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Cytotaxonomical Diagnostics of Species from the Genus Cricotopus (Chironomidae, Diptera)

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CYTOTAXONOMICAL DIAGNOSTICS OF SPECIES FROM THE GENUS *CRICOTOPUS* (CHIRONOMIDAE, DIPTERA)

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INTRODUCTION

The cytogenetical method proves to be particularly effective in the differentiation of species of order Diptera. It is possible to separate phenotypically similar species of the Simulidae using the number of chromosomes, the degree of conjugation, the particularities in the centromere region and the structural characteristics of the chromosomes (SIMONENKO 1966; SHERBAKOV 1966; GRINCK and CHUBAREVA 1974; PETROVA 1972; KACHVORJAN and CHUBAREVA 1974). Some clarity has been reached in the systematic position of the species of the Chironominae subfamily (NESTROVA 1967, 1969; KEYL and STRENZKE 1956; KEYL 1957, 1959, 1960, 1961). However in this respect the representatives of the Orthocladiinae subfamily are still poorly studied, although the karyological method could help in the differentiation of the species in this subfamily, which are hard to discern morphologically from the outside. There are some karyological date only about *Cr. vitripennis* (BEERMANN 1952). In this work the author reported about diploid set of this species, about the form of the chromosomes and about special feature of the chromosomes structure. By the species *Acr. lucidus* is established, that the Puffs formation is different not only during the ontogeny development, but in the different cells in one and the same larvae stage (MECHELKE 1961). There is established a connection between structure rearrangement and activity of the definite puff (MECHELKE 1960; PANITZ 1965).

We have concentrated on the *Cr. gr. silvestris* group, widely found in the lakes of the Black Sea, taxonomically understudied, the karyological characteristics of the species of this group being unknown at all. Larvae of the group have been found in the lakes of Shabla, Dourankulak, Beloslav, Varna and Mandra (TSVETKOV 1955) but it has not been pointed exactly which species of the group have been found, the reason being their external morphological similarity (small differences exist between the pupa and imago).

The present paper attempts to verify the extent of external morphological similarity as reflected in the karyology of the species and the possibility to differentiate the species by applying the cytogenetical method as well as to throw some light on the philogenetic relationship between the two species. It is necessary to this end to draw cytological maps.

MATERIAL AND METHODS

The larvae material has been collected in the spring time of 1973, 1974 from the lakes of Dourankulak, Bourgas, Mandra and from a canal of the Atanasov salt lakes. It has been collected from stones and vegetation. Inspite of the different place of living no morphological differences have been observed and according to the key of PANKRATOVA (1970) they are related to the silvestris group. There exists a great similarity among adults hatched in laboratory conditions, but applying the key of HIRVENOJA (1973) it has been found that the imago of larvae collected from stones is *Cr. silvestris* and that of larvae collected from vegetation is *Cr. ornatus*.

The fixation and karyological processing of the larva is done after a method described earlier (MICHAILOVA 1975.) As polytene chromosomes of both species possesses a clearly expressed centromere region the definition of the form of the chromosomes has been made by measuring each arm separately, the ratio between the long and short arm has been calculated and the form has been defined using the Tables of LEVAN *et al.* (1964). Karyologically *Cr. ornatus* (24 ♂♂ and 87 ♀♀) and 135 *Cr. silvestris* (33 ♂♂ and 102 ♀♀) larvae have been analysed.

RESULTS AND DISCUSSION

The karyological analysis of larvae defined after the key of PANKRATOVA (1970) has shown the existence of two forms — one with a diploid set $2n=4$, the other with a diploid set of chromosomes $2n=6$. Correlating the external morphological examination to the cytogenetical analysis establishes that the form having a diploid set of chromosomes $2n=6$ is *Cr. ornatus* and the one with $2n=4$ is *Cr. silvestris*.

I. Description of the chromosomes.

Cricotopus silvestris (FABRICIUS 1794).

The diploid set of chromosomes is $2n=4$. «B» and heterochromosomes are not found. The first and the second pair of chromosomes are submetacentric. The ratio of the arms for the first chromosome is 1,82, for the second - 2,95. There is authentic difference between the lengths of the two chromosomes (Table 1). The karyological particularity of the species is the uncomplete conjugation between the homologes accompanied in some places by disparity of the bands and in some sections by presence of homozygote inversion. These particularities in the karyology of the species resemble the one established at some hybrid forms of species from the genus Chironomus (GOLDSCHMIDT 1942) and the Genus Anopheles (KITZMILLER 1967), the uncomplete conjugation with the disparity of the bands likewise has no exact position. These particularities prove the hybrid origin of the species.

The above features make the composition of cytological maps difficult. The map was composed using species with well uncoiled chromosomes and with a maximum homologity of the bands in both pairs of chromosomes (Fig. 1).

