

Cytoecological Study of Indigenous Populations of the Malaria Mosquito
in the Territory of the U.S.S.R.

I. Identification of a New Species of *Anopheles* in the *maculipennis*
Complex by the Cytodiagnostic Method.*

by

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Despite excellent knowledge of the biology of the Palaearctic *Anopheles maculipennis* complex, the taxonomic status of the members of this complex remains controversial.

Such a situation is due mainly to the astonishing morphological similarity of the "*maculipennis*" varieties in larval and adult stages of development. In Russian literature, one polytypic species - *Anopheles maculipennis* Meigen - is recognized, with the following sub-species: *An. m. maculipennis* Meigen, *An. m. messeae* Falleroni, *An. m. melanoon* Hackett (*subalpinus* Hackett & Lewis), *An. m. labranchiae* Falleroni, *An. m. atroparvus* Van Thiel and *An. m. sacharovi* Favre (Gutsevich et al., 1970). On the basis of non-hybridization in the laboratory, some scientists distinguish these forms as independent sibling species (Bates, 1940). In principle, such an approach is correct since, for a biological species, reproductive isolation is the main criterion of independence (Mayr, 1971). However, in this particular case, isolation was not fully proved since hybridization was carried out exclusively with *An. m. atroparvus*, colonised in the laboratory, and since the results of laboratory hybridization frequently do not reflect the actual situation in nature. In our view, only a study of the interrelations existing in nature between members of the Palaearctic *maculipennis* complex can provide a decisive answer regarding their taxonomic status. Such a study is very difficult by means of the existing method for identification of the *maculipennis* varieties according to egg characteristics.

The technique of cytodiagnosis of *An. m. atroparvus*, *An. m. maculipennis*, *An. m. messeae* and *An. m. sacharovi* according to the structure of the polytene chromosomes, proposed by the Italian scientist Frizzi in 1947, permits one to distinguish these varieties in both the larval (chromosomes of the salivary glands) and the imaginal (chromosomes of the ovarian nurse cells) stages. The use of cytodiagnosis permits one to investigate regular features of the population structure, the adaptation potential, and the degree of reproductive

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isolation of each of the varieties of the complex. Such a study is the more necessary because of the high resistance of certain varieties of *maculipennis* to insecticides.

As a result of our investigations of the chromosomal structure in populations of *An. m. maculipennis* and *An. m. messeae* along the Ob river, in the Tomsk Region, a definite degree of inversion polymorphism in each of the varieties was revealed, and it was shown that there exist between them marked differences in major fixed rearrangements of the inversion type (Kabanova et al., 1972a; b; Stegnii et al., 1974). Cytogenetic analysis of a large number of specimens of *An. m. maculipennis* and *An. m. messeae* in sympatric areas revealed not a single indigenous hybrid. This led us to conclude that there existed a strict reproductive isolation and specific independence of these varieties (Stegnii et al., 1973). Comparison of *An. m. maculipennis* from West Siberia with the West-European *An. m. maculipennis* described by Frizzi (1956) revealed important chromosomal differences between them which cast doubt on the identities of these varieties.

It was necessary to study the native populations of *An. m. maculipennis* from different geographical regions, especially the Caucasian variety.

Material and Method

The varieties of the *maculipennis* complex were distinguished on the basis of comparative analysis of the structure of polytene chromosomes from salivary gland cells of stage IV larvae. The larvae were dehydrated and fixed, at the place of collection, in an acetic alcohol mixture (3 parts of 96% alcohol and 1 part of glacial acetic acid). The salivary glands were isolated by means of an MBS-1 microscope in a drop of fixative, diluted with water (1:15). They were stained (5-15 min) with lactoaceto-orceine (2g of orceine + 50 ml of glacial acetic acid + 50 ml lactic acid), differentiated in successive 60, 30 and 15% solutions of lactic acid (1 min in each) and squashed in 45% acetic acid. Stained chromosomes were photographed by means of the MΦH-10 attachment through an MBI-3 microscope onto a MIKRAT-300 film.

