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Chromosomes and Systematics in the Chironomidae

By Hans Bauer

At the beginning of his review of "Chironomid Research - Its Importance for Limnology and Biology", Thienemann (1939) raised the question as to whether it was worthwhile devoting one's life to the study of such a small systematic group. And at the end of his review he leaves the answer to the reader, who, crushed by the mass of the problems results, might well wonder for his part whether the strength of one or a few workers was sufficient to deal with all these problems.

Thienemann's paper makes it clear that a great deal of attention has been paid to those sides of Chironomid biology that are associated with ecology. This is not surprising since here we see Thienemann's own work. On the other hand it is obvious from his review, how comparatively slight is our knowledge of general biological questions. I have been trying for several years to fill one of these gaps in our knowledge. As mentioned in Thienemann's work, "the Chironomidae have assumed special importance for chromosome research". As Diptera, the Chironomidae are known to have in their salivary glands unusually large chromosomes, the study of which has yielded important information for many cytological and genetic problems. But apart from their importance for the theoretical disciplines, it is precisely the study of these chromosomes that offers the possibility of overcoming a difficulty which limnology also has to battle.

Fauna-ecological works on Chironomidae are inhibited by the fact that in their larval state (which is precisely the most important for hydrobiological research) the detectable morphological characteristics are insufficient to provide a certain determination of species. It is only possible to fix their membership of a group, in which was grouped those species between which no definite distinction is possible. The extent of these groups varies: in addition to those in which perhaps only one species is included, there are others in which species from more than one genus have to be allotted.

Lenz (1927) tried to reduce the extent of these determinative difficulties. He uses "the term "type" for the distinguishable larvae and pupa forms". According to him, "this seems all the more desirable since the larval form recognisable morphologically as a special type also occurs ecologically as a unit or type. How far these types in the area of study treated actually only represent each a particular species is a secondary question" (Lenz, 1927).

If this agreement between the morphology of the types and their biotype were in fact to be admitted, then determination of the species would be unnecessary for the hydrobiologist. Such a relationship, which doubtless did correspond with the material available to Lenz, will however, as Pagast (1940) has indicated, be limited in its range of validity as observations are widened, and thus lose its general significance. To check it, species determination will again be needed. The safest method hitherto has been the

breeding of the imago, a procedure that is often tiresome or (with one year forms) not possible at certain times. This is where there comes in the possibility of making species determination directly from the larvae by studying their salivary glands chromosomes.

As is known, the salivary gland chromosomes are richly membered structures that show a constant pattern of strongly staining bands of varying condition and thickness, plus constrictions and thickening, for each particular chromosome. Nucleoli appear singly and multiply at certain places in the individual chromosomes. There can be distinguished principal and secondary nucleoli, and nucleoli of special types, e.g. the so-called Balbiani ring. Normally each cell contains giant chromosomes in haploid number, since the homologous chromosomes coming from the male and female parent pair completely in length, mostly without visible boundary. The special values attributed to these richly membered structures as a characteristic for species determination is based on the fact that, unlike the other bodily features, they do not stand in any direct functional connection with particular abilities and that therefore in each case similarities in their structure directly indicated a relationship factor. Convergences that so often cloud natural systematics, also hardly play any part with them.

The chromosome pattern of related species may show differences in the number, shape and fine structure of the chromosomes. We have considerable information today on the reason why such differences arise. Without discussing this question in detail here (for a more comprehensive treatment see Bauer and Timofeeff-Ressovsky, 1943), brief reference must be made to few facts in order to render comprehensible hat follows in this paper. The chromosomes of almost all organisms have a site, the centromere, which is physiologically and often morphologically particularly differentiated, and which guarantees the order mitotic movement of chromosomes. It divides the chromosomes into two arms, which may range from equal in length to extremely unequal. When the centromere is median in position, we have a V-shaped chromosome; where it is almost [1)] terminal, we have rod shaped chromosomes (Textfig. 1a, b)

([1)] Chromosomes with a terminal centromere do not persist).

The transformation of chromosomes is effected essentially by chromosome mutations, which represent a change of position of chromosome sections. For the evolutionary variation of the chromosome pattern, of many possible and experimentally obtainable changes in position, simple inversions, pericentric inversion and reciprocal translocation are important. Simple inversions are rotations of sections of an arm, where sites originally close to the centromere are moved away from it and vice versa. With a large number of such inversions within an arm the fine structure can be completely altered while the original chromosome shape in maintained (Textfig. 1c). With pericentric inversions the centromere lies in the section rotated. If it is not exactly median in position, this leads to an alteration in the ratio of lengths of arms, thus the chromosome form as in Textfig. 1d, e. In the limiting case, by one or several such pericentric inversions a rod shaped chromosome can be formed a V-shaped one.