First chromosome. It is provisionally divided into 27 sections. A characteristic feature of the chromosome is that it bears three Balbian rings and one nucleolus .In some species an accumulation of large heterochromatic beads has been observed which is a result of vigorous winding into a spiral of the chromosome. A similar phenomenon has been found out with species Cnephia lapponica by PETROVA (1972) and with genus Prosimulium by RALCHEVA (1974). Typical for the chromosome are heterochromatic bands in sections 3/4, 4/5, 5/6, 13/14, 15/16 and 19/20. Conjugation between the heterochromatic band in section 5/6 and the centromere region of the second chromosome can be observed. It is probable that not only morphological but also genetic similarity exists between these heterochromatic regions. After zone 6/7 has uncomplete conjugation accompanied by disparity of the bands. The absence of conjugation is caused by various extent of expressiveness of the bands and changes in their number in one of the homologs. Similar phenomenon has been observed by KEYL (1957) with *Ch. thummi.* There is uncomplete conjugation between section 7/8 and 8/9 due to homozygote inversion. The absence of conjugation in zone 9/10 is not accompanied by disruption in the continuity of bands in two homologs. From section 20/21 to the centromere region a disruption of the conjugation is observed. It is accompanied by disparity of the bands. Sometimes the homologs interlace each other. The centromere region appears to be a well expressed dark band in the section 22/23. The third Balbian ring follows up. The chromosome ends with three heterochromatic bands (Fig. 1, Plate I, 1).

Second chromosome. It is provisionally subdivided into 17 sections and has one Balbian ring. A characteristic feature of the chromosome is a

TABLE 1

Length of the chromosomes and correlation of theirs armes in Cricotopus silvestris

Chromosome	*n*	$\bar{X} \pm m$	p	d	md	td	P	L	R	L/R
I	30	556,3 ± 15,39	84,31	—	—	—	—	359,± 13,23	197,3± 11,91	1,82
II	30	239,3 ± 10,73	58,80	M_1-M_2= 317	18,76	16,90	0,001	178 ± 9,80	60,3 ± 5,23	2,59

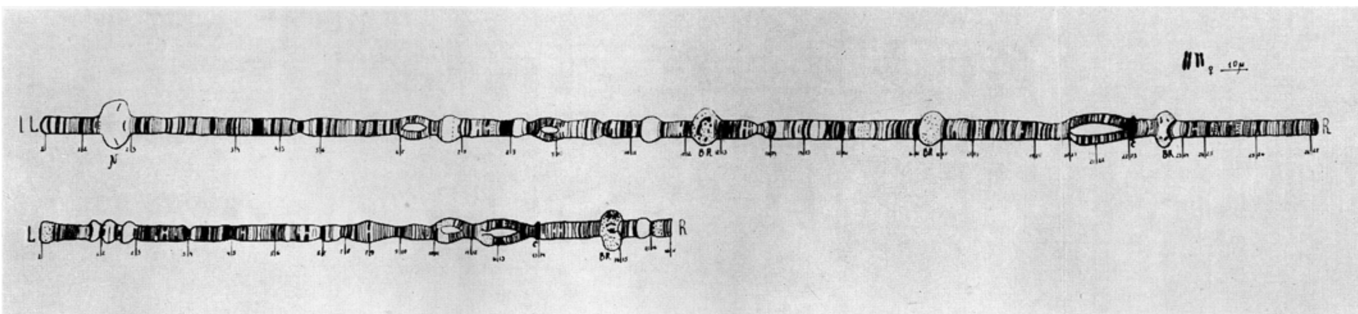

Fig. 1. — *Cricotopus silvestris* - Salivary chromosome map.