From each population, a sample consisting of an average of 100 larvae was analyzed. In the Tomsk Region, during the 1972-1973 seasons, more than 5000 specimens were examined. For the present work, we used samples from the following localities: Irkutsk (Meget village), Krasnoyarsk (Startsevo), Tomsk (Kolarovo, Rybalovo, Kargasok, Prokhorkino, Larino, Svetlaya Protoka), Tiumen (Uteshevo), Gorno-Altaiisk (Mangerok); from the regions of Chità, Solikàmsk, Cheboksàry, Tuapsè; from Moldavia (the towns of Beltsy and Faleshty; Armenia (Idgevan); Georgia (Sheshelety).

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Results

We studied populations of *An. m. maculipennis* and *An. m. atroparvus* collected in a number of localities of the Caucasus and Moldavia, and continued the study of the Siberian varieties of *An. m. maculipennis* and *An. m. messeae* from a large part of the territory of the Soviet Union (Fig. 1).

Comparative analysis of polytene chromosomes of *An. m. maculipennis* from populations collected in the Caucasus and Moldavia revealed their absolute identity with the West-European *An. m. maculipennis* described by Frizzi (1956). The Siberian variety clearly differs from the Caucasian and Moldavian *An. m. maculipennis* by a whole series of fixed chromosomal inversions; this permits one to regard it as a completely independent type, which we have referred to as *Anopheles maculipennis* species.

Description of the polytene chromosomes of *An. maculipennis* sp.

Like other members of the *maculipennis* complex, *An. maculipennis* sp. has 3 pairs of synapsed polytene chromosomes, with a chromocentre (fig. 2). Schematic comparison of *An. maculipennis* sp. with other varieties is shown in Fig. 3.

Chromosome I (sex) - one-arm; in *An. maculipennis* sp. its distal end differs strikingly from all Palearctic "*maculipennis*". The sector enclosed in brackets is inverted as compared to chromosome I of the *An. m. maculipennis* (= * *An. m. atroparvus*).

Chromosome II consists of two arms. The left arm (IIL) is not shown in the diagram because it is identical in all members of the Palaeartic *maculipennis* complex. The right arm (IIR) of *An. maculipennis* sp. has an inverted gene sequence in the middle part of the chromosome, as compared to IIR of *An. m. maculipennis* (= *An. m. atroparvus* = *An. m. messeae*). This inversion is somewhat shorter than the heterozygous floating inversion of IIR found in *An. m. messeae*; that is to say, *An. maculipennis* sp. and *An. m. messeae* have only one common breakage point in the distal part of the chromosome.

Chromosome III also has two arms. The right arm (IIIR) of *An. maculipennis* sp. differs very sharply from IIIR of *An. m. maculipennis* (*An. m. messeae*) by two overlapping inversions, the intermediate structure being the IIIR of *An. m. atroparvus*. As a result of such a rearrangement, the sector a-b in *An. m. maculipennis* sp. is shifted in relation to *An. m. maculipennis* from the distal part of the arm to the central. The left arm (IIIL) in *An. maculipennis* sp. is also markedly different from that in all other members of the complex. The sector c-d in *An. maculipennis* sp. is displaced and reversed as compared to *An. m. maculipennis* (= *An. m. atroparvus* = *An. m. messeae*). Such an arrangement of bands has arisen as a result of two successive overlapping inversions. The intermediate form (Fig. 3, IIIL B), shown schematically, has not been found in any of the Palaeartic varieties of the complex.