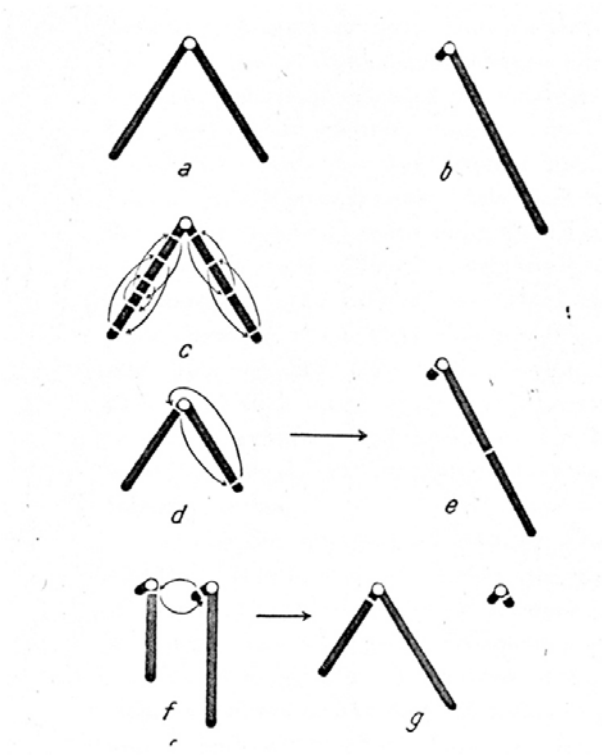

Fig. 1. Shape and change of chromosomes (see text) a, b V- and rod-form chromosomes, with median and subterminal spindle attachments (white circles) respectively. c Through successive simple inversions the order of the segments of the arm are altered. d, e Pericentric inversions lead to change in the position of the spindle attachments and thereby to changes in the form of the chromosomes. f, g Reciprocal translocations of whole arms of rod-shaped chromosomes leads to V-shapes of very different size. - The arrow gives the direction of the rearrangement, with the limits of the chromosome segments involved marked by the white spaces.

Changes in number come about through reciprocal translocations. These consist in the exchange of terminal sections of two arms of different chromosomes. Whereas, in translocations experimentally induced, the sections exchanged may have any length, in the evolutionary process almost exclusively only those reciprocal translocations in which almost the whole arm is exchanged can persist. If a translocation occurs between two rod shaped chromosomes (Fig. 1f, g) such that the short arm of one isexchanged for the long arm of the other, then two new chromosome are formed, one having a large and the other a very small V-shape. The latter, which certainly often does not represent a vital part of the hereditary mass, may then be lost without prejudicing the vital function of it possessor. The number of chromosomes is thus reduced by such reciprocal translocations. Within small related groups the lower number of chromosomes will thus almost always be that of the derived, younger members.

Changes in the number and sites of the nucleoli cannot always be related directly to chromosome mutations. Precisely the comparative study of chironomid chromosomes has shown that many chromosome sections are potential sites for nucleoli formation, but that normally only one or a few actually form a nucleolus. There are reasons for assuming that, in association with chromosome mutations, but probably also directly through gene mutations, hitherto non-participating sections become functionally active as formers of nucleoli, while, vice versa, the sites which formerly produced nucleoli may become inactive.

Due to the processes mentioned, chromosome mutation and reorganisation of nucleoli, the essential differences in the chromosome pattern of the various species arise. Three new informative examples of this may be quoted.

Among the largest representatives of our Chironominae are *Tendipes plumosus* and the two *Camptochironomus* species *C. tentans* and *C. pallidivittatus*. They often occur in the same biotype. No distinction is possible between them at the larval stage by means of conventional diagnostic features; they have the same labial structure and tubular appendices on the 10th segment, and thus all belong to the *plumosus* larva type. However, there do exist certain differences between *Tendipes plumosus* and the *Camptochironomus* species. Thus the latter are slimmer (smaller length-breadth ratio of the segments), they mostly show a dark red colour and when disturbed they remain longer in the spirally rolled position. However, these distinctions are generally only clear when one has the two types of larva side by side.

