THE SALIVARY GLAND CHROMOSOMES OF *CULEX PIPIENS MOLESTUS*

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ABSTRACT

The polytene chromosomal pattern together with the somatic and meiotic chromosomes of the autogenous Tehran strain of *Culex pipiens molestus* are described. The first and third chromosomes are metacentric, the second one is slightly submetacentric. Each chromosome has well-defined, large puffed characteristic centromeres. Randomly distributed asynaptic regions on the polytens chromosomes are indicative of gene differences on homologous arms, which occasionally involve the centromeres. Puffs are sometimes noticeable on the chromosomal arms. A permanent puff is located on the first and third chromosome. The morphology of the first meiotic metaphase chromosomes is characteristic of the species. The existence of interchromosomal and intrachromosomal connectives, and also stickiness of the ends of the salivary chromosomes to one another, is the major difficulty in the preparation of the salivary gland chromosomes of *Culex*, which has been overcome by the technique for the mosquito chromosome spreads.

INTRODUCTION

Since the first attempt made for the description of the salivary gland chromosomes of *Culex pipiens* (Berger, 1936), other workers have tried to demonstrate the polytene chromosomes of *Culex* (Sutton, 1942; Kitzmiller & Clark, 1952; Kitzmiller, 1954; Kitzmiller & Keppler, 1961 & Kanda, 1964), yet, the full credit goes to Dr. Donnhöfer of Johannes Gutenberg University of Germany, who published a detailed account of the salivary gland chromosomes

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of *Culex pipiens* in 1968 and demonstrated clearly the morphology and position of the centromeres in the chromosomes, which are the most important land-marks of the chromosomal complement of the species. While describing a technique for the staining of the salivary gland chromosomes of *Culex* (Amirkhanian, 1968) photomicrographs of the salivary gland chromosomes of *Culex p. molestus* was demonstrated for the first time, until the completion of the map, which is introduced in the present paper.

The preparation of the polytene chromosomes of *Culex*, in comparison with the other dipteran chromosomes, presents more difficulties, due to numerous and tenacious interchromosomal and intrachromosomal connectives, which prevent the spreading of the chromosomes in squash preparations.

Sharma et al (1969) and Kanda (1970) described independently, the salivary gland chromosomes of *Culex pipiens fatigans*, the principal vector of *Wuchereria bancrofti*, but with considerable discrepancies in the banding pattern of the same species of different strains of *Culex*. It is suggestive, that the technique of the preparation of the chromosomes, the interpretational error of the person concerned in the mapping of the chromosomal pattern, and even the conditions for rearing the larvae and the mosquitoes might be the main causes for such discrepancies. Thus it is suggested that for mapping the salivary gland chromosomes of mosquitoes, the work be carried out under the advice of an expert group of cytologists, using similar conditions of rearing the mosquitoes, collected from different areas, and same technique for the preparation of the chromosomes. Further more, for the sake of clarity and to prevent confusion in the identification of the species through the banding pattern, it would be sufficient to indicate in the map the most distinct banding patterns as "instant" land-marks, as presented by Sharma et al (1969).

In the present study, which is by no means an exhaustive one, endeavour has been made to present the most important land-marks on the chromosomes of *Culex* with a particular emphasis on the structure of centromeres.

MATERIALS & METHODS

The laboratory colonized Tehran strain of *Culex pipiens molestus* which was mass inbred for about 95 generations, was used to prepare the salivary gland chromosomes as well as somatic and meiotic chromosomes. In order to obtain good salivary chromosomes the larvae were reared at 16-18°C from the beginning of hatching up to the 4th instar stage.

After dissecting the glands in 0.65% sodium chloride on the slide, and immersing in a drop of hydrolisis-fixative solution on the slide for 4-6 minutes, they were washed by few drops of Carnoy's and staining proceeded according to the technique of Amirkhanian (1968). The somatic and meiotic chromosomes were prepared from the brain cells and the testes respectively. The photomicrographs taken from the temporary slides were enalrged and the mapping of the chromosomes proceeded according to the method of Dennhöfer (1968).

