in region B. Zone 53 contains 3 small puffs with dark bands and, in region B, 2 dotted bands. Zone 54 is a relatively large, long puff with 3 strong bands in region A. Zone 55 contains 3 dotted bands in region D, and ends with a dark band. Zone 56 contains a large puff with a relatively dark band in region B. Zone 57 contains 2 puffs which have a series of dark bands, and ends with a light band in region D.

**CENTROMERE (C<sub>3</sub>).** This zone (58) consists of a long, spherical bulb. In regions C and D, 4 relatively dark, strong bands divide the centromere into 3 parts. Region B contains 3 light bands, and region A 2 dark bands. Region E contains a dark band followed by 2 light bands in region F and a dark band in region G.

**LEFT ARM (3L).** The free end of this arm consists of a light puff with a strong band in region 70B and 2 dark bands in region A. Region 69A contains a small characteristic puff with 2 dotted bands and a double band. Zone 68 contains a large puff and, in regions B and C, a series of strong bands. Zone 67 contains a small puff with a strong band in the middle. Zone 66 contains puffs in regions A and C, each with a heavy, strong, dark band, and B also with a double band. Region 65B contains 2 relatively strong, dark bands. Region 64B contains a small dotted band. Region 63B contains 2 large, dotted bands, while region 62C contains 2 dotted bands and a characteristic dark band. Re-

gion 62A contains a double band. Region 61C contains a characteristic puff with 3 double bands. Zone 60 contains a small puff, with 2 double bands in region B. Zone 59 contains a relatively elongated puff, with double bands in regions B and C and a characteristic band in region A.

### DISCUSSION

In comparison with the results obtained by Kitzmiller (1961) and Dennhöfer (1968) in subspecies *pipiens*, and Sharma (1969) and Kanda (1970) in subspecies *quinquefasciatus*, the lengths of the salivary gland chromosomes are shown in Table 1. There are large differences in the measurements of the subspecies *quinquefasciatus* reported by Sharma *et al.* (1969) and Kanda (1970) but the measurements for the subspecies *pipiens* reported by Kitzmiller and Keppler (1961), Dennhöfer (1968), and by the present writers are in fairly close agreement.

The centromere areas in the 3 chromosomes consist of large spherical puffs, fibrous and weakly stained, with different kinds of bands. After squashing the centromeres, the large single spherical puffs shown in figures 2 and 3 take different

shapes according to the location of the strong bands as shown in Figure 1. The centromere of chromosome I in 9B has a strong, heavy, dark one and a strong, dark one, which form its waist and divide it into 2 bulbs. The centromere in chromosome II contains a narrow region, 30B, characterized by 3 heavy, dark bands. This narrow region forms a waist and divides the centromere into 2 bulbs. The centromere in chromosome III contains 2 strong regions, 58C and 58D, each of which has a pair of relatively strong bands which divide the centromere into 3 bulbs. Many preparations had all the centromeres in one side of the nucleus, but there was no evidence that they were connected together to form a chromocenter.

The following is a comparison of some of the more obvious differences in the maps by Tewfik, Dennhöfer, Kanda, and Sharma.

Chromosome I, right arm, region 1B; dotted band between two light bands (Tewfik); two thin, light bands between two moderately light bands (Dennhöfer); light bands (Kanda); dark bands (Sharma). Region 1C; double band in middle of characteristic puff (Tewfik); three light bands (Dennhöfer); a strong

TABLE 1.—Lengths (in microns) of salivary gland chromosomes of *Culex pipiens* subspecies in five studies.

Chromosome		<i>Quinquefasciatus</i>		Kitzmiller	<i>Pipiens</i>	
		Sharma	Kanda		Dennhöfer	Present
I	1R	97	140	165	140	119
	C1	15	...	20	20	18
	1L	82	110	150	120	102
	Total	194	250	335	280	239
II	2R	130	250	225	250	252
	C2	18	...	20	30	32
	2L	125	200	170	205	200
	Total	273	450	415	485	484
III	3R	140	270	225	275	280
	C3	20	...	30	50	44
	3L	162	230	200	230	205
	Total	322	500	455	555	529

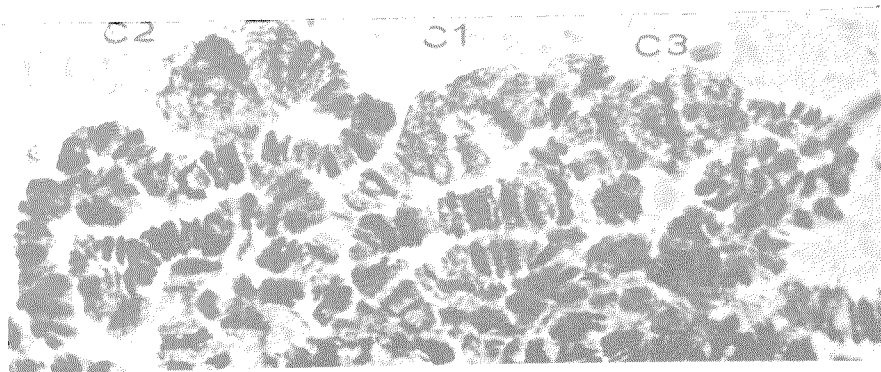


FIG. 3.—Photomicrograph of the centromeres of the salivary gland chromosomes of *Culex pipiens* before complete squashing. They are single, spherical, large puffs with strong bands; in C1 two strong dark bands form its waist, in C2 three heavy dark bands form its waist, in C3 two regions each with a pair of relatively strong bands form its two waists. The three centromeres are close together and are located on one side of the nucleus.

band (Kanda); no band (Sharma). Zone 3; double band in region C (Tewfik & Dennhöfer), no double band (Sharma & Kanda). Zone 5; three double bands, one in region A and two in C (Tewfik); two close double bands in B (Kanda); no double bands (Dennhöfer & Sharma).