In addition to fixed chromosomal rearrangements, *An. maculipennis* sp. is

* The equals sign indicates identity of the chromosomal structures in different species; in this particular case - in *An. m. maculipennis* and *An. m. atroparvus*.

characterized by 8 heterozygous (floating) inversions, found at frequencies of from 1 to 20% in particular populations. In addition to inversions described previously (Stegnii et al., 1974), three others were noted: in IIIR, IIR and IIL arms. In the distal part of IIR, there is an inversion which, earlier, we took for a region of non-specific telomeric pairing. The locations of all the floating inversions in *An. maculipennis* sp. are shown by brackets in Fig. 2.

An. m. maculipennis is characterized by exceptional homozygosity. In Caucasian populations, not a single floating inversion was found; in Moldavian populations, only one heterozygote was found for an inversion in the left arm of chromosome II.

Morphology of the Eggs

Comparison of the morphological features of the eggs revealed that *An. maculipennis* sp. can be readily identified by egg characters. The eggs of *An. maculipennis* sp. differ sharply from the eggs of both *An. m. maculipennis* and other varieties (Fig. 4). They are narrower shaped, the tips are dark and pointed, and the surface is much smoother than those of the eggs of *An. m. maculipennis* and *An. m. messeae*. The length of *An. maculipennis* sp. eggs is approximately the same as for *An. m. maculipennis* and *An. m. messeae* but they are noticeably narrower in the middle part. In our opinion the relation of the width of the middle part of the egg between the floats, to the length (in *An. maculipennis* sp. this relation is $12 \pm 1\%$, in *An. m. maculipennis* and *An. m. messeae* it is $17 \pm 1\%$) can be a reliable criterion for distinguishing *An. maculipennis* sp.

Distribution and Ecological Attributes

An. maculipennis was found in a large area of the Soviet Union (see Fig. 1), while in Western Siberia it was found wherever we searched for it. In some places, *An. maculipennis* sp. was found in larval biotypes together with a majority of *An. m. messeae* (Tiumen, Krasnoyarsk, Cheboksary); in other places it predominated entirely (Mangerok village, in the Gorno-Altai autonomous district, in the North of the Tomsk Region).

In some villages of the Tomsk Region where, as far as possible, all Anopheline breeding places were examined, we noted an ecological difference between *An. maculipennis* sp. and *An. m. messeae*. For example, in the village of Killarovo, in one larval biotope *An. m. messeae* predominated (95%); in another breeding site, situated 50-60m. from the former, *An. maculipennis* sp. was prevalent (98%). This difference persisted throughout the whole summer of 1973. Preliminary data from the ecological study of *An. maculipennis* sp. larvae show that they can exist in breeding places quite typical of *An. n. messeae*. Thus, in the breeding site at the eastern end of the village of Mangerok, only larvae of *An. maculipennis* sp. (100% of the specimens) were found. The pond was very polluted by humus, aquatic plants were absent and the larvae lived only around the shore of the pond. A pond of the same kind, populated exclusively by larvae of *An. maculipennis* sp., without admixture of *An. m. messeae*, was found in the village of Kargasok (Tomsk Region).

Discussion of the Results

Hacket and Barber (1935), when studying varieties of *An. maculipennis* in the Soviet Union, discovered a new type of egg, previously not found in Western Europe. Egg-batches were found in considerable quantities in the neighbourhood of the town of Orekhovo-Zuyevo, and in the Volga river basin. Since, in these areas, *An. m. messeae* predominated, the authors took these eggs for a variation of *An. m. messeae*. Judging by the photograph of the eggs, they were very similar to the eggs of *An. maculipennis* sp. Later, a number of researchers (Markin, 1938; Karpovich and Dobrynina, 1941; Pestriakova, 1954), when studying *An. maculipennis* in the Ural region, and in Western Siberia, thought such egg-batches belonged to *An. m. maculipennis*, because of the similarity of the exochorion pattern of the eggs (two dark transverse bars); they pointed out, however, differences of the eggs of this variety as compared to the Caucasian one: a slimmer, daintier shape and a smooth surface. Still, one inconsistency remained unexplained: the range of the southern variety of *An. m. maculipennis*, widespread in the Caucasus, was bounded in the North by the Terek and Kuban rivers (Beklemishev, 1944); then they reappeared in the northern part of the country, in the Ural region and Western Siberia. It was thought that *An. m. maculipennis* preferred elevated terrain, in particular the Valdai and Ural mountains. But this did not agree with the fact that *An. m. maculipennis* was found in the West-Siberian depression. Beklemishev (1944) also noted great differences in the biology of the Caucasian *An. m. maculipennis* as compared to the northern variety, which would point to a possible independence of these varieties.