Cytological distinction between *Tendipes plumosus* on the one hand and the *Camptochironomus* species on the other can be readily made on the basis of nucleoli relationships. All three species have normal number of chromosomes as prevails in the Chironominae: 3 long and 1 short chromosomes. Now *T. plumosus*, like *T. thummi, T. dorsalis* and others, have only one large principal nucleolus, which appears on the short chromosome. The photo (Fig. 1) shows this on a living nucleus. As I have already described (1936), the homologous partners of the small chromosome tend to pair incompletely or not all. Such a case, with pairing absent, is shown in Fig. 1. The two small unpaired chromosomes are separately located on the nucleolus, a pattern arising from the fact that each partner chromosome forms a nucleolus and these two fuse together. The *Camptochironomus* species, which can only be distinguished from each other by the fine structure (which will not be discussed in further detail here), can be distinguished by the fact that each nucleus has two nucleoli, each located on one of the long chromosomes. The photos Fig. 2a and b show a nucleus of C. *tentans* photographed alive under two focuses. In 2a the three long chromosomes can be seen for the most part in sharp focus. The chromosome with one end at top left shows at this site the one dumbbell shaped nucleolus. The second nucleolus can be seen clearly in Fig. 2b on the large chromosome at the bottom of the photo. It has a submedian position and is formed as a large drop shape body especially towards one side. At the bottom of the same photo, partly covering the large chromosome, is another structure. This is the short chromosome, which is, however, heterozygous for a large inversion often present in this species and hence does not have normal stretched shape, but forms an irregular ring. Through the clear difference in the number of nucleoli and the nature of the airing of the small chromosomes, this example shows that a distinctive separation, which is hardly possible on the larvae, can be easily effected by reference to salivary gland chromosomes.

In the two further examples there are difference in the number of chromosomes. One relates to larvae of the *Cryptochironomus defectus* group. The two species compared have nor yet been bred so that their assignment to an imaginal species has not yet been effected. Both show the same differentiation of the salivary gland cell in four large cells close to the excretory duct and a large number of smaller cells. One of the species, species 1 (to which I had inadvertently given the species name *defectus*), I gave (1936) the number of chromosomes as n = 3, and illustrated one of the chromosomes from the large cell. A general view of the chromosomes from one of the smaller cells is show in Plate XLVIII, Fig. 3 (live) and Fig. 4

(stained). On the photo of the living nucleus one can clearly distinguish the three chromosomes, which are substantially slimmer than those from the large cells, and it can be seen at the same time that one nucleolus is formed medianly in the lower chromosome in the photo and one subterminally in the sharply focussed chromosome lying in the centre, and that both nucleoli are fused. From Plate XLIX, Fig. 4 it is seen that all three chromosomes have approximately the same length. This species was frequently in the mussel zone in the N.E. basin of the Plover See (close by the Hydrobiological Institute). Species 2 of the *defectus* group, of which only one specimen was hitherto available for study, was found by Mr. Laskar on a sandbank in the middle of the Plover-See. It is markedly distinguished from the other by the fact that it has only two chromosomes(2), of which one is approximately twice the length of the other (Plate XLIX, Fig. 5, from a large cell). This finding not only represents a good example for practical possibility of distinguishing the chironomid species, but also has evolutionary and cytological interest. 2)

(2) Thus the chironomids too have a representative of the lowest known number of chromosomes, such as has only been found for some rhabdocoeles and coccids. The number n = 1 given for *Apus* only, is certainly wrong, even though my material was inadequate to determine the real number. The classical examples of *Ascaris megalocephala bivalens* and *univalens* should no longer be referred to in this connection because of the very much higher normal somatic number of chromosomes).

Compared with the other, the two-chromosome species exhibits the derived chromosome pattern. Even with the 3-chromosome type, compared with the normal pattern of the chironomids, a combination of the short with one of the long chromosomes must have occurred in a similar manner to that shown later in this example. The length ratio of the 2 chromosomes of Species 2 must be understood again only in the sense that there has been a combination into two of the three more or less equally long chromosomes assumed to have been present originally. A combination of this sort, as referred to briefly earlier, may consist not simply of a linking together of whole chromosome; rather must chromosome mutations, especially pericentric inversions, have occurred, by which in those which were later combined the centromeres have been displaced to one end of the chromosome. Only then can the two chromosomes have been joined by reciprocal translocation. Direct observations on mitotic chromosomes are difficult for all the chironomids, since partition figures can only rarely be found (mostly shortly before the pupal stage in the gonads and imaginal discs). However, from the position of the heterochromatic section distinguished by special staining and structural properties (cf. Bauer,1936), the position of the centromere can be concluded (see also Wolf, 1947). For *C. defectus*, sp. 1., on the basis of the heterochromatin a more or less median position of the centromeres, i.e. V-shaped chromosomes, can be assumed. Species 2, heterochromatin sections are found in the places indicated by arrows in Fig. 5, Plate XLIX, median in the long and terminal in the shorter chromosome. Here again one may conclude with great probability that the large chromosome is V-shaped and the smaller one rod shaped. Thus it is not improbable that prior to the joining of two chromosomes, all three had become rod shaped by pericentric inversions. Comparative studies on different species of *Cyptochironomus* promise further information on possible intermediate stages between the forms observed and will thus offer suggestions for a natural organisation of the genus.

