A CYTOTAXONOMIC STUDY OF THE MOST COMMON LARVAL CHIRONOMIDAE IN A SERIES OF SALINE WATERS IN THE SOUTHERN INTERIOR OF BRITISH COLUMBIA

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ABSTRACT

A preliminary cytotaxonomic study of the common Chironomidae in a series of saline waters in the southern interior of British Columbia has been undertaken. The banding pattern of the salivary gland chromosomes, once it had been described, was used as a taxonomic criterion and as an indicator of the relationships between the groups involved. In order to obtain associated stages in the life cycle, the larvae were reared in individual vials.

The polytene chromosome analysis revealed seven well defined larval species. The subsequent morphological analysis showed that five of these larval species could usually be separated by their external morphology. However, two cytologically distinct species are morphologically indistinguishable.

Recent work on sibling species in <u>Drosophila</u> and <u>Chironomus</u> (Diptera) has shown that sibling species have salivary gland chromosomes with an identical banding pattern but, differ from one another in the frequency of inversions. The present study suggests that the morphologically identical larvae mentioned above are sibling species in larval morphology but are clearly separable by chromosome analysis. The fact that they occur together in the same lake tends to eliminate the view that they are distinct populations of a single species. That there may be more than one species involved in those here considered to be a single taxa, should however not be forgotten.

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INTRODUCTION

Chironomid larvae, like all Diptera, have striking polytene chromosomes which are enormously enlarged and have a banding pattern which has been shown (Beermann, 1956) to be constant within different larval tissues. In addition, the banding patterns of larvae from a single interbreeding population are often found to differ from one individual to another. These differences may consist of translocations, and perhaps duplications, but are most often inversions. An inversion is a chromosome aberration in which a segment exists in reverse relationship to the rest of its chromosome. It results when the chromosome arm breaks twice and the chromosome piece becomes inverted, end to end, before the breakage points rejoin. An inversion does not normally arise as the result of chromosome breaks in the somatic tissue (eg. salivary gland) but instead arises usually in germ cells (Wallace, 1966). Such inversions are assumed to be of great evolutionary significance since they cause the fixation of various gene combinations permanently. The polytene chromosomes allow one to easily recognize these features and to assess with extreme accuracy, differences between populations and species.

The banding pattern of the giant chromosomes, once it has been described, may be used as a taxonomic criterion and at the same time, in favourable cases, as an indication of the relationships between groups, either species, races or populations. Work in this latter category has been done using <u>Drosophila</u> (see Dobzhansky, 1951; Patterson and Stone, 1940), but very little has been reported outside this taxon, and even in this genus, while different wild populations are often found to differ, in most cases the selective advantage of various genotypes in their habitats is not understood since nothing is known on the larval biology.

The Holarctic species of Chironomid, <u>Chironomus tentans</u> Fabricius, has proved to be an extremely interesting species for population studies in the wild. Acton (1959) has demonstrated that the banding patterns in European populations differ from those in North America, yet interbreeding between flies from the two regions is possible. Similarly, he has shown great variation between populations of <u>C. tentans</u> in Great Britain (Acton, 1956). This however, is the only species on which extensive data is available.

It is desirable to extend our knowledge of the forces which act on populations in order to understand the process of evolution in detail. The Chironomidae appear to be ideal material for these studies since the larval biology can be accurately studied and their habitats are frequently quite distinct and easily defined. Further, British Columbia seems a particularly favourable area which to undertake a study of this sort, since it offers a wide range of aquatic habitats (Northcote and Larkin, 1963).

Recent studies on the inland waters of Southern British Columbia (Scudder, unpub.) show a considerable range of salinity and have revealed that a great part of the benthic fauna, in many, is made up of larval Chironomidae. In some lakes, the population density of such larvae may attain a figure of 5000 per square meter: they contribute the bulk of the biomass within most lakes.

The majority of the chironomid larvae of these lakes are red ones, but to date the species have not been identified. Complete and accurate identification is a prerequisite for any ecological study and certainly for one which is concerned with the study of closely related species and populations within these.

The identification of chironòmid larvae in general is rather difficult and this is true for the British Columbia fauna. The best known keys to the North American Chironomidae are those of Townes (1945) and Johannsen and Townes (1952), but these are both concerned with the adults. Besides the fact that parts of these are also now out of date due to the work of Sublette (1960;1964a;1964b) ; they are purely descriptive and do not contain data on the larvae. The only keys available to the larvae are by Johannsen (1937a;1937b) and Roback (1957) which deal mainly with material collected east of the Rocky Mountains. As a result, many British Columbia species are not included in these works (Hamilton, 1965).