Our cytogenetic research confirmed that the northern variety had nothing in common with *An. m. maculipennis* proper. The chromosomal structure of *An. maculipennis* sp. differs sharply from that of *An. m. maculipennis*. In chromosome III, the phylogenetic link between them can be traced only through intermediate chromosomal forms. In this sense, *An. maculipennis* sp. stands somewhat apart also from other Palaearctic "*maculipennis*". Among Nearctic species, *An. earlei* is the nearest to *An. maculipennis* sp. In particular, they present a similar pattern of bands in IIR, and differ by a small inversion in IIIR. The IIIIL of *An. earlei* has that intermediate structure which serves as the basis from which the banding pattern of *An. maculipennis* sp. is derived (Kitzmilller and Baker, 1965).

Fixed chromosomal rearrangements in *An. maculipennis* sp. are so different from those in other forms of "*maculipennis*" that it is difficult to imagine any possibility of hybridization between sympatric populations. In fact, as a result of cytogenetic analysis of more than 5000 specimens of *An. m. messeae* in areas where they are sympatric not one hybrid was found. Reproductive isolation between these varieties permits one to regard them as good biological species; sympatry, as shown when comparing their chromosomal structure, is apparently a secondary situation.

The geographical range of *An. maculipennis* sp. has not yet been clearly traced. In the East, it may be found beyond Krasnoyarsk; in the North, it may spread to the distributional limits of the entire *maculipennis* complex. In the West, *An. maculipennis* sp. has been traced by us up to Cheboksary; it would seem that in the north of the European part of the U.S.S.R. it is *An. maculipennis* sp. that is widespread, and not *An. m. maculipennis*, as believed hitherto.

Apparently, the area occupied in the Palaearctic region by the *An. maculipennis* sp. is second only to that occupied by *An. m. messeae*; this correlates with the degree of inversion polymorphism. *An. m. messeae* is a very polymorphic variety; in the central region, along the banks of the Ob river, about 80% of the specimens in the populations of *An. m. messeae* are inversion heterozygotes (Kabanova et al., 1973). Polymorphism of *An. maculipennis* sp. is much less; the occurrence of inversions in different populations fluctuates between 1% and 20%. Other Palaearctic varieties of "*maculipennis*", both according to data obtained by Frizzi (1956), and according to our own observations, are characterized by a low degree of polymorphism, or are altogether homozygous. Since the degree of inversion polymorphism reflects the adaptive potential of the species (Da Cunha, 1960), *An. maculipennis* sp. in this respect is apparently ecologically more plastic than *An. m. maculipennis* and *An. m. atroparvus*. The phenomenon of ecological dissociation of *An. maculipennis* sp. and *An. m. messeae* which we found in adjoining larval biotopes permits us to assume that, despite territorial sympatry, these varieties do not compete with each other, but occupy different ecological "niches".

Thus *An. maculipennis* sp. can be regarded as an independent species in the Palaearctic *maculipennis* complex; this view is supported by the following arguments:

1. fixed chromosomal differences distinguish it from all other forms of "*maculipennis*";
2. floating inversions are characteristic of this species alone;
3. the egg morphology is distinctive;
4. the distribution and morphology are distinctive;
5. *An. maculipennis* sp. is reproductively isolated from sympatric *An. m. messeae*.