While with the *Cryptochironomus* species conclusion regarding the joining of

chromosomes can be drawn only from the relationship of number and lengths, the two Sergentia species, *S. longiventris* and *S, coracina* off more detailed information due to a structural peculiarity.

S. longiventris has the normal pattern of Chironominae: 4 chromosomes, including the short one. This is characterised by a striking peculiarity - it is always thinner than the long chromosomes. Plate XLIX, Fig. 6, shows the chromosome pattern. The thin smaller chromosome is at the left of the photo, with one end slightly overlying one long chromosome. Near the other end it has a loose site. This represents the formation site of a special nucleolar structure, such as is present similarly in the Balbiani ring of *Tendipes thummi* (Bauer,1935) and which has, in contrast to the principal nucleolus, a granular instead of homogeneous texture. A true nucleolus occurs on one of the long chromosomes, on the lower left chromosome inFig. 6, Plate XLIX. Its site is also characterised by loosening of the chromosome section which forms it. In this illustration it is found at the place where the short chromosome overlies the long one. The nucleoli themselves, which are not visible in Fig. 6, Plate XLIX are shown in Fig. 7 (alcohol material), where in a section from nucleus there are shown only the two chromosomes which carry nucleoli. The bulbous, sharply delimited principal nucleolus is on the long chromosome, while on the short one, whose lesser thickness is marked here, is the Balbiani ring with no sharp limits.

S. coracina has only three chromosomes (Bauer 1936). Among them appears a very remarkable one, consisting of a shorter thin section and a long broad section. In my old material from 1932 this division could not be detected owing to the use of historical techniques then in vogue. This chromosome is shown at the top of the photo in Fig. 8, Plate XLIX, which shows a whole nucleus. Its thick end is here turned over and covers the mid-section. Fig. 9 shows it in full length under increased magnification. It is obvious that this thin chromosome section is nothing more than a free small chromosome of *S. longiventris,* which in *S. coracina* is attached to one of the long ones. Apart from remarkable thinness, this is seen from the similarity in length and particularly from the occurrence of the Balbiani ring also in *S. coracina.* Only here, which is most probable, it is subdivided, or similar structure of smaller size has perhaps been newly formed. For toward the middle of the chromosome a small, second one follows on the real Balbiani ring (Fig. 8, Plate XLIX). This difference indicates that transpositions have occurred inside the small chromosome, through which have arisen the structural differences of the two species. There are likewise to be assumed for the long chromosome whose structural sequence show only a few likenesses. Here again the differences can most simple be detected from the nucleolar pattern. While *S. longiventris,* as stated, has only 1 nucleolus on the shorter of the long chromosomes, with *S. coracina* the two uniformly thick ones have nucleoli; one of them has two, the other one only (Fig. 8, Plate XLIX). A conclusion regarding the form of the mitotic chromosomes can be also be drawn here from the appearance of heterochramatic sections. There are shows by arrows in Fig. 8. Their more or less median position indicates that all three chromosomes are V-shaped. For *S. longiventris,* the V-shape of the large chromosomes could be shown directly by spermatocyte divisions. The position of the heterochromatin section in the composite chromosome of *S. coracina* shows that the thick section, which in its length corresponds to the shortest large chromosome of *S. longiventris*, must be very substantially altered. Before or simultaneously with the attachment of small chromosome, the centromere must have been displaced near to one end.[3)]

([3)] If the fusion had taken place with one end of the unchanged large chromosome, then the

composite chromosome would not have been capable of persisting. In animals heterozygous for this chromosome, where there was a possibility of exchange of factors between the point of attachment of the small chromosome and the centromere, half the progeny would die (see. Bauer and Timofeef-Rassorsky, 1942). The new chromosome arrangement would thus very rapidly disappear in the population).

The chromosome pattern of *S. coracina* is thus revealed to be a derivative one, which naturally does not mean that the species has derived directly from *S. longiventris. S. longiventris* may naturally have differentiated itself substantially by gene mutations from the extinct or yet unknown phylogenus.

These two *Sergentia* species offer remarkable information in two directions. Firstly, cytologically:, the constant thinness of the small chromosome of *longiventris* could be based on a reduced number of longitudinal elements building up chromosome. The salivary gland are, we know, bundles of stretched out chromonemata (cf. Bauer, 1935, 1936). Due to a factor effective only in this chromosome the multiplication of these longitudinal elements by division could have been inhibited and lag behind the two other chromosomes.