The European literature on larval classification is more complete and several keys have been written (Lipina, 1928; Thienemann, 1924;1929; Chernovskii, 1949; Bryce, 1960) using morphological features, in particular the head capsule. In 1956, it was shown by Acton that cytological features can be used in conjunction with important external characters to accurately identify certain larvae of <u>Chironomus</u>. Since then, a comprehensive cytotaxonomic key to the European species of Chironomus has been compiled by Keyl (1959), using methods first suggested by Bauer (1945). The banding patterns of the polytene chromosomes of the salivary glands are inherited directly and are not affected by environment, endogenous activity, or the age of the larvae. They are therefore, a particularly reliable and useful character for the identification of the larvae.

The present study is thus concerned with the accurate identification of the most common red larval Chironomidae in the series of interior saline waters mentioned earlier. Due to their reliability in delimiting species, particular attention was given to the compilation of maps of the larvae salivary gland chromosomes. The species once recognized by these chromosomes, then can be separated by coincident morphological features. In this way, a key to the larvae has been compiled. Such a key, permitting a reliable and rapid separation of the red Chironomidae larvae, will make possible further detailed study on the fauna of selected lakes, which in time will unravel some of the factors governing the distribution and evolution of these taxa.

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MATERIALS AND METHODS

Larvae were collected , at various intervals extending over a period of 12 months, from a series of saline lakes and water bodies in the southern interior of British Columbia (Table 2). These larvae were separated from the bottom mud with a fine mesh net and transferred to large thermos jugs of lake water. In the laboratory a random sample was taken for squashes and the others were reared at 20[°] C. in large plastic trays. The larvae were fed on powdered nettle leaves.

In order to obtain associated stages in the life cycle, late fourth instars with enlarged prothoracic segments were transferred to individual large shell vials containing about 3/4 inch of lake water. Most adults emerged within two weeks when this technique was employed. The males were killed with ethyl acetate vapour a few hours after emergence and pinned or preserved with their larval and pupal exuviae in 70 % ethyl alcohol. The preserved material was made into slide mounts following the method suggested by Schlee (1966).

To prepare the polytene chromosomes, the salivary glands were dissected out on a slide and hydrolyzed in 1 N HCl at room temperature. They were stained in warm 1 % synthetic aceto-orcein for 7 to 10 minutes. After squashing, the dry ice technique (Conger and Fairchild, 1963) was employed to make permanent preparations, with Euparal as the mounting medium. To identify the nucleolar regions either acridine orange (MacInnis and Uretz, 1966) or Feulgen-Fast Green (Semmens and Bhaduri, 1941) stain was used. The latter was the best method,

especially when the glands were pre-fixed in formol-acetic-alcohol (see Basrur, 1957).

A large number of squashes were made of each species (Table 2). The reared adults were always preserved with their larval and pupal exuviae. Likewise, the larval head and abdominal segments were kept in numbered vials after the salivary glands were removed for staining. In this manner, an implicit association between larvae and adults of the same species was always assured.

A series of idiograms was prepared to show the standard chromosome complement of each species. The relative lengths of the chromosomes and the distances between banding landmarks are shown in percentage total complement length (% T.C.L.). The % T.C.L. was averaged from measurements of camera lucida sketches of 2 nuclear complements from 3 different larvae. The chromosomes are numbered I to IV or I to III in decreasing order of length. The other morphological aspects of the salivary gland chromosomes are shown in composite photographs.

When possible, the larvae were sexed and the head capsule and posterior abdominal segments preserved in 3:1 alcohol/acetic acid fixative. To observe the gonads, the contents of the eight posterior segments were squeezed out onto the slide by firm pressure along the integument. The larvae were classified as males if the gonads were small, spheroid bodies with a smooth non-granular appearance. In females, the gonads were large sausage-shaped bodies with a granular texture. In the early instars, the reproductive organs are essentially similiar in the male and female and no separation was possible.

The external morphology of the larval head capsules and posterior abdominal segments was recorded by photomicrography and camera lucida drawings. The preserved adults were sent to Dr. D.R. Oliver¹ and Dr. J.E. Sublette² for final identification.

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RESULTS

A. Cytology

a. General Features of the Polytene Chromosomes

For the cytological analysis, the standard arrangement of each chromosome was determined and a series of composite photographs were made (Plates 1 to 7). In addition, a series of camera lucida maps was prepared to show the distinguishing features and prominant landmarks in their respective places on each chromosome (Figs. 1 to7). Some regions (eg. the chromosome tips), were more completely mapped than others. The positions of landmarks and prominant banding sequences are shown by numbered arrows. The approximate distances between these arrows and the comparative lengths of chromosome arms were measured from camera lucida drawings and converted into percentage T.C.L. (see materials and methods). For example, on chromosome I of Species II, there is a "centromere" at arrow 4 (Sp. II-I-4). This point is 1.9 T.C.L. units from the nucleolus and 16.8 (5.6+9.3+1.9) units from the left tip. Finally, a cytological summary of each species is given in the form of an idiogram (Fig. 8), along with a gross chromosome description. Table 1 explains the conventions and abbreviations used in constructing the maps and idiograms.