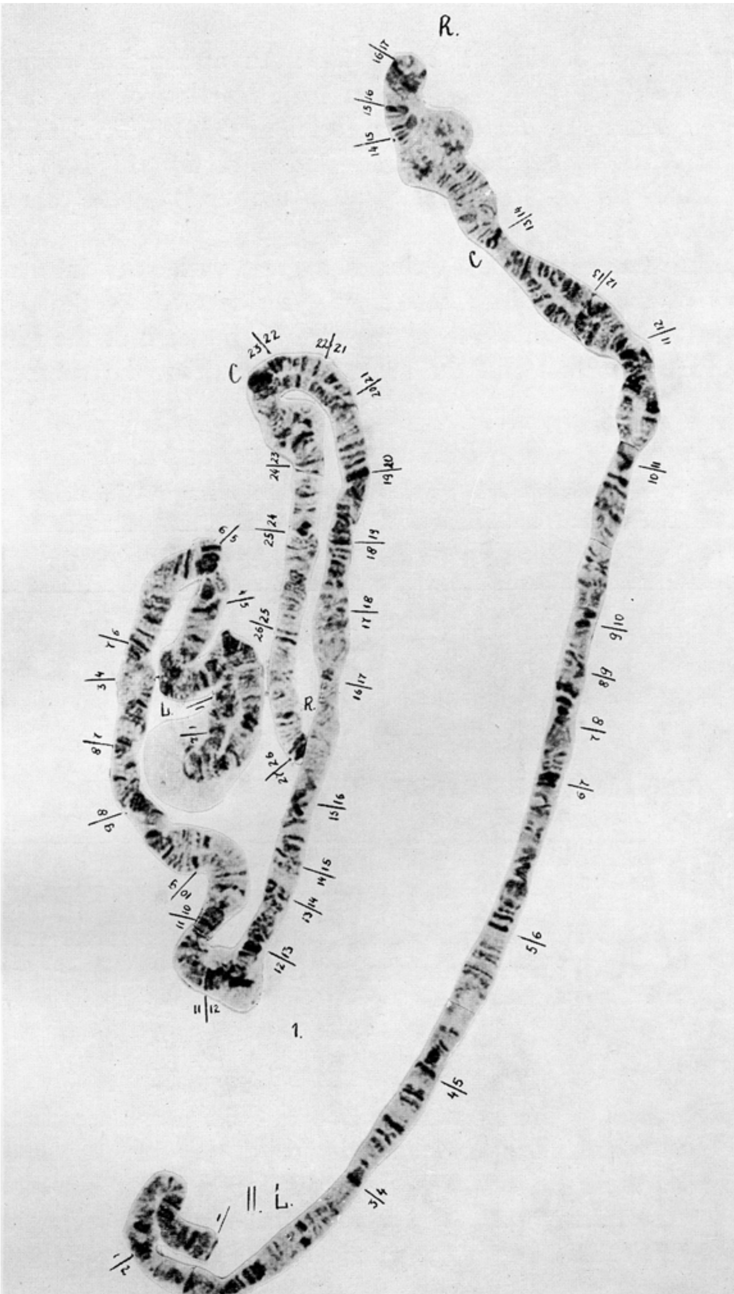

Plate I, 1. I. — Salivary chromosomes of *Cricotopus silvestris.*
Plate, I, 2. II. — Salivary chromosomes of *Cricotopus silvestris.*

broadening between sections 1/2 and 2/3. An uncomplete conjugation is observed between the sections 2/3 and 3/4. Markers of the chromosome are the heterochromatic bands in the sections 3/4, 4/5, 5/6, 6/7, 8/9 and 9/10. The centromere region is situated in section 13/14. The heterochromatic bands in section 16/17 mark the end of the chromosomes (Fig. 1, Plate I, 2).

A special feature of the species is a great variability in the external morphology of the larva. In a few cases the side teeth of the submentum are very poorly developed, which is true also of the teeth of the mandibulae. In some species the first and the second side teeth of the submentum are

TABLE 2

Length of the chromosomes and correlation of theirs armes in Cricotopus ornatus.

Chromosome	*n*	$\bar{X} \pm m$	p	d	md	td	P	L	R	L/R
I	30	359,7 ± 9,40	51,53	—	—	—	—	238,3 ± 10,5	121,4 ± 5,12	1,96
II	30	290,7 ± 8,38	85,92	M_1-M_2 = 69,0	12,6	5,48	0,001	147,4 ± 5,22	143,3 ± 4,33	1,02
				M_2-M_3 = 100,7	12,6	7,44	0,001			
III	30	190,0 ± 9,34	51,21	M_1-M_3 = 169,7	13,7	12,26	0,001	150,0 ± 5,78	46,7 ± 3,90	3,21

emerged. These external morphological peculiarities correlate with variability with respect to the distribution of the unconjugated section in the chromosomes.

Cricotopus ornatus (MEIGEN 1848).

The diploid set of chromosomes is $2n=6$. « B » and heterochromosomes have not been found. The first and the third pair of chromosomes are submetacentric, the ratio between the arms for the first chromosomes is 1,96 and for the third - 3,21. The second chromosome is metacentric with aim ratio - 1,02. Table 2 shows the values of the lengths of the chromosomes and their arms.

First chromosome. Provisionally divided into 24 sections. The heterochromatic bands in section 1/2, 5/6, 7/8, 11/12 and 18/19 are a diagnostical feature of the chromosome. There is a narrowness between sections 12/13 and 13/14 - a feature which can immediately identify the chromo-