RESULTS

The salivary gland chromosomes of *Culex pipiens molestus* consists of three pairs of long armed polytenic chromosomes, with characteristic banding pattern. Randomly occurring asynaptic regions are seen on each pair of the chromosome, which occassionally involve the centromeres (Fig. 1). The free ends of the chromosomes are mostly attached to one another, which gives a ring appearance to the chromosomes, thus making the identification of the ends difficult. (Fig. 2). The interchromosomal connectives between different arms of the chromosomes, present the major difficulty in the spreading of the chromosomes when prepared by the ordinary staining techniques.

The differentiated swollen region in the middle of each chromosome has a characteristic shape of a double bulb, which constitute the puffed-centromeres. These puffs which usually have a crown-shaped mosaic appearance, are the most characteristic "instant" land-marks of the species. The first chromosome, which is the shortest one, has the smallest puffed-centromere, with an average length of 12.5 μ. The second chromosome has a centromeric puff of 17.5 μ in length, and the third one, which is the longest chromosome, has an average centromeric length of 25 μ.

DESCRIPTION OF THE CHROMOSOME ARMS

CHROMOSOME 1.

Right arm (1R) About 107.5 μ in length. The end of this arm in 1A starts with rounded tip and two thick bands, 1B has four thick bands, 1C starts with three spaced bands then a dotted one and ends with a double band. Heavily staining double bands are seen in 2A, 3B and 4A. Dotted bands are seen in 5B, 6A, 6C, 7B followed by darkly staining bands at 7C and 8A & B. A characteristic puff at 8C is in the middle of two darkly staining thick bands with tapered ends, followed by four thick darkly stained bands at 8D.

Centromere : About 12.5 μ in length, characteristic, with two granulated swellings of different sizes, 9A covers the small one and the 9B, 9C the large swelling. After the staining, when the chromosomes are seen under the miscroscope, without excessive pressure applied on the coverslip, the centromeres appear as semispherical in shape occupying a volume and the granulated regions are somewhat regular mosaic shaped. Between the two swellings at the beginning of 9B, there is a slight constriction which is covered by square-shaped regular mosaics all-round. At 9B & 9C the large swelling is covered by somewhat hexagonal mosaics.

Left arm (1L). — About 95 μ in length. 10A is the region of the tapering, dark staining end of the centromere. At 10B the three, thick and darkly staining bands are characteristic. 11A, 11B and 12A are regions of thick, darkly staining bands, inside of these bands are occupied by many dotted bands. At

15C there is a puff, on both sides of which are many dotted thick bands. At 16C is the free end of this arm a characteristic darkly staining thick band.

CHROMOSOME 2.

Right arm (2R). About 198.7 μ in length. The free end of this arm at 17A is tapering without any bands, followed by three bands at 17B, then a dotted one, and then a double band. 17C is the region of three double bands, which is followed by a dotted, then a full band followed by dotted then a double band at 18A. There is somewhat a constricted region at 28B followed by a swollen portion consisting of dotted bands and ending with two rather thick dark bands. *Centromere* : About 17.5 μ in length. Consists of two bulb-shaped swellings differing in size with characteristic band pattern. The waist between the two swellings has two wavy somewhat dentated bands at 30B. The small bulb at 30A and the large bulb partly in 30B and mainly in 30C have diffused staining dotted lines radiating from the poles of the bulbs. At the tapering ends of the bulbs there are two bands at both sides.

Left arm (2L). About 157.5 μ in length. 31A starts with three spaced bands, followed by two double bands at 31B and some dotted bands at 31C. The inversion present at 32B is characteristic of the species kept in our insectarium. Heavily staining region with closely attached bands at 34A is followed by a series of dotted and thin bands intermittenly up to 38C where an interspecific inversion is present. The tapering end of the free end of this arm is marked by two double bands and a single one at 41B.

CHROMOSOME 3.

Right arm (3R). About 191 μ in length. Well defined free end of this arm has a thick semicircular band at the tip then a thicker one, followed by a thin band at 42A. At 42B two double-banded region is followed by a single band at the middle of 42C, then followed by a series of dotted bands. Two thick bands at 46B and three bands at 47A are followed by a well defined characteristic puff at 47B, then followed by a thick band at 47C. Another puff at 54A is followed by a series of thick bands at 54B & C, 55C, followed by a series of dotted bands up to the centromere.