The chromosomes of each species are orientated in the equatorial plane with the short arm facing left. This arrangement was arbitrary as gonial mitosis was not studied. Chromosomes are also arranged according to their length, the longest being at the top. No attempt has been made to describe inversions or changes from the standard form.

A number of specific terms have been used in this section to describe certain features of the chromosomes. Certain loci on chromosome IV always appear puffed or greatly expanded into a large loop around the chromosome (ie. Sp.II-IV-2; Sp.VII-IV-2). These constant large puffs are called Balbiani rings (see Beermann and Clever, 1964). After staining with aceto-orcein, chromosomes I to III frequently have large clear protrusions which are constant for the species (ie. Sp.II-I-3; Sp.II-III-6; Sp.III-III-3). These areas stain green with Feulgen-Fast Green and fluoresce red after staining with Acridine-Orange. On the basis of these results, these loci are called the nucleolar organizers. Species II, III, V, and VI have large heterochromatic swellings at specific sites along their chromosome arms. In Sp. V these regions remain attached even after squashing. Assuming these areas may be analgous to the chromocenters of Drosophila polytene chromosomes, they will be called "centromeres". Bauer (1936) has called these regions heterochromomeres. Gonial mitosis has revealed that if the centromeres are not within these heterochromatic swellings, they must be very close to them. These terms and assumptions will be expanded upon in the discussion which follows this section.

Three additional points need to be mentioned. First, all the chromosomes are shown in the homozygous condition, such that the homologues are intimately paired. In some species, small inversion

loops were observed. The significance of these inversions is explained in the discussion. Second, no gross differences between male and female banding patterns were observed. It is not suggested that they do not occur but rather that their detection would be extremely difficult and of lattle practical value in a study of this type. Third, a large number of figures have been prepared to accompany the relatively brief text. It was felt that a simple diagram could best describe some of the complex physical ärrangements of the chromosome parts. Some references are referred to many times throughout the text; consequently, all figures and plates have been gathered together for convenience in a single section at the end of the thesis.

b. Description of Standard Chromosome Complements

Species I

The general aspect of the four standard chromosomes of Sp. I is shown in Plate 1. Chromosomes I and II each have a prominent nucleolar organizer near the center. Separation of these two chromosomes is best achieved by comparison of the banding sequences near these nucleoli (Sp.I-I-3; Sp.I-II-3) and the tips.

Chromosome III is shorter, but like I and II appears to be metacentric. The nucleolus of chromosome III is not obvious but the banding sequence at III-2 on the left arm serves as a good reference.

Chromosome IV has three large Balbiani rings. It is also

recognized by its short relative length, and the presence of two deep heterochromatic patches directly to the left of points IV-3 and IV-4.

The chromosomes are free of one another; there is no conclusive morphological evidence on the position of the centromeres. The gross banding pattern is shown in figure 1.

Species II

The general aspect of the four standard chromosomes is shown in Plate 2. Two pictures of chromosome III were needed to show the complete banding pattern of both arms.

The three long metacentric chromosomes have a large heterochromatic swelling near their centers. These sites are generally referred to as the "centromeres" (see Basrur, 1957), or heterochromomeres (see Bauer, 1936).

Chromosomes I and II have a nucleolar region near their "centromeres", while chromosome III carries the nucleolus near the right end. An adequate separation of chromosomes I and II can be achieved by following the major banding sequence in relation to the "centromere" and nucleolus. Chromosome III can be identified by the position of the nucleolus and from the banding landmarks.

The short acrocentric chromosome IV is easily recognized by its short length. It has a darkly staining "centromere" on the left tip and a Balbiani ring and nucleolus on the right side (Fig. 2). Two heterochromatic bands are also characteristic of this chromosome (Plate 2-IV-1-2).

Some intact nuclei suggest weak ectopic pairing occurs between

the four "centromeres" but this is usually lost when the nucleus ruptures. The other important banding sequences are best learned from the chromosome map (Fig. 2).

Species III

Species III has only three chromosomes (Plate 3). This aspect alone allows rapid separation from the other species studied (Fig. 8). All three chromosomes are metacentric and have a small heterochromatic "centromere" near their center.

Chromosome I can always be recognized by the three pairs of heterochromatic bands near the free end of the left arm. The middle pair of these bands is shown at point I-2 on plate 3.

The position of the nucleolar organizer allows for a good separation of chromosomes II and III. The standard map (Fig. 3) shows other points which can be used for identification.

Generally, the salivary gland chromosomes of this species appear much larger, both in length and width, than the other species studied. The chromosomes are always free of one another. Speculation on the incorporation of chromosome IV is impeded by the scarcity of puffing in the chromosomes. If chromosome IV was incorporated into the other chromosomes, one should be able to observe the large Balbiani rings which are so characteristic of this chromosome. However, the broken pairing between regions III-4 and III-5 suggests parts of chromosome IV may be within this segment. Species IV

The gross features of the four chromosomes of this species are shown in Plate 4. The standard banding sequence between regions III-3 and III-4 is partially covered by an inversion.