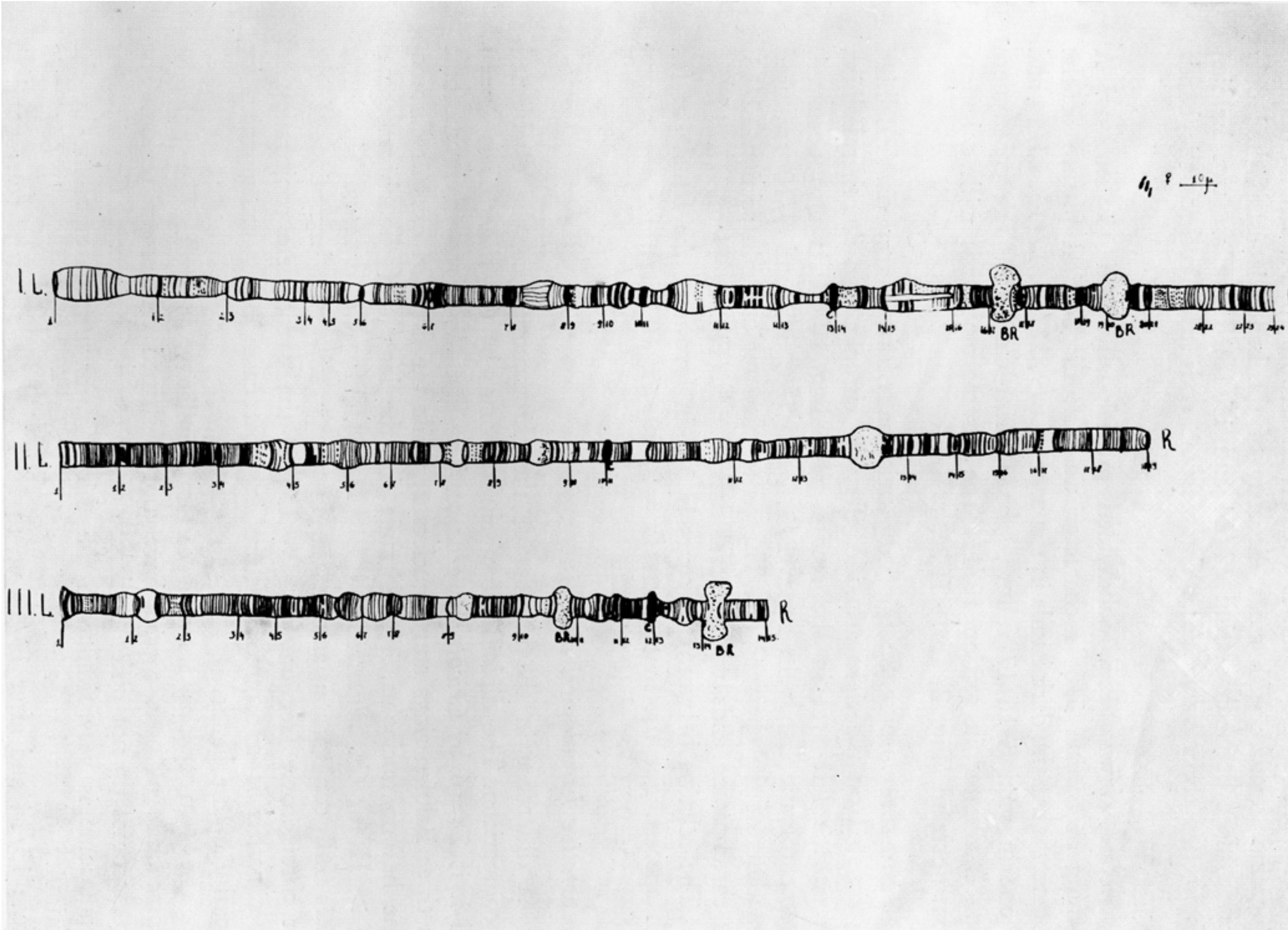

Fig. 2. — *Cricotopus ornatus* - Salivary chromosome map.

some. The centromere region is situated in section 13/14. An uncomplete conjugation together with some disparity of the bands is established only in some place between section 13/14 and 15/16. There are two Balbian rings in this chromosome. The chromosome ends with euchromatic bands (Fig. 2, Plate II, 1).

Second chromosome. Subdivided provisionally into 19 sections. A widening in section 5/6 is characteristic. The diagnostical feature of the chromosome are the dark bands in the zone of 8/9, 11/12, 12/13, 13/14 and the one in section 16/17 followed by a point-like band. The centromere region is situated in section 10/11. The chromosome ends with a heterochromatic band (Fig. 2, Plate II,2).

Third chromosome. Subdivided provisionally into 15 sections. A Balbian ring situated on the right arm identifies the chromosome. Another Balbian ring on the left arm in the section preceding 10/11 is also observed. Heterochromatic bands in zones 2/3, 3/4, 5/6, 8/9 and 9/10 are used as markers of the chromosome. The centromere region is in section 12/13. The chromosome ends with several dark bands (Fig. 2, Plate III, 1).

The relation between the sexes is 3:1 in favour of the female or for both species. For the first species $x^2=0{,}029<x^2$ (0,05,I); for the second species $x^2=0{,}764<x^2$ (0,05,I).

Comparative karyological analysis.

A difference in the number of the chromosomes can be established by comparison. The diploid set of chromosomes by *Cr. silvestris* is $2n=4$ and it is $2n=6$ by *Cricotopus ornatus*. The first *Cr. silvestris* chromosome has three Balbian rings and a nucleolus, while the first *Cr. ornatus* chromosome has no nucleolus. The formula for the length of the chromosomes is I > II for *Cr. silvestris* and I > II > III for *Cr. ornatus*. The latter shows full conjugation among the homologs with the exception of the first chromosome (section 13/14 - 15/16) the uncomplete conjugation in *Cr. silvestris* is a common feature in most cases being accompanied by disparity in the bands.