In honour of the distinguished Soviet scientist and malariologist V. N. Beklemishev, the authors propose the new species be named *Anopheles beklemishevi* Stegnii & Kabanova sp.n.

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Figure 1.

Distribution of separate members of the *Anopheles maculipennis* complex in the territory of the U.S.S.R. a = *An. beklemishevi*; b = *An. m. messeae*; c = *An. m. maculipennis*; d = *An. m. atroparvus* 1. Beltzy. 2. Faleshty. 3. Tuapse. 4. Tbilissi. 5. Erevan. 6. Cheboksary. 7. Solikamsk. 8. Tumen. 9. Gorno-Altaysk. 10. Tomsk. 11. Krasnoyarsk. 12. Irkutsk. 13. Chita.

Figure 2.

Polytene chromosomes in *An. beklemishevi* (enlarged 10 x 40). IL (X) - first (sex) chromosome; IIR and IIL - right and left arms of chromosome-II; IIIR and IIIL - right and left arms of chromosome-III; C - chromocentre. Regions of floating inversions are shown in brackets.

Figure 3.

Schematic comparison of chromosomes in members of the *maculipennis* complex (enlarged 10 x 90). IL - first chromosome: A - *An. beklemishevi*; B - *An. m. maculipennis/An. m. atroparvus*. IIR - right arm of second chromosome: A - *An. beklemishevi*; B - *An. m. maculipennis/An. m. messeae/An. m. atroparvus*. IIIR - right arm of third chromosome: A - *An. beklemishevi*; B - intermediate form; C - *An. m. maculipennis/An. m. messeae/An. m. atroparvus*.

Figure 4.

Eggs of *An. beklemishevi* (a) and *An. m. maculipennis* (b). (enlarged 8x).