Chromosome I has a nucleolus near its center. The right side of this nucleolus always has a repeating 1-2-2-1 banding sequence (Fig. 4-I-4). The nucleolus is located near the left end on chromosomeII. Three heterochromatic bands separate the nucleolus from the tip region (Fig. 4-II-2). The nucleolar region on chromosome III is bocated near the base of the left arm. It is relatively easy to locate the left arm of this chromosome because it always has " 3 heavy groups" adjacent to the tip. Each heavy group is inturn composed of 3 dark heterochromatic bands (Plate 4-III-1-2).

Chromosome IV is considerably shorter than the others, and has two prominent Balbiani rings. The right end of this chromosome appears to have a nucleolar swelling but this may be an artifact due to deterioration of the larvae before squashing.

The chromosomes are always free of one another. The position of the "centromeres" could not be determined.

Species V

The salient features of the polytene chromosomes of Sp. V are shown in Plate 5. Apart from the relative lengths of the chromosome arms and the presence of heterochromatic "centromeres", the most striking feature is the extensive ectopic pairing between the "centromeres". This association is not broken by squashing.

There is no distinct expanded nucleolar region in chromosome I. There are, however, two distinct banding areas on the long right arm which allow one to orientate the chromosome (Plate 5-I-3). The nucleolus is on the right arm of chromosome II, near the "centromere". Chromosome III has the nucleolus near the tip of the short arm. In many slides, a "gap" area appears just to the right of the "centromere". These features and the obvious banding sequence allow rapid identification of these metacentric chromosomes.

Chromosome IV begins with an extensive heterochromatic "centromere" (Fig. 5-IV-1). The single prominent Balbiani ring is always followed by a deep heterochromatic patch. The distinct subterminal swelling at the right end of this chromosome suggests puffing also occurs in this region.

To emphasize the ectopic binding which exists between these chromosomes, the "centromeres" are shown attached in the idiogram (Fig. 8).

Species VI

The general aspect of the four standard chromosomes of this species is shown in Plate 6. All four chromosomes have conspicuous heterochromatic "centromeres" but only chromosome II has a definite nucleolar region.

Chromosome I has a distinctive heterochromatic band in the flared end of its short arm. The banding sequence near the right tip also serves as a reference. The right end of chromosome II has a thin dark band followed by two dark reverse "C" shaped bands (Fig. 6-II-5). A shorter left arm and an easily recognized banding sequence near the "centromere" are characteristic of chromosome III. Standard chromosome IV is short and acrocentric, with a large heterochromatic "centromere" on the left tip. The first Balbiani ring (IV-3) is always associated with a wide heterochromatic band. The other Balbiani ring is typically located near the right tip.

The three long chromosomes are metacentric. The banding landmarks are much more distinctive on the longest chromosomes I and II. There is not evidence of ectopic pairing between the "centromeres".

Species VII

The large salivary glands of Sp. VII gave excellent squashes with good separation of the chromosomes. The morphology of chromosomes I to IV is shown in Plate 7. Two pictures of chromosome IV are provided to demonstrate the full banding sequence after the right Balbiani ring (IV-4). The "centromeres" are not heterochromatic and could not be located.

Chromosomes I and II have a distinctive nucleolar region near their centers. Chromosome II is distinguished from the former by a banding arrangement on the right tip (Fig. 7-II-6). The landmarks on chromosome III are best located with the help of the map.

Chromosome IV has three Balbiani rings. The two closely associated rings on the left arm are separated by a simple double band. The relatively bong piece between region IV-2 and IV-4 has a specific pattern and is terminated by a large Balbiani ring and heterochromatic patch (Fig. 7-IV).

The large discrete chromosomes which characterize this species, never show morphological evidence of ectopic pairing.

c. Chromosome Key to the Fourth Instar Larvae of Chironomidae in the Southern Interior of British Columbia