The two species show a certain similarity as well. The polytene chromosomes of both species have a well shaped centromere region. The chromosomes do not differ in degree of polyteny. The second *Cr. silvestris* chromosome is very similar to the third *Cr. ornatus* chromosome by the continuity of its bands – sections 1/2, 3/4 – 4/5 (inverted) 6/7, 7/8 before 13/14 and after 13/14 up to 16/17 of *Cr. silvestris* correspond to the bands of *Cr. ornatus* in the section 1/2, 5/6 before 9/10, 10/11, before 12/13 and from 13/14 up to 14/15. The continuity of the bands

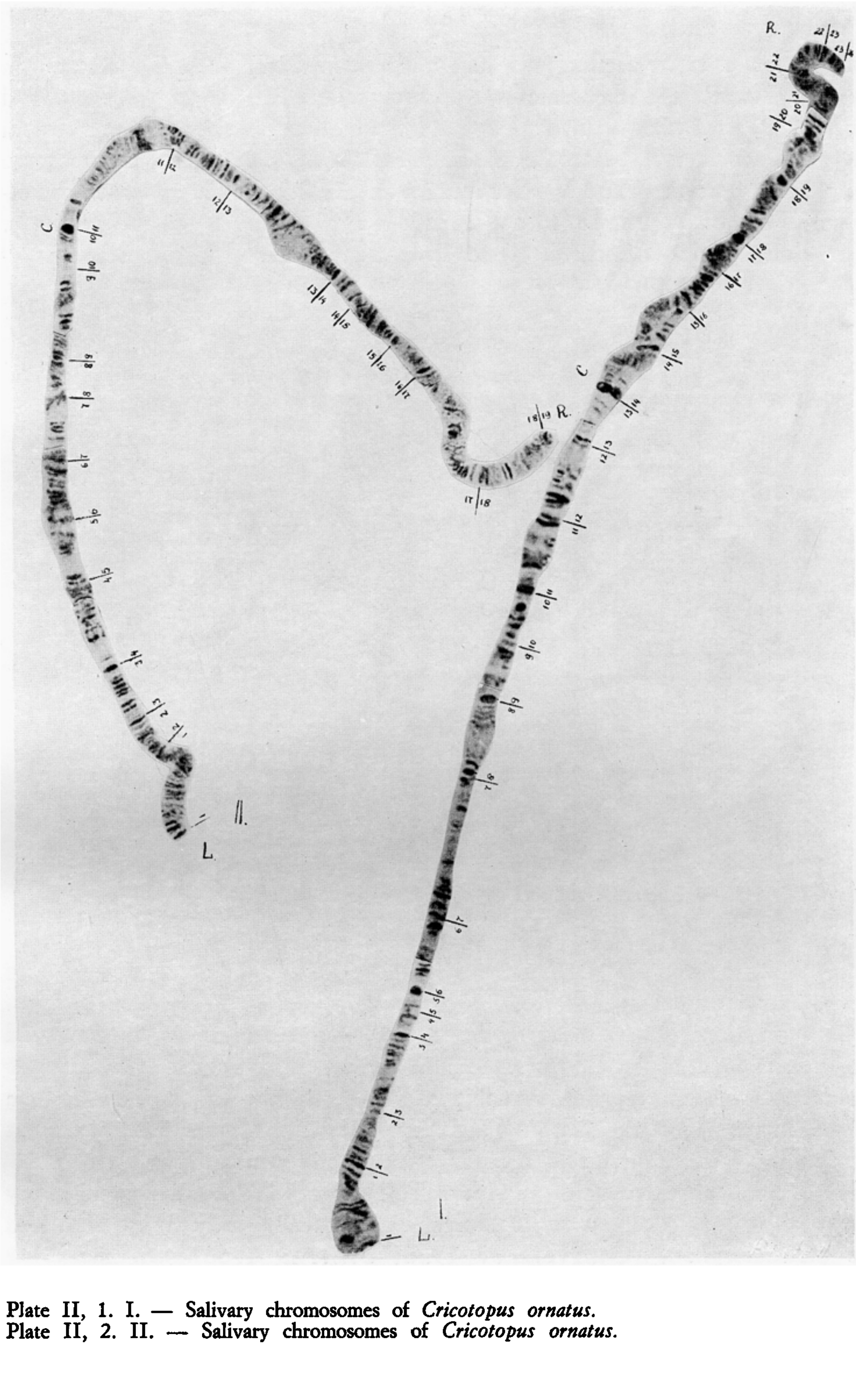

Plate II, 1. I. — Salivary chromosomes of *Cricotopus ornatus*.
Plate II, 2. II. — Salivary chromosomes of *Cricotopus ornatus*.

of *Cr. silvestris* in section 4/5 and after is identical with *Cr. ornatus* in section I and after, the same is true for section 5/6 of *Cr. silvestris* and section 2/3 of *Cr. ornatus* (Plate IV). Identical sections can be found between the first chromosome of *Cr. silvestris* and the second chromosome of the *Cr. ornatus* (Plate V). Section 25/26, 18/19 by *Cr. silvestris* correspond to section 17/18, 18/19 and 11/12 - 12/13 by *Cr. ornatus* respectively.