Centromere. About 25 μ in length. Two unequal swellings are separated by a constriction, covered by three wavy thick bands between 58C & B. The bigger swelling is further devided into two unequal bands separated by a regularly set squared mosaics between 58B & A, which is somewhat crown-shaped. At 58A there are two wavy moderately stained bands. The beginning of the centromere at 58A is marked by two well defined thick bands.

Left arm (3L). About 188.7 μ in length. Close to the centromere end, at 59B, two dark double bands are followed by a series of dark singie bands up to 61B, where two dark and thick bands are situated. At 62B, there is a well defined

puff on either side of which two dark bands are apparent. Following the puff, many dotted as well as single bands are present between the zones 63A and 65C, after which, three closely situated thick bands are seen at 66A. The club-shaped free end of 3L at 70C, has four dark bands near the tip of the chromosome followed by one dotted band, then two dark and thick bands, followed by two dotted and two dark ones.

DISCUSSION

As pointed out by Kitzmiller & Laven (1969), a considerable amount of the "behind the scene" differentiation has taken place in mosquitoes; populations having reached a high level of differentiation while still masquerading behind relatively similar morphology. Thus several groups of sibling species exist with similar morphological appearance but different physiologically, and often with reproductive barriers among them.

Although morphological studies in *Culex pipiens* complex have so far revealed two subspecies, *Culex pipiens pipiens and Culex pipiens fatigans* (Laven, 1969), there exist several autogenous populations that present different gene frequencies for the autogeny trait and some are even 100% autogenous (Laven, 1969). Since most of these autogenous populations are found within the range of typical *pipiens* and with which they are morphologically identical, according to Laven's description, "*molestus*" is meaningless as specific designation, because it includes several populations which are themselves species. Thus the designation of "*molestus*" as such in the present paper is indicative of an autogenous strain of *Culex pipiens* complex of Tehran.

Some isolating mechanism in certain places prevent gene flow partially or fully betwen the two forms of pipiens and fatigans (Lavan, 1969), but in laboratory conditions they copulate freely. Barr, 1957, has reported that the two species hybridize in nature from Southern U.S.A. to Southern Canada. The *molestus*, a *pipiens* form, which needs no blood meal to produce a first batch of eggs, is also able to copulate with *Culex pipiens pipiens* and produce an intermediate form. In a preliminary study on autogeny in *Culex pipiens* complex, Zein El-din (1956) has introduced some experimental facts on the hybridization of autogenous and unautogenous colonies of Culex from different areas of Egypt. Further studies have been carried out by Aslamkhan & Laven (1970), on the mechanism of inheritance of autogeny in the *Culex pipiens* complex, with interesting conclusions.

The explanation given by Dennhöfer (1968) and Sharma *et al* (1969) for the randomly occurring asynaptic regions on the polytene chromosomes of *Culex*, that may be due to pressure exerted on the coverslip during the squashing procedure, could not be regarded as convincing since the presence of the asynaptic regions, when present, could be seen on the chromosomes under high resolution of microscope, even when the nuclei are not well squashed. Furthermore, the hydrolisis-fixation technique used for the preparation of chromo-

somes (Amirkhanian, 1968) which affects all regions of chromosomes uniformly and spreading is achieved easily without severe pressure on the coverslip, the asynaptic parts are well-defined as separate entities from the paired regions. Thus it is the frank opinion of the writer, that the asynaptic regions, with even similar banding pattern, are due to gene differences on homologous chromosomes, which ought to be elucidated in future experiments. It could be postulated, that one of the reasons might be due to intermingling of the two strains of *molestus* and *pipiens* in the laboratory or in nature. The second and the most probable reasoning for gene differences at the asynaptic regions could be correlated with the logical conception, that a relatively low "natural mutation rate" for morphological characters, but an "adequate rate" for the physiological adaptive characters in Culex (Kitzmiller & Laven, 1969), creates such differences in certain gene loci, that affects the chromosomal pairing, thus producing asynapsis.