Or alternatively a substance produced by the chromosomes could be quantitatively less in this chromosome, as a result of which the longitudinal elements present in the same numbers in the others were not so widely expanded, and hence would be thinner as a bundle. The fact that with *S. coracina* the section corresponding to the small chromosome is thinner, excludes the first interpretation. According to this, there would otherwise have to be assumed at the point of transition from the thick to the thin part, forked chromosomes, and these could not be established. If therefore the thinness is due to altered production of a substance, this is still most peculiar. In general it should be assumed that for such changes, one or a few hereditary factors in the chromosome are responsible. But, in the composite chromosome of *S. coracina*, these should not be limited in their effect precisely to the section of the original smaller chromosome, but should be either act also on the remainder of the chromosome or be limited or eliminated in effect by influences proceeding from this remainder. But since the boundary between the two parts is sharp, the cause of reduced production of substance in the thin section cannot be due to a few hereditary factors of this type, but must rather be based on a change over the whole length of that section of chromosome exhibiting this phenomenon. We have no idea as to what the nature of this change can be. There is a parallel example with an analogous regional variation of substance pattern only in *Drosophila virilis*, in which one strain occurs with a constantly paler stained small (6th) chromosome (Fujii, 1940). With this stain two sections which are joined (by exchange of factors) to other chromosomes retain these properties over the whole length. For *Sergentia*, to prove these conclusions fully, we would need to find inversions in the composite chromosome of *S. coracina*, in which their sections were interpolated between thick ones, but really clear examples which would have to be transcentric inversions, can hardly be counted on because of their rarity.

The second piece of information from study of these two species is an ecological nature, and in contrast to the assumption of Lenz, referred to earlier, shows that where there is a possibility of morphological separation between hitherto indistinguishable larvae, ecological differences may also appear.

In his work Norwegian mountain lakes, Lenz gave the following details on the

distribution of *Sergentia*. *S. longiventris* has hitherto been known only from 2 Norwegian lakes (Tjernosen and Nedre Sjodalsvand). S. *coracina* (*profundorum*) has been bred from one Holstein, one Mecklenburg and two Polish lakes. Larvae of undetermined species have been found in a number of Finnish, North German and Alpine lakes. Since then Prof. Thienemann (unpublished work) has shown *S. longiventris* by breeding test to be very widespread in the Mittersee near Lunz.

During a stay at the Lunz station this spring, which was to be devoted primarily to cytological study of *S. longiventris*, we looked for *Sergentia* also in the Untersee and found larvae there, in conformity with Lenz's statements, but in very small numbers. Cytological study off 11 specimens taken showed the remarkable fact that they were all the species *S. coracina*, whereas the numerous larvae from the Mittersee, as might have been expected from Thienemann's breeding test, were all found to be *S. longiventris.* Investigation of alcohol specimens from Prof. Thienemann's collection confirmed the occurrence of *S. coracina* in the Schrimmersee, and the material cytologically studied earlier proved their occurrence in the Schoolsee.

The finding that, in two connected and closely neighbouring lakes like the Mittersee and Untersee at Lunz, both *Sergentia* species are found strictly separated (according to determinations so far carried out and which need to be further checked) is most remarkable.

If it is also considered that *S. coracina* in the Untersee is a form found at depth, just as in the German lakes, which - as far as has been found to date - hardly ever rise above the 15 m. level, whereas *S. longiventris* both in the Norwegian lakes, particular Tjernosen, and in the Mittersee, develops in mass at a depth of one or few metres, then it is seen that the two species are adapted to very differing conditions of life. Thus *S. longiventris* appears to be a shallow water form, the wider distribution of which determined, apart from the usual ecological conditions of lakes, probably also substantially by the (low) temperature. Its distribution as hitherto know only in the high mountains ranges of Norway and in the Alps renders feasible the assumption that this species is a glacial relict. *S. coracina* on the other hand, being adapted to lake depths, show wide distribution even from the scanty details available from specimens which have been bred, and it may be expected that this is the species involved generally in the North German, Finnish and other water ways in plain country. A further remarkable difference between the two species is shown in their development in mass. *S. longiventris* appears to form predominantly groups with large number of individuals, while *S. coracina* has often few individuals and so occurs only as an accompanying form. Lenz felt some uncertainty when establishing the type of Sergentia - lake and tried to divide it into two sub-types (*Tanytarsus* - *Sergentia* lake and *Stictochironomus* - *Sergentia* lake). After what had been said earlier it is enlightening to note that *Sergentia* occurs in two different types of lakes and that only *S. longiventris* is the actual index form for lakes of Pjernosen and the Mittersee. These deserve the *Sergentia* (*longiventris*) lakes. Naturally, to confirm these conclusions, much more material will need to be available for study. These three examples probably suffice as evidence that one can revise Thienemann's sentence (as quoted on p. 996) and write :
"Chromosomes have gained very special importance for the study of Chironomids". To use successfully the process of investigation based thereon, it is, however, first of all necessary to carry out a systematic investigation of Chironomids, a task which beyond the power of any individual. The simple investigation technique, which no longer has anything in common with the despised microtome technique of classical cell investigation, makes this process capable of

being readily used by any hydrobiologist [4)].