Both species are characterized by a high grade of chromosome poly-

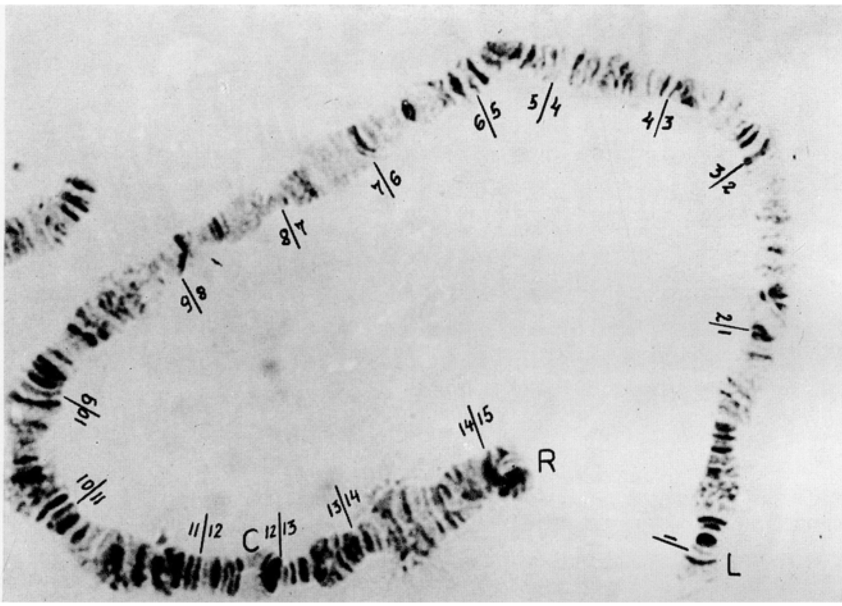

Plate III, III. — Salivary chromosomes of *Cricotopus ornatus.*

morphism, the one of *Cr. silvestris* being significantly higher. The percentage of homokaryotypes is 2,96 for *Cr. silvestris* and 22,52 for *Cr. ornatus.* The aberrations in *Cr. silvestris* in chromosome 1 and 2 are concentrated in their right arms, in chromosome 3 in the left arm, while by *Cr. ornatus* the aberrations of the chromosome 1 are in both arms, and of chromosome 2 are in the right arm (Fig. 3). The larvae of *Cr. silvestris* show a great variability with reference to morphological symptoms.

Species of chromosomes with a comparative high polymorphism by heterozigots are accepted as apomorphous and those without modifications as plesiomorphous (CHETVERIKOV 1926; STEFFAN 1966). On the other hand species with a smaller number of chromosomes are considered philogenetically younger (STEFFAN 1966).