Furthermore, polymorphism based on chromosomal asynapsis, which seems to be rare or absent in other species, could nevertheless permit an approach to some biological problems of an interesting nature within the *Culex pipiens* complex.

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LEGENDS TO THE FIGURES

FIG. 1. Photomicrograph of the polytene chromosomes of *Culex pipiens molestus*. Asynaptic centromeres are presented by letters C1, C2 and C3 of chromosomes 1, 2 and 3 respectively. The asynaptic regions on the chromosome arms, with similar banding pattern on each homologous chromosome are presented by arrows. The terminal ends of the chromosomal arms are shown by the letter 'e'.

FIG. 2. The polytene chromosomal complement of *Culex*, the terminal ends of the arms are fused to one another thus giving ring appearance to the chromosomes.

FIG. 3 & 4. The poletene chromosomes of *Culex*, with interchromosomal connectives between the arms. In FIG. 4, the connectives between the centromeres are apparent.

FIG. 5. The polytene chromosomal complement of *Culex pipiens molestus* from the salivary glands of the fourth instar larva. The centromeres are presented by letters 'C', and the right and left arms by letters 'R' and 'L' respectively. The parts of the arms are shown by letter 'e'.

FIG. 6. Meiotic chromosomes of *Culex* from testes of male mosquito. Showing anaphase and metaphase stages of spermatogonial cells. The small chromosomes are migrated to the poles first. At the right corner, somatic metaphase from a brain squash preparation.

FIG. 7. The morphology of the first meiotic metaphase from testes of *Culex pipiens molestus*.

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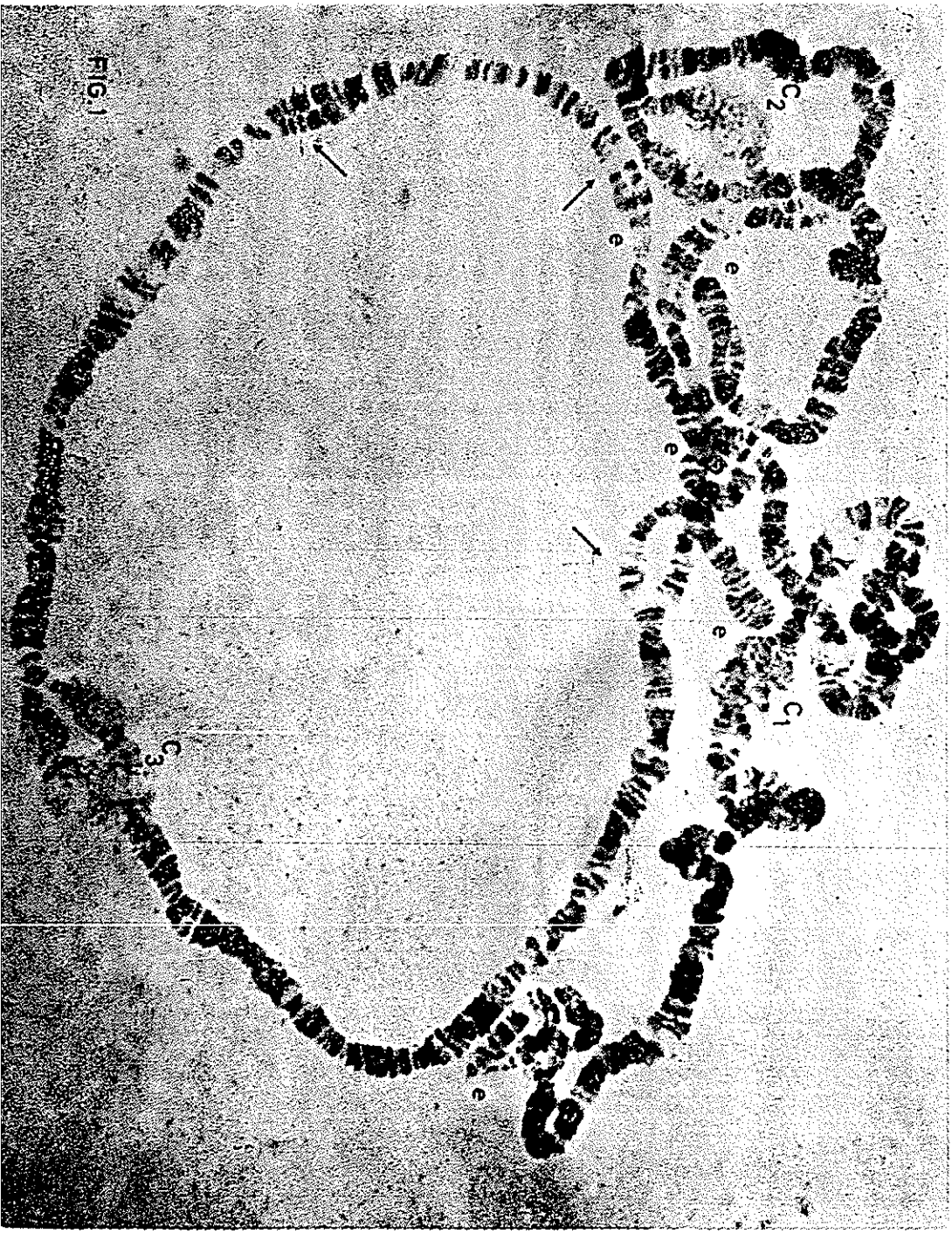