([4)] The technique of carmine-acetic acid preparation has been described in detail by Bauer and Timofeef-Ressovsky (1939) for *Drosophila*. Here we give only a few further details important for Chironomidae. To determine the species according to chromosome count it is sufficient to examine fresh preparations stained with aceto-carmine. For this purpose the salivary glands obtained by decapitation on a concave ground glass slide are dropped in alcohol glacial acetic acid (absolute or 96%, 3 : 1). (This give better preservation of the nuclei and less staining of the cytoplasm. However, this can be eliminated and aceto-carmine used direct). Suck off after 3-5 minutes and add aceto-carmine. After 1/2-1 hour the glands are mounted between slide and cover glass and examined. If gland contains too much secretion, the cells can be flattened by squashing as needed. For species not previously investigated, permanent preparation are preferable, the production of which may seem to involve grater difficulty but does not in fact do so. Since the secretion does not permit uniform squashing and in addition because of its stickiness often adheres to the slide and cover glass so that the gland are torn, it is advisable to dissect out the cells. This can be done easily with two preparation needle, holding the gland still by the middles with one needle and with the other, introduced flat, pressing from inside against the individual cells which (in Chironomidae) are seated around the edge of the secretion-mass like the dividers in a filing cabinet. This preparation made in 45% acetic acid stained pale pink with carmine-acetic acid. The cells are then brought on to the albumen slide with a pipette. Further treatment as has bee described for *Drosophila*.)

However some limitation is appropriate here: the "quality" of the chromosomes is a function cell size. Since, in general, this again is a function of body size (this naturally holds only for those cells which do not further divide after embryonic development, among which belong the salivary gland as well as many larval tissues and organs), the largest chironomids, i.e., primarily the *Chironomaria*, with their salivary glands composed of a few large cells, are also best suited for study. The least suitable are the small *Orthocladiinae*. The *Tanypondinae* are also suitable for use only in the largest species (*Anatopynia* etc.) because of their small salivary glands, which is connected with their predatory way of life and absence of tubular structure. Since, however, in hydrobiology it is precisely the *Chironominae* which are used as the principal form to define lake types, the less suitability of the others subfamilies is of smaller importance.

When finally descriptions of the chromosome complement of a larger number of species is available, then not only will the advantage of the cytological method of determination be demonstrated but there will also be the opportunity (as was mentioned earlier) of drawing conclusions about systematic relationships.

But here too prior clarification of what we may expect is needed. Systematic relationship is an expression of the similarity in complement of hereditary factors, i.e., of certain allelomorphic features. Chromosome mutations, at least in so far as they are capable of remaining alive under natural conditions, represent, on the other hand, only alterations in arrangement, not in the composition of the gene complement. A differentiation between several species from an original one would be conceivable wholly without any participation by structural alterations of the chromosomes. But since, in addition to gene mutations, chromosomes mutations continually appear, they would superimpose on any difference due to gene mutation. They would do this even if they coincided accidentally with various gene mutations. But since they also play a part in the separation - or isolation - of gene

combinations, their participation in species differentiation must be greater than would be expected purely on statistical grounds. In general, therefore, the chromosome structure of two species will be all the more different, the further they are removed from each other. However, here it is a question of an average of difference, the extent of which may individually vary considerably. This connection must be closest for the most frequent chromosome mutations, the simple inversion. Rare chromosome mutations, pericentric inversion and reciprocal translocations of the whole are will only very infrequently characterise the higher groups (Phyla etc). Rather are they, purely statistically, more probable with in these groups. If they have one been present in an initial species, then they characterise the species derived from it with great certainty. According to experience provided by cytological and genetic comparison of the *Drosophila* species, in each case structural differences, even though of slight extent, have to be reckoned with between two species, even though they may by very closely related.

On the whole, therefore, chromosome investigation, in addition to the distinction of species, will offer certain possibilities of throwing light on systematic relations, naturally without in any way reducing the importance of systematics based on morphology, as an expression of the composition of hereditary factors.