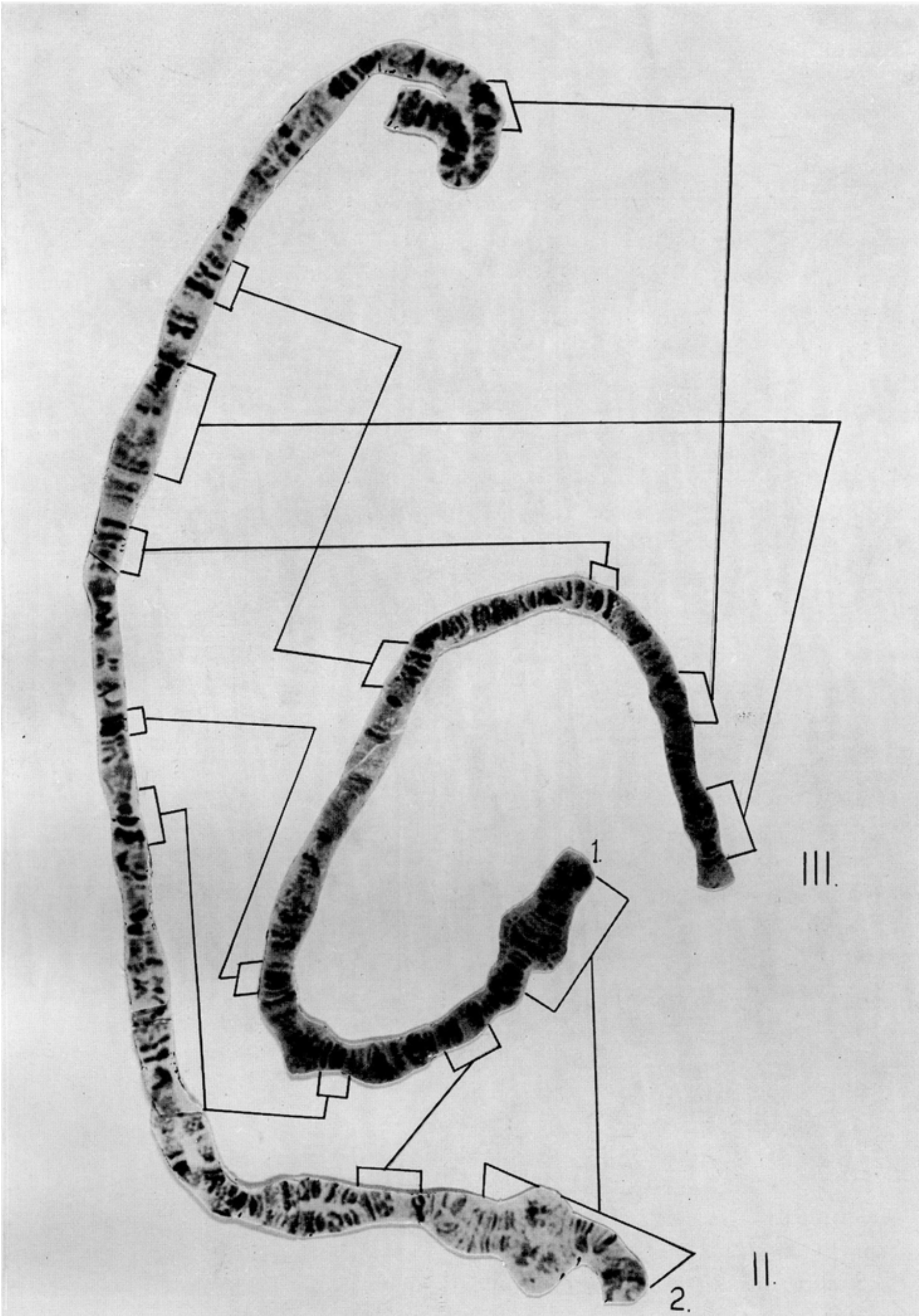

Plate IV. — Comparison of the II. Salivary chromosomes of *Cr. silvestris* with III. Salivary chromosomes of *C. ornatus*. 1. *Cricotopus ornatus*. 2. *Cricotopus silvestris*.

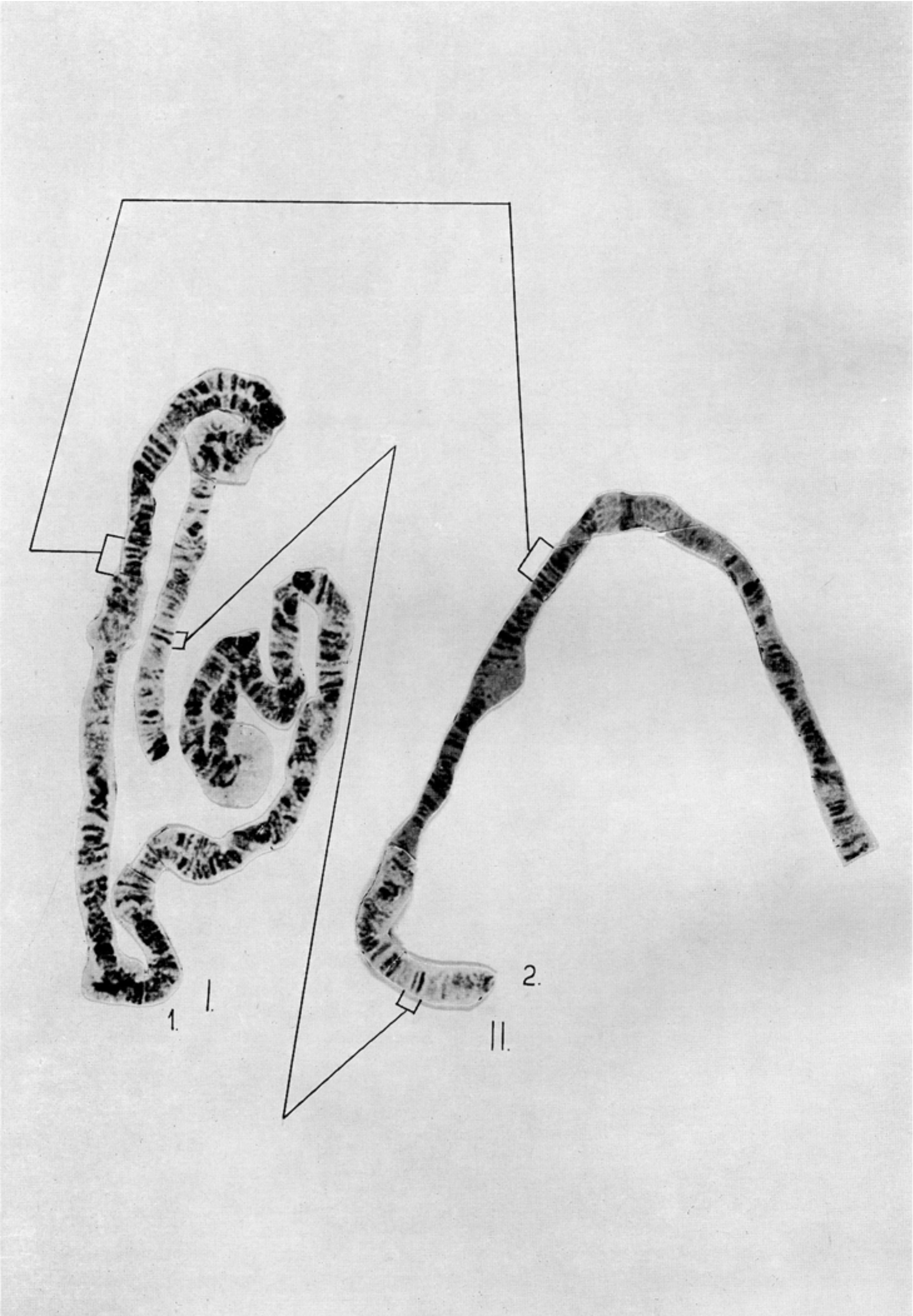

Plate V. — Comparison of the I. Salivary chromosomes of *Cr. silvestris* with II. Salivary chromosomes of *Cr. ornatus*. 1. *Cricotopus silvestris*. 2. *Cricotopus ornatus*.