FIG. 1

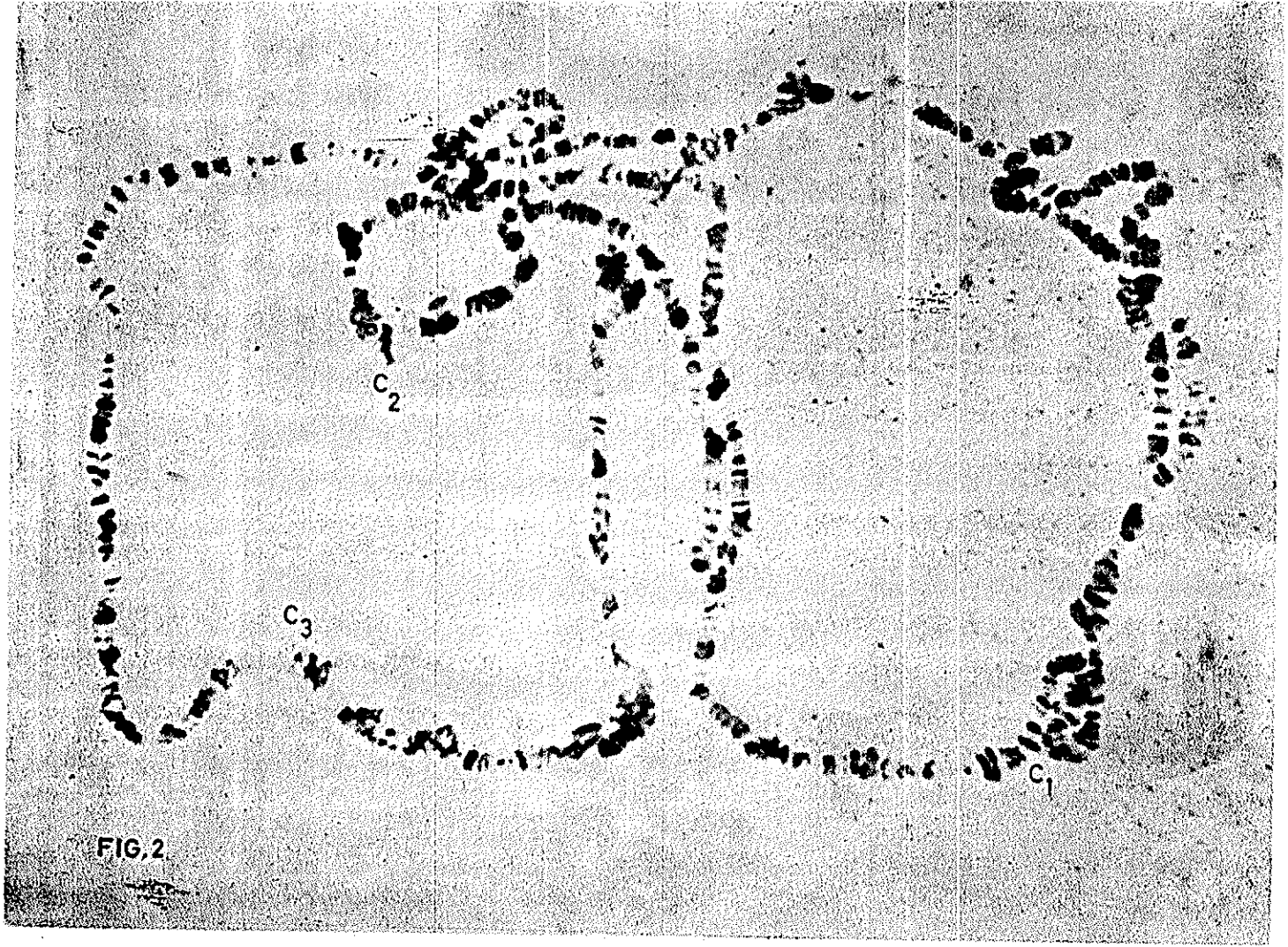

FIG. 2

FIG.3