In the left arm of chromosome I; dotted bands in regions 10C, 12B, 13A and B and a characteristic band in 10C (Tewfik); dotted bands in regions 12C, 15C and 16C (Sharma); no dotted bands (Dennhöfer & Kanda).

Chromosome II, right arm, zone 20; a large puff with a heavy, dark band in region B (Tewfik); 4 strong bands in region A and 2 strong bands in region B (Dennhöfer); a strong band (Kanda); 2 strong bands in region A, one in B, and two in C (Sharma). Zone 24, a dark area, with many heavy, strong bands in region C (Tewfik); a light area with a strong band in region C (Dennhöfer); a light band between two strong bands in regions A and B (Kanda); a strong band in region A (Sharma). Zones 28 and 29; 2 characteristic puffs with strong bands and a dotted band in region 28D (Tewfik); a characteristic band in region 28C (Dennhöfer); strong bands (Kanda & Sharma).

The left arm of the second chromosome, zone 39; a dark area with strong bands from region A through D (Tewfik); a characteristic band in region D (Dennhöfer); two strong bands and a dark one in region D, a characteristic light guitar puff with two dark bands in regions C and A (Kanda); a light area with light bands (Sharma). Zone 36, region A; a characteristic small puff with a dark band in the middle, a series of dark bands in regions D and C (Tewfik); a light area in region C (Kanda); dotted bands in regions C and D (Sharma). Zone 31, a dotted band in an elongated puff (Tewfik); this is missing in the other maps.

Chromosome III, right arm; in region 43C a double band; dotted bands in regions 46B and 48A; 2 dotted bands in region 52B; three dotted bands in region 55D (Tewfik); these bands are missing in the other maps.

The left arm; a dark area with a characteristic puff in zone 69 and two dotted bands and a double band in region 69A; a dotted band in region 64B, two in region 63B, and two in region 62C; a double band in region 62A (Tewfik); these bands are missing in the other maps.

There are large differences in the mea-

surements of the chromosomes of *quinquefasciatus* as reported by Sharma *et al.* and Kanda (Table 1); 194 and 250  $\mu$  respectively for the first chromosome, 273 and 450  $\mu$  for the 2nd, and 322 and 500  $\mu$  for the 3rd.

There are also significant differences between the shapes of the centromere areas in the 3 chromosomes. They are large, single, spherical puffs, fibrous and weakly stained, with different kinds of bands in the present study of *pipiens*, but in the form of double bulbs in *pipiens* studied by Dönhöfer and *quinquefasciatus* studied by Sharma *et al.* and Kanda.

These differences are probably to a great extent related to the technique used in the preparation of the salivary gland chromo-

somes, i.e. the degree of squashing, the size of the nucleus, staining, and fixation.

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## EFFECTIVENESS OF SEVERAL PYRETHROID VAPORS AGAINST *Aedes aegypti* (L.) AND *Musca domestica* L.<sup>1</sup>

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**ABSTRACT.** Insecticides of low volatility were vaporized with a small electric thermal generator as an alternative to dispersing them as aerosols. Prothrin<sup>TM</sup> (5-(2-propynyl)furfuryl *cis, trans*-( $\pm$ )-2,2-dimethyl-3-(2-methylpropenyl) cyclopropanecarboxylate) and the combination of res-

methrin and *d-trans* allethrin gave 100 percent knockdown of *Aedes aegypti* (L.) in one-half hour. However, only Prothrin produced 100 percent mortality at one-half hour, possibly because it was the most volatile material tested.

Vaporization of insecticidal chemicals is an alternative to dispersing them as aerosols. For example, Sullivan *et al.* (1940) found that substances with low volatility could be dispersed by spraying them (in

a suitable solvent) onto a surface heated to 375° C. Likewise, the vapor from filters treated with lindane (0.09-0.16  $\mu$ g/liter air) gave excellent kill of free-flying house flies, *Musca domestica* L., exposed for 30-60 minutes (Quarterman and Sullivan, 1953). Vaporized dichlorovos was effective in killing flies in aircraft (Maddock *et al.* 1961). Prothrin<sup>TM</sup> (5-(2-propynyl)furfuryl *cis,trans*-( $\pm$ )-2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate) and allethrin (100  $\mu$ g) were vaporized by an electrical device (Ogami *et al.*, 1970). In this experiment Prothrin killed

<sup>1</sup> Mention of a pesticide as a proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the U.S. Department of Agriculture.

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