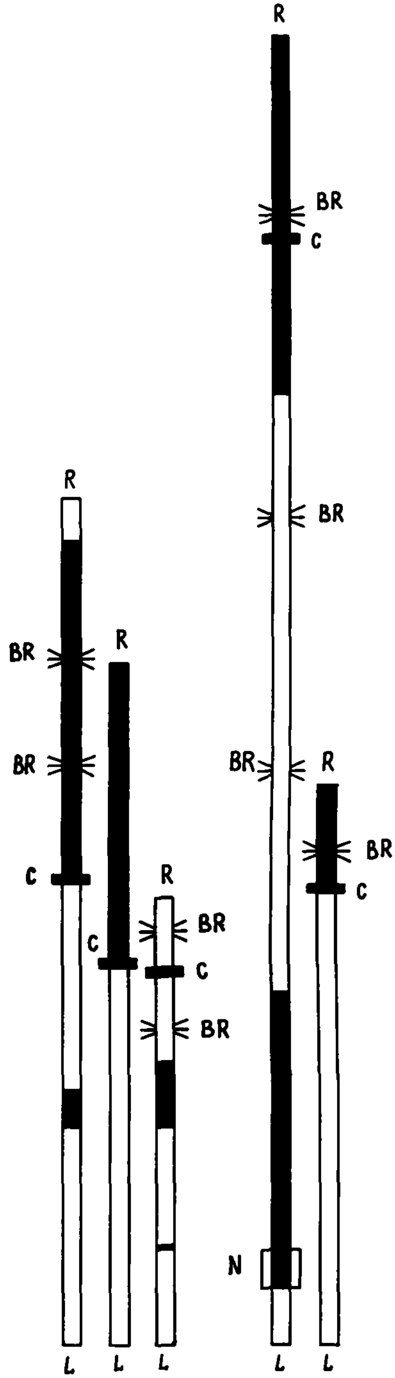

Fig. 3. — Schema of the distribution of the aberrations in *Cr. silvestris* and *Cr. ornatus*.

On the basis of this and the data from the karyology and morphology of *Cr. silvestris* we accept that it is more apomorphous, than *Cr. ornatus*. But although the two species are morphologically very close a genetical resemblance has also been noted (the great identity of *Cr. silvestris* chromosome 2 and *Cr. ornatus* chromosome 3 is may be due to their belonging to one group), we cannot suggest that *Cr. silvestris* has evolved from *Cr. ornatus*, as the homology has been observed only in some section. On the other hand the karyological analysis proves the hybrid origin of *Cr. silvestris*. In order to make the philogeny completely clear it is necessary to examine cytogenetically other species of this group.

CONCLUSIONS

The results of the present study can be summarised as follows:

1) The application of the cytogenetical method make possible to discern as early as the larva stage two very close species: *Cr. silvestris* and *Cr. ornatus*.

The larvae of the species *Cr. silvestris* found on the stones of the Black Sea lakes have diploid set of chromosomes $2n = 4$.

The larvae of the species *Cr. ornatus* found in the vegetation of the Black Sea lakes have a diploid set of chromosome $2n = 6$.

2) Information about the karyology of the species *Cr. silvestris* and *Cr. ornatus* is given for the first time. The cytological maps have been compiled for the first time.

3) The hybrid origin of *Cr. silvestris* is proved karyologically.

4) The smaller diploid set of chromosomes of *Cr. silvestris*, the high grade of chromosome polymorphism, the great variability in the external morphology of the larvae of the species show their philogenetical young age.

5) Both species show a statistical relation between the two sexes 3:1, the female sex prevailing.

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SUMMARY

The structure of the polytene chromosomes in the salivary gland of two hard for distinguishing species *Cricotopus silvestris* Fabricius and *Cricotopus ornatus* Meigen was studied. For the both salivary chromosomes mapes are worked out. Using the karyological

analysis is established the hybrid origin of the species *Cricotopus silvestris*.

The comparative-karyologic analysis of the taxons showed some common features in their karyotypes. There is some resemblance between II chromosomes of the species *Cr. silvestris* and III chromosomes of the species *Cr. ornatus* and between I chromosomes of *Cr. silvestris* and II chromosomes of *Cr. ornatus*. At the same time these species are well distinguished karyologically according to the number of chromosomes (the species *Cr. silvestris* has $2n=4$, but the species *Cr. ornatus* has $2n=6$) and the chromosomal polymorphism - *Cr silvestris* is more polymorphic. These date indicate the philogenetic jouth of the species *Cr. silvestris*.