Figure 1

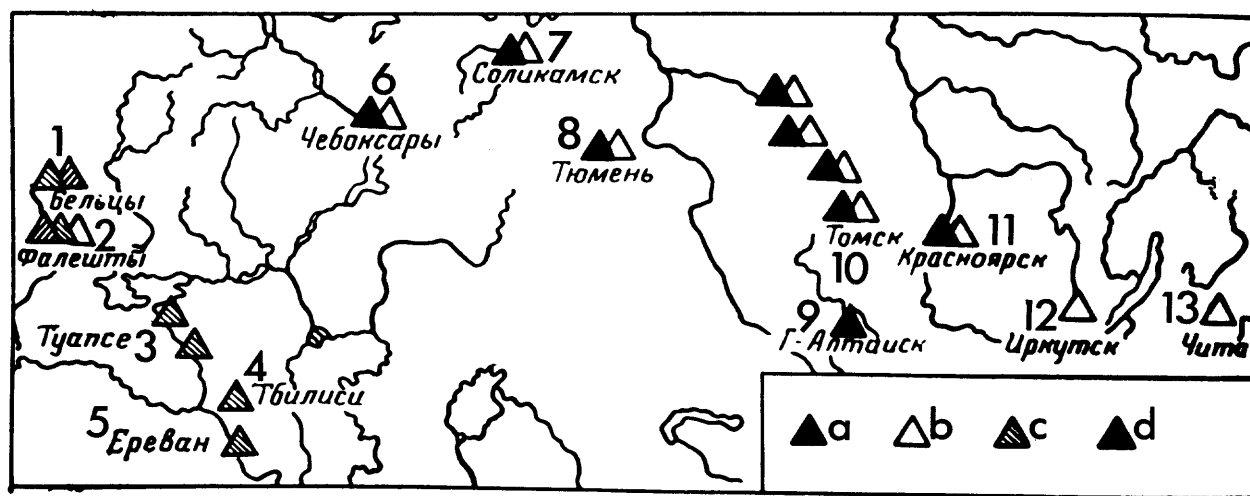


Figure 2

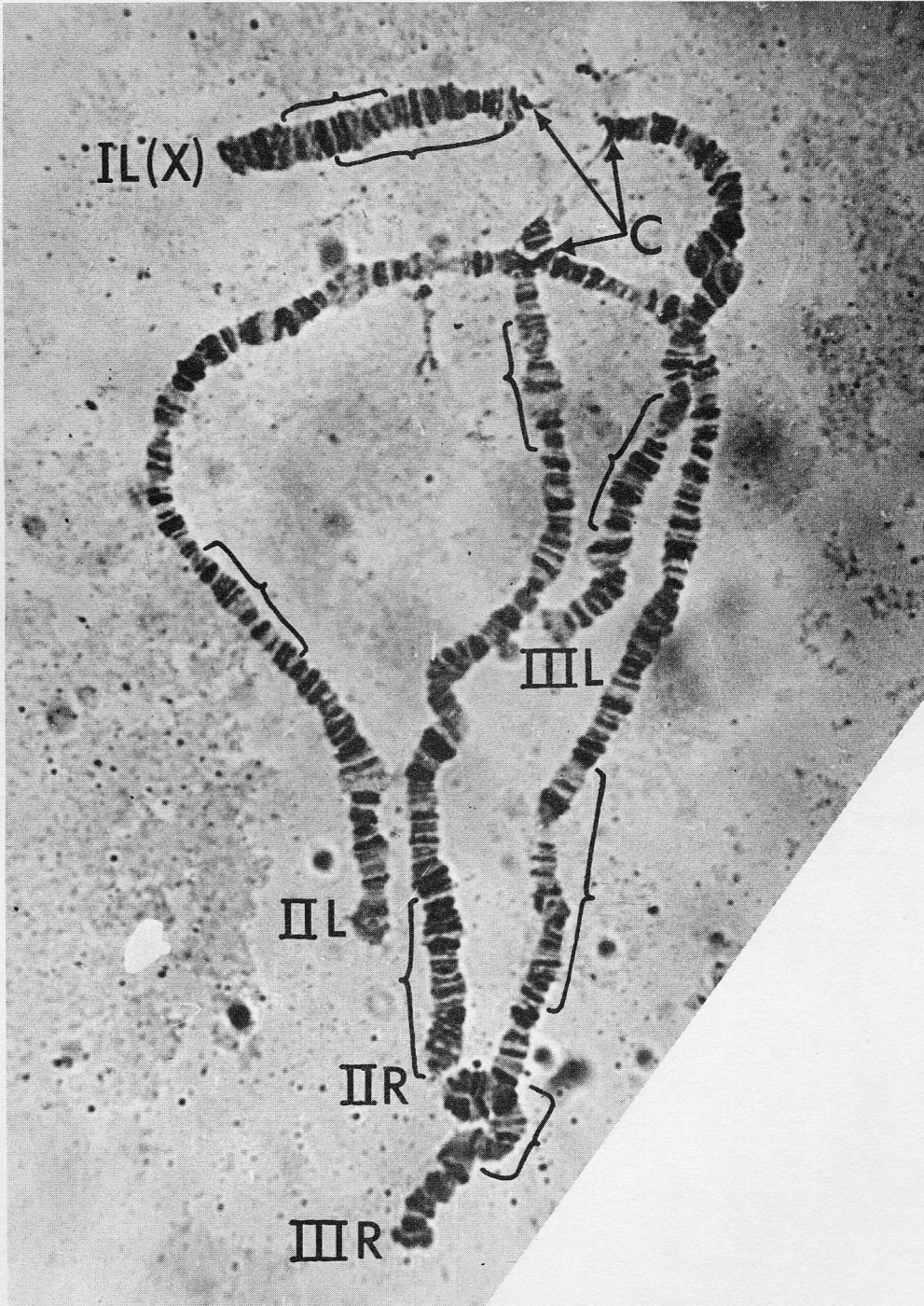