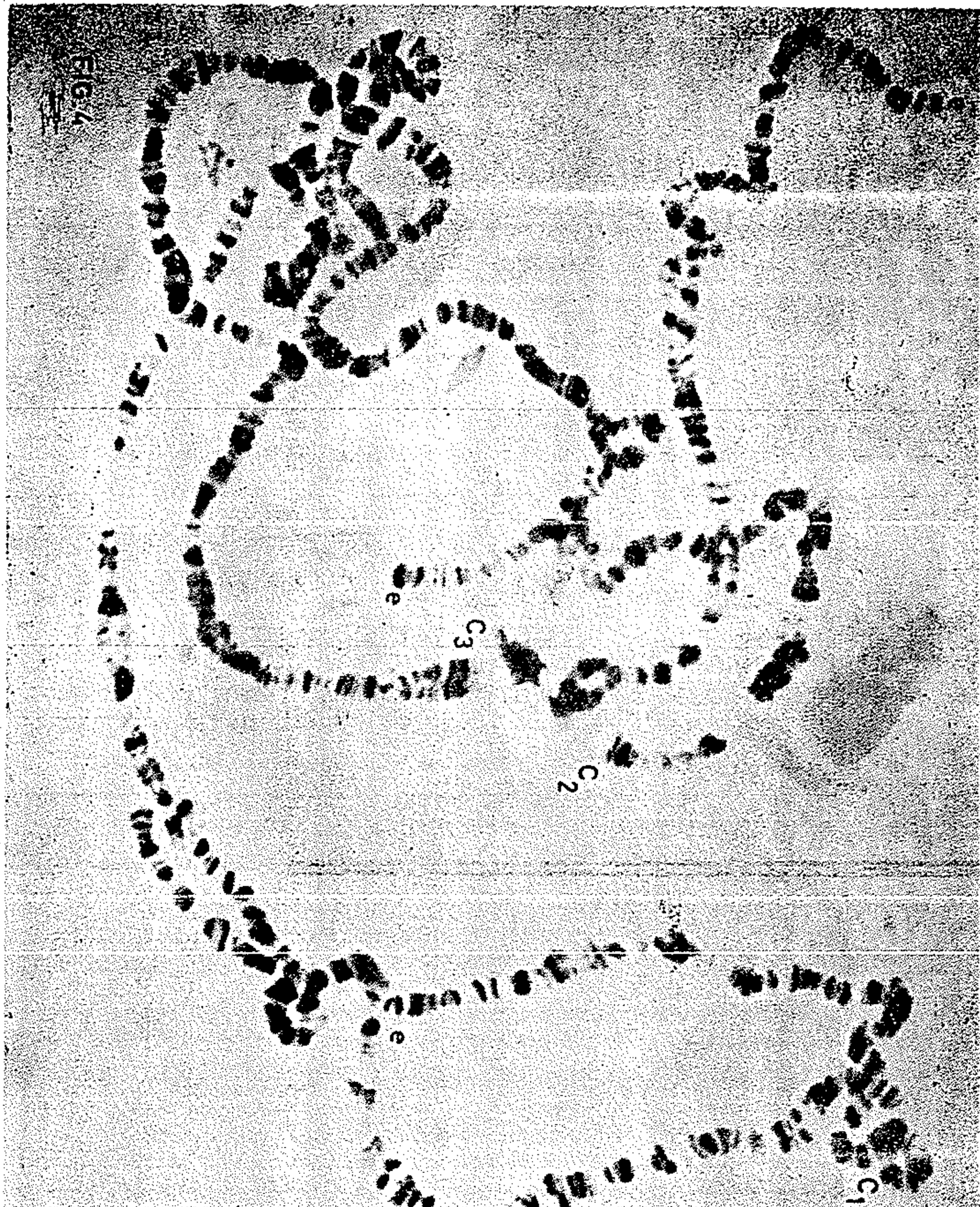
FIG 4
C1
C2
C3
e
e

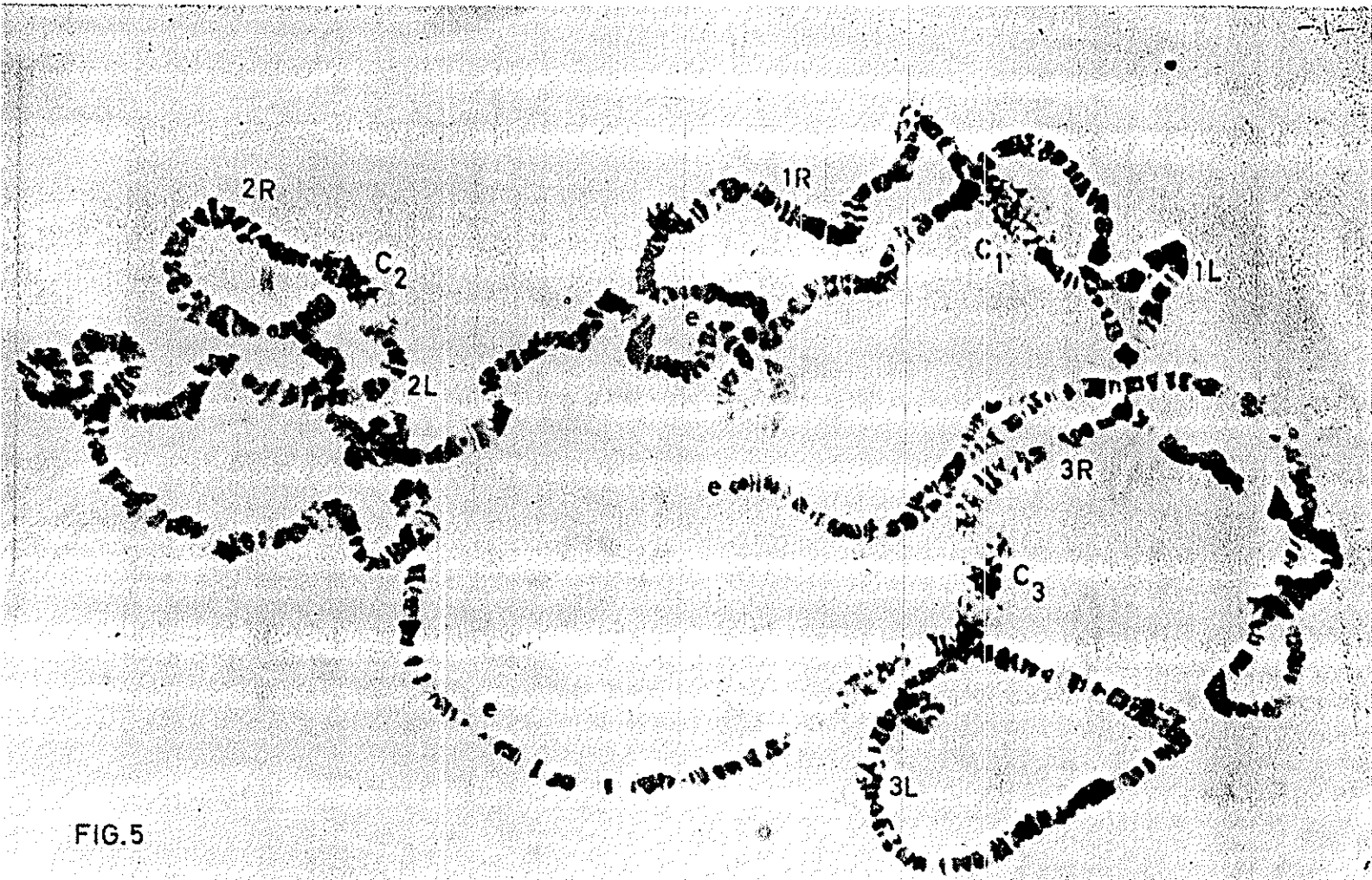