1. Salivary gland nuclei with 3 polytene chromosomes (Fig. 8-3; Fig. 3; Plate 3).....Species III Salivary gland nuclei with 4 polytene chromosomes (Fig. 8-1 to 7; Plates 1,2,4,5,6,7).....2 2. Chromosomes with dark heterochromatic "centromeres" Chromosomes without dark heterochromatic "centromeres"; (Fig. 8-1,4,7).....5 3. Chromosome I with distinct nucleolar organizer very close to "centromere" (Fig. 8-2); chromosome III with distinct nucleolar organizer close to the right end (Fig. 2-III; Plate 2).....Species II Chromosome I without distinct nucleolar organizer close to "centromere" (Fig. 8-5,6).....4 4. Chromosomes always attached by their heterochromatic "centromeres" (Fig. 8-5); with nucleolar organizer on left arm of chromosome III(Fig. 5-III).....Species V Chromosomes never attached by their heterochromatic "centromeres" (Fig. 8-6); without nucleolar organizer on left arm of chromosome III (Fig. 6).....Species VI 5. Chromosome III with distinct nucleolar organizer near its center (Fig. 8-4); with distinct nucleolar organizer near end of chromosome II(Fig. 4-II)......Species IV Chromosome III without distinct nucleolar organizer near its center (Fig. 8-1,7)......6 6. Chromosome II with 3 distinct dark bands at the right tip and with a dark pair of bands to the right of the nucleolar organizer(Fig. 7); chromosome IV without overlapping Balbiani on left arm (Fig. 7); left arms of chromosome I and II with distinct pattern(Plate 7)....Species VII

Chromosome II without 3 distinct dark bands at the right tip and without a dark pair of bands to the right of the nucleolar organizer(Fig. 1); chromosome IV withooverlapping Balbiani on left arm (Fig. 1); left arms of chromosomes I and II with different banding patterns then above (Plate 1).....Species I

B. External Morphology

a. General Features of the Larvae

The following account is intended only to supplement the cytological section. When full grown, the larvae of the species examined were from 10 to 25 mm. long, cylindrical and blood red in colour. The prognathous head capsule was ovoid in transverse section in all the larvae except Sp. III. In this species the mid-posterior caudal margin of the head was wide and gave the head capsule a "pear-shaped" appearance (Fig. 9c). The pigmentation on the head capsule was generally constant within each species studied. More will be said on this feature in a later section.

The two eyespots were always separated from one another and never reniform. In Sp. II and VII, the ventral eyespots were weakly bilobed (Fig. 11b,g).

Striated paralabial plates were a constant feature; only the shape and the degree of overlap over the labial plate changed from species to species. The labial plate was pigmented and had numerous teeth along its anterior border. The shape, size and number of teeth vary between the species. The lateral teeth and accessory tooth of the mandible are also different in each species (Fig. 11 a to g).

The antennae have a large, thickened basal segment, an upper segmented flagellum and a blade (Fig. 11-ii). The basal segment has a ring organ (companiform sensillum) at its proximal

third. Only species III has a distinct Lauterborn organ on the upper segments (Fig. 11c-ii). The antennal ratio of each species is found in Table 3.

The distinguishing aspects of the anal segments are shown in Figure 10. Species II has one pair of short abdominal gills extending down from the junction of segments 10 and 11. Species IV, V and VII have two pairs of long abdominal gills on the 10 th. segment and one pair of short lateral appendages extending along from the border of the 9 th. segment. All species have 4 anal gills and a pair of pseudopods (posterior prolegs) on the last segment. The shape of the anal gills varies very slightly between species. Species I has a pair of anal papillae, each with a brush of 7 setae. The other groups have a single papilla with 6 to 8 long setae. A general separation of all the prominant features is given in Table 3.

b. Head Capsule Pigmentation

The distribution of pigment on the upper and lower surfaces of the head capsule was different in each species studied. The pigment appears concentrated into definite patches, in between which the head capsule is either colourless or significantly paler in colour than the pigmented areas. A complete separation of each species is best achieved by direct comparison with the drawings in Figure 9 (a to g) and the morphological key at the end of this section. The drawings were made from larvae with swollen prothoracic

segments and must be considered as the fourth instar stage.

In most species the patches were regular and only the density of the pigment changed within the group. This change is particularly striking on the ventral aspect of the parietal plates in Sp. IV and Sp. V (Fig. 9d, e). Species VI has a characteristic dorsal "fenestra" on the anterior face of the fronto-clypeus. In Sp. III, the ventral suture of the parietals is not evident.

c. Morphological Key to the Fourth Instar Larvae of Chironomidae in the Southern Interior of British Columbia

- 1. Larvae without abdominal gills (Fig. 10a,c,f).....2 Larvae with abdominal gills (Fig. 10b,d,e,g).....4
- 2. Distinct Lauterborn organ present on antennae (Fig. 11c); body of 13 segments; ventral aspect of head (parietals) without median longitudinal suture (Fig. 9c); head capsule "pear-shaped" (Fig.9c).....Species III

3. Head capsule with dorsal fenestra (Fig. 9f); with single anal papilla (Fig. 10f); mandible with 4 dark coloured teeth (Fig. 11f); labium with one mediolateral and 10 lateral teeth (Fig. 11f).....Species VI

Head capsule without a dorsal fenestra (Fig. 9a); with two anal papillae (Fig. 10a); mandible with 3 pale coloured teeth (Fig. 11a); labium with 2 median and 12 lateral teeth (Fig. 11a).....Species I

4. Larvae with one pair of abdominal gills (Fig.10b).....<u>Species_II</u> Larvae with two pairs of abdominal gills (Fig.10d)....5