Figure 3

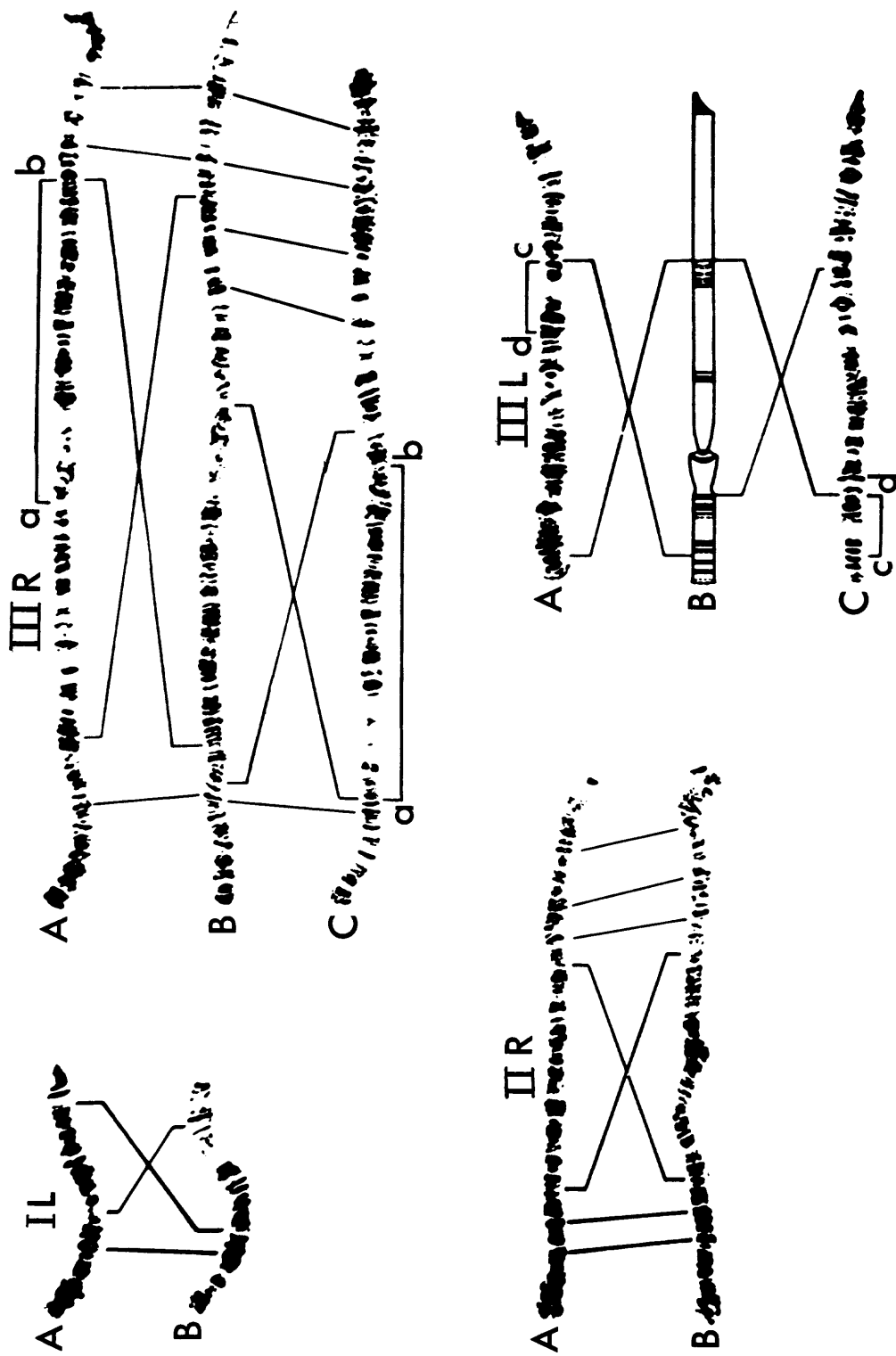


Figure 4