Addition after second correction

Since this manuscript was sent in (summer 1942), the *Sergentia* material was increased by several visits to Lunz. The mitotic chromosomes are now known for both species: for *S. longiventris* the set consists of three V-shaped and one rod shaped chromosomes, while *S. coracina* has only three V-shaped chromosomes. (All chromosomes have uniform thickness in the metaphase). The number of larvae taken in the Untersee has been increased to 28, all *S. coracina*. One larva of *S. longiventris* was caught in the channel above the waterfall - other species of fauna from the Mittersee are already known from this spot.

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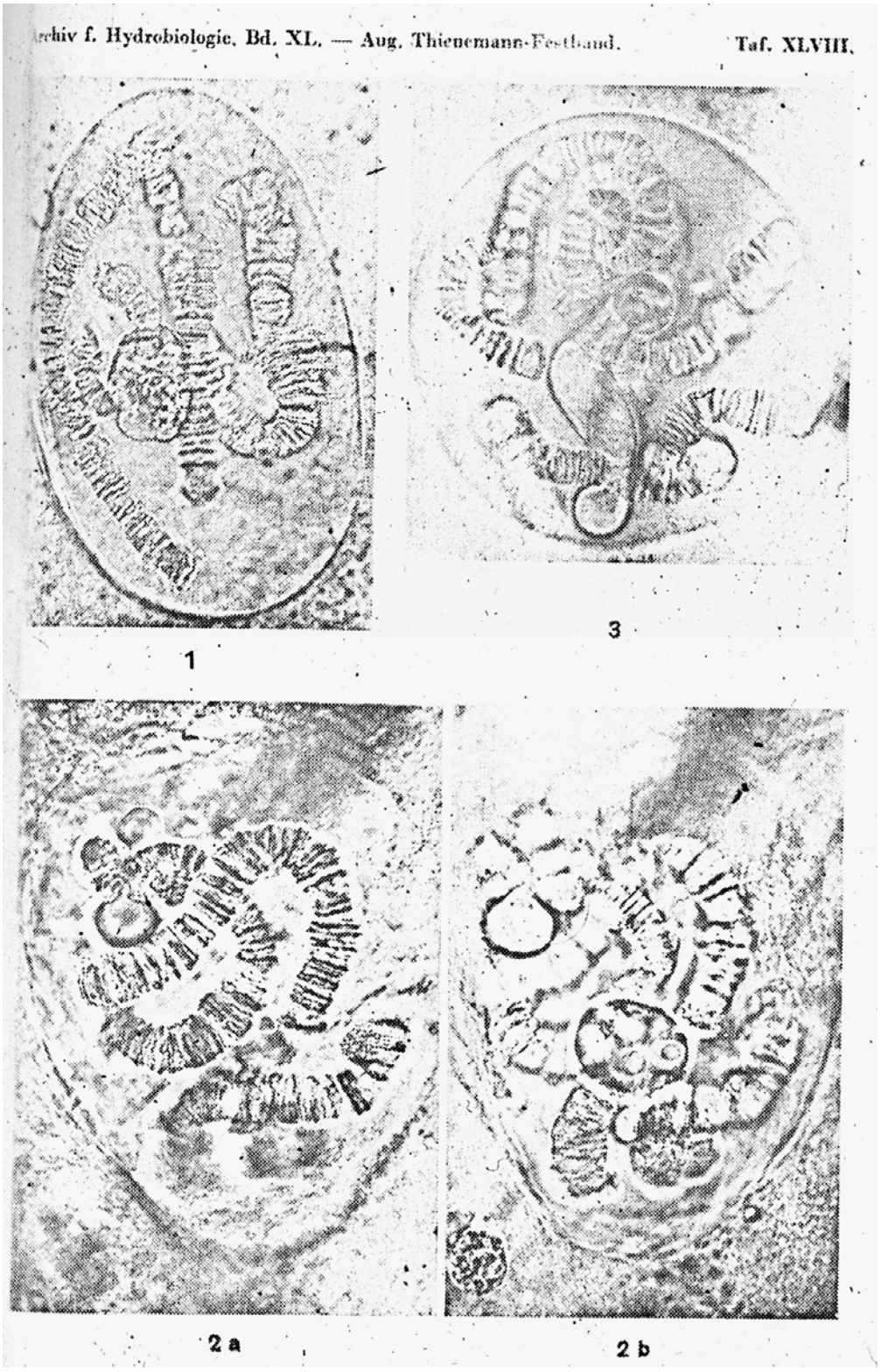
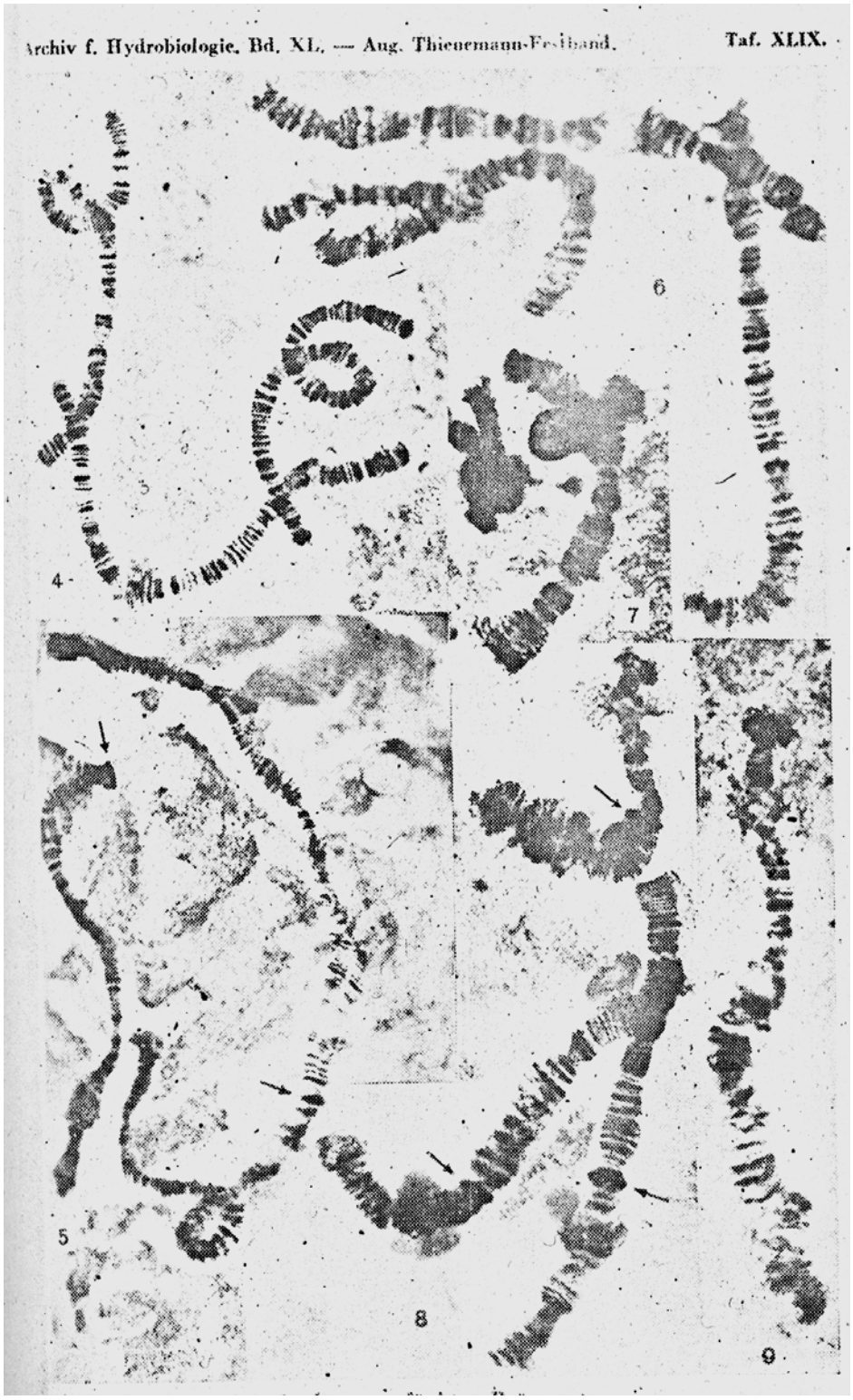
Tafel XLV111



Photographs of the living salivary gland nuclei

Fig. 1. *Tendipes plumosus.* Magn. 500x
Fig. 2. *Camptochironomus tentans*. Nucleus at two different focal levels. Magn. 500x.
Fig. 3. *Cryptochironomus defectus*-group, spec. 1. Magn. 500x
Fig. 1 and 3 are Leica photographs made by Herr Dr. F. Hustedt and it is appreciated his being available. All other photographs were made with the small microphotographic attachment of Zeiss.

Tafel XLV1X



Photographs of stained preparations

The arrows in Fig. 5 and 8 mark the sites of heterochromatic segments.

Fig. 4. *Cryptochironomus defectus*-group, species 1. Magn. 360x.

Fig. 5. Species 2. Magn. 180x.

Fig. 6. *Sergentia longiventris*. Entire chromosome complement. Magn. 540x.

Fig. 7. The same. A part of the nucleus, showing the nucleolus chromosome. Magn. 540x.

Fig. 8. *Sergentia profundorum* (?*coracina*) Chromosome complement. Magn. 540x

Fig. 9. The same. The chromosome created by fusion. Magn. 580x.