5.	Head capsule with a black wedge-shaped mark mid- dorsally (Fig. 9g); antennal ratio 13:14:7:7:3	
	(Fig. 11g) <u>Species</u>	VII
	Head capsule without black wedge-shaped mark mid- dorsally Fig. 9d,e); Antennal ratio 30:11:4:3	
	(Fig. 11 d,e)Species	IV
	Species	V

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DISCUSSION

In many taxonomic problems, particularly those concerned with the evolutionary trends within small groups (ie. species-groups or genera), it is often difficult, using conventional methods, to make decisions as to which are the best characters to utilize. Presumably it is the best procedure to select a character which changes very little, and is constant for a species. It is argued that the polytene chromosomes are a valid and reliable criterion by which to judge speciation and evolutionary relationships.

The composite photographs which accompany this study show the chromosomes in the predominant homozygous condition. Although each chromosome appears to be a single structure, it is actually two chromosomes tightly paired throughout their entire length. Bridges (1935) was able to represent the paired strands separately for the chromosomes of D. melanogaster. Since the chromosome bands appear as a single structure, it can be assumed that pairing results from a band-to-band attraction. It would follow therefore, that if the chromosome banding patterns of the parents are identical, the offspring should have chromosomes that are intimately paired throughout their lengths. Each band on the paternal chromosome would pair exactly with the homologous band on the maternal chromosome. The next step would be to associate this fundamental chromosome banding pattern with a given larval form and then with a given adult. This has been done in this thesis on larval identification.

It is perhaps appropriate at this point to return to a basic difficulty which exists in relating life stages. The removal of the salivary glands always terminates the life of the larva. Since the adults do not possess polytene chromosomes, it is impossible to have a giant chromosome slide that corresponds directly with a given adult. The association between larva and adult can only be inferred.

In this present study, the possibility of error in relating life stages is almost negligible in view of the large number of comparisons made and the method of rearing the adults. Each adult was reared separately in a large shell vial. The larval exuviae from all the individually reared males were always compared to corresponding larvae that were preserved after salivary gland removal. A further precaution was exercised in assigning the cytological species numbers (I to VII) rather than generic names.

Throughout this report, the "fundamental Pattern" is interpreted as that banding arrangement which is most common within the species: it was determined in relation to the total number of slides that were analyzed. Since chromosome rearrangements (eg. inversions) always arise from pre-existing patterns, variations can always be related back to the fundamental arrangement.

Perhaps it is relevant to mention here a specific example from the present study. In 3 of the slides of Sp. VII, an inversion loop was observed on the left end of chromosome I. The uninverted region in this loop was easily identified and corresponded to a

homologous region in the fundamental pattern (VII-I-2). Since this arrangement occurred in only a small number of larvae (3 in 99), the alternate pattern is regarded as fundamental.

The present study has shown that the red Chironomidae in the lakes mentioned previously is composed of seven cytologically distinct species. The idiograms (Fig. 8) are graphic representations of the gross features of the polytene chromosome complements of the seven species. There is little doubt that five of these seven larval species are distinct species as they differ in the ordinary taxonomic criteria as well as their cytological features. For example, one species (Sp. III) has only three chromosomes while other species have four. The latter are also distinguished by large heterochromatic "centromeres" on the chromosomes (eg. Sp. II and Sp. VI). Thus, distinct chromosome types are easily recognized by their morphology, the three chromosome type for example having a distinct Lauterborn organ on the antenna and 13 segments on the body (see Table 3). However, two cytologically distinct species (Sp. IV and Sp. V), cannot be separated as yet by their larval morphology. Except for small variations in head capsule pigmentation, these species are morphologically indistinguishable as larvae: the adults may or may not prove morphologically distinct.

The traditional approach to the systematics of the chironomids, the approach used by Meigen (1803), Thienemann (1929), Townes (1945), and others, is based solely on the external morphology of the adult form. This follows from the general finding that adults show a greater degree of differentiation than the larvae, and consequently

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their characters are likely to remain more important for species determination. As a result in many families the knowledge of the larval form is still very limited (Gordon, 1955).

Hamilton (1966) recently completed a survey of the Chironomidae in Marion Lake, British Columbia. In this study, 31 of 51 identified species were "definitely" or "almost definitely" associated with their larval forms. The larval key presented with Hamilton's work is based on the examination of only two or three larvae from each species. Larvae from populations outside the lake were not compared. It should have been apparent that identification on the basis of only a few isolated samples sometimes can lead to erroneous criteria for a species. The limitations which must be imposed on such a key could nullify its use on other populations and other species.

Two species in the present study serve to emphasize this point. Species IV and V cannot be separated by larval external morphology (see Key I, p. 19). A taxonomist working only on these larvae would therefore call them one species. However, cytological examination reveals quite readily that we are dealing with two distinct species (see Fig. 8). Perhaps if several hundred of these larvae were carefully observed, a morphological difference could be found. The chance of finding reliable characters by examination of only a few larvae would be very small.