FIG.5

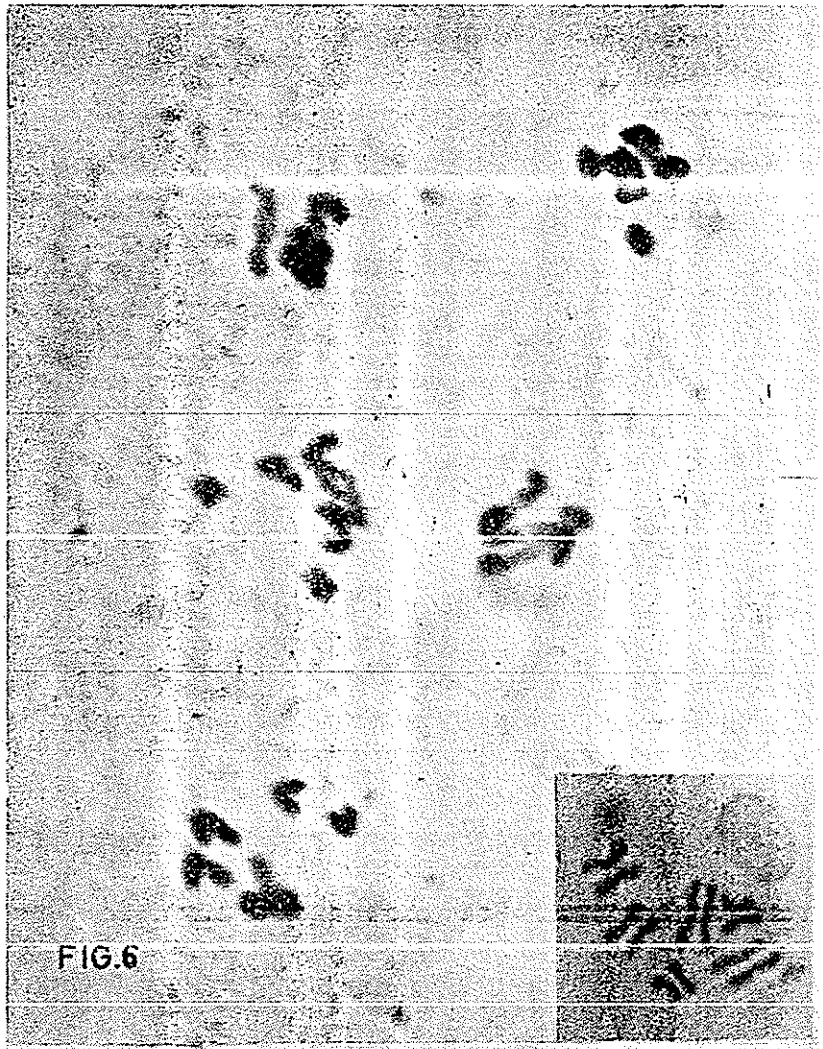

FIG.6

FIG.7

SALIVARY CHROMOSOME MAP OF
CULEX PIPIENS MOLESTUS
(TEHRAN STRAIN)