The pigmentation of the head capsule has received little attention in the larval keys mentioned above. Pigmentation appears to develope first in places to which muscles are attached and later

in areas of mechanical stress (Chernovskii, 1949). Acton (1956) has indicated that pigmentation of the head capsule may be of value in separating at least some species of <u>Chironomus</u>.

In the seven species studied, the appearance of pigment on the head is constant within each species. Moreover, a general separation can be accomplished using this feature with other accessory criteria. The one possible exception is between Sp. IV and Sp. V which have almost identical head capsule pigmentation (see Fig. 9d,e).

Most of the other morphological characters referred to in this work are in general use as taxonomic indicators in larval keys. Bryce (1960), for example, makes extensive use of the features of the antennae and hypostomal (labial) plate. Hamilton (1965) separates the subfamily <u>Tanypodinae</u> by the retractability of the antennae and the eyespot separation. Johannsen (1952) also uses the retractability of the antennae, but Chernovskii (1949) uses neither of these features for the initial separation of the European Tanypodinae .

For the sake of simplicity in separating the broad groups, only the gross features of the head and posterior body segments are shown. It was felt that these features can be used in the field to readily separate the larvae into appropriate groups. In a thorough morphological survey, these criteria could probably be supplemented by a number of less obvious but also reliable characters.

In the present investigation, an attempt has been made to employ techniques of which the above criticism cannot be made. The polytene chromosomes are a basic character and give an accurate

identification of each larval species. These enormously enlarged chromosomes have an intricate series of cross-striations along their lengths. Dobzhansky (1951) suggest that these striations are composed of "stainable discs" which may or may not correspond each to a single gene. The constant pattern that they form may reflect the gene arrangement in the chromosomes. Because of the precision with which these chromosomes are replicated, it is possible to construct a "chromosome map" using the bands as referencespoints. Painter (1934) and Bridges (1935) were among the first workers to publish maps of the salivary gland chromosomes of Drosophila. Since that time many detailed linkage maps have been prepared to show the sequence and locations of the genes for a number of mutant and normal characters in Drosophila (ef. Bridges et al, 1936; Dobzhansky and Sturtevant, 1938; Wharton, 1942). The salivary gland chromosome banding patterns of many other Diptera have also been determined. For example, Basrur (1959,1962) and Dunbar (1959) have done extensive work on the Simuliidae, and Bates (1940) has worked with the Culicidae. A great store of information has also been amassed for the Chironomidae. (eg. Beermann, 1952; Rothfels and Fairlie, 1957; Keyl and Keyl, 1959). Thus it can be said with some confidence, that the salivary gland chromosomes are an important tool for species analysis.

The precise replication of chromosomal material is further demonstrated by comparing the giant chromosomes of two different larval tissues. Pavan and Breuer (1952) showed that the banding pattern of salivary chromosomes and malphigian tubule chromosomes are nearly identical in the fungus fly (<u>Rhynchosciara</u>). The 26

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differences that occur in the chromosomes of these two tissues concern the frequency and position of the "puffs". These puffs or expanded regions have recently been evaluated by Beermann and Clever (1964). The puffed regions appear to be actively engaged in RNA production, and are controlled either directly or indirectly by the internal hormone balance. At any one time, different tissues have different puffs, but cells of one tissue will have only one particular pattern.

It may be well to consider at this point another application of chromosome banding patterns as a criterion for a species, especially groups of sibling species. Such sibling species occur in <u>Chironomus</u> (eg. <u>C. tentans</u> and <u>C. pallidivittatus</u>) and in <u>Drosophila</u> (eg. <u>D. pseudoobscura</u> and <u>D. persimilis; D. melanogaster</u> and <u>D. <u>simulans</u>). These sibling species have salivary gland chromosomes with an identical banding pattern but differ from one another in the frequency of their inversions. It could be argued that these small chromosome changes are not significant enough to necessitate the separation of these populations into two species. However, breeding experiments can provide evidence for such a separation.</u>

Beermann (1953) found that it was possible to mate <u>C. tentans</u> and <u>C. pallidivittatus</u> in the laboratory but, the viability of the eggs was greatly reduced. Later, Acton (1956) found this greatly reduced interfertility was characteristic of natural populations and only a few viable hybrids occur when these two species are found in the same pond.

Similarly in <u>Drosophila</u>, Lancefield (1929) has found that crosses between the sibling species <u>D. pseudoobscura</u> and <u>D. persimilis</u> give F_1 hybrids that are sterile as males, but fertile as females. The sterility is due to a profound modification of the process of spermatogenesis such that the spermatids degenerate. Dobzhansky and Sturtevant (1938) demonstrated that natural populations of the above species are mixtures of individuals with different gene arrangements in their chromosomes. In chromosome III for example, sixteen different arrangements are found in natural populations. All the arrangements appear to be related by overlapping inversions. The complete collection of arrangements, however, is never found in any one natural population of each species.

One might ask whether two species are indeed "good" species if a viable hybrid can be formed between them. The simplest and most consistant explanation for hybrids is common descent. Wallace (1966) suggests that the amount of chromosomal pairing in a hybrid fly is roughly correlated with the similarity of the parental species. The above problem is best approached by using the Hardy-Weinberg Law (see Li, 1955). If the flies mate at random with respect to chromosome types, the frequencies of the banding patterns or gene frequencies will in the absence of strong selection, remain constant from generation to generation. Suppose for example, an inversion heterozygote (Aa) occurs in only 1% of the population and the corresponding homozygotes occur regularly with frequencies AA/50 : Aa/1 : aa/49. If these frequencies are maintained in succeeding generations we can assume the homozygotes are interbreeding freely (separate species) and the heterozygotes can only be maintained by chance matings or rare back-crosses. On the other

hand, if the frequencies had been AA/25 : Aa/50 : aa/25 , the heterozygotes would be difficult to explain on the assumption that AA and aa belonged to different species. This latter example is obviously and example of inversion combinations within a single interbreeding group, whose frequencies agree with the Hardy-Weinberg Law. Nevertheless, it is still difficult to predict at what point two closely related populations will actually become true species, but by applying the Hardy-Weinberg Law to the frequencies of the banding patterns, it is possible to objectively decide the significance of hybrid formation between two closely related species.

The present study suggests that the species here called Sp. IV and Sp. V are sibling species in larval morphology, but are clearly separable on chromosome idiograms. The sibling species studied to date by previous authors (see Lancefield, 1929; Bates, 1940; Rizki, 1951; Dobzhansky, 1951) show virtually identical banding patterns in the polytene chromosomes. And further, the studies on these genera show that one species does not have two fundamentally different chromosome banding patterns.

Hence, these Chironomids must represent two distinct species or two distinct populations of a single species. The fact that they occur together in the same lake tends to eliminate the second choice and so we must assume them to be two separate taxa. Of course, only a program of breeding can unquestionably decide their status.

The fact that the larvae are morphologically indistinguishable may merely represent a stage of evolutionary conservationism.

Dobzhansky (1951) suggests it may be possible that in some groups the external morphology has reached so high an adaptive level that changes which might be expected are discriminated against by natural selection. Finally, it cannot be ruled out that a small genetic difference within a species may be reflected as more than one morphological pattern: many cases of morphological polymorphism are known. As such, only chromosomal analysis could be used to identify the species.

In summary, seven distinct species are recognized in the lakes studied; five of them clearly on both chromosome and larval morphology, two of them on chromosome patterns alone. That there may be more than one species involved in those here considered to be a single taxa, should however not be forgotten.
a	= antenna
abg	= abdominal gill
ag	= anal gill
ap	= anal papillae
Ъ	= blade
bs	= basal segment
fc	= fronto-clypeus
la	= lateral appendage
lo	= Lauterborn organ
lp	= labial plate
m	= mandible
р	= parietal sclerite
plp	= paralabial plate
pp	= posterior proleg
ro	= ring organ

*LOCATIONS :-Series A - Springhouse Area

> -Westwick Lk. -Rush Lk. -Boitano Lk. -Sorenson Lk.

Series B - Riske Creek Area

-Box 17 -Box 89 -N.Op.Box 4 -N.Op.Cres. -Op.Box 4 -Phalarope -Racetrack -Rock

Series C - Gang Ranch Area

-Long Lk. -Long E. #2 -Long E. #4 -Long E. #5

Series D - Kamloops Area

-Lac du Bois -Lac du Bois #1 -Kamloops #1

SPECIES	COLLECTION	LOCATION *			_	SLIDES MADE	TOTAL SLIDES
	Day/Mon/Yr	Α	В	С	D		
	9/6/66		Х			21	
· 1	1/ 8 /66		X			5	
i i	16/10 /66		Х			9	41
	3/ 5/67		X			6	
	9/6/66	X	Х			24.24	
	1/8/66	Х	X			78	
	5/8/66			X		2	175
	16/10 /66	Х	Х			26	
	3/ 5 /67	Х	Х			25	
	91.6 /66	Х	Х			5	
	8/7/66				Х	3	
	1/ 8 /66	Х	X			23	
	5/8/66			X		4	60
	23/ 8 /66				Х	2	
	16/10 /66	Х	X			12	
	3/ 5 /67	Х	Х			11	
	9/6/66	X				3	
	1/8/66	Х	Х			27	
IV	23/ 8 /66				Х	12	
	16/10 /66	X				13	76
	3/ 5 /67	X	Х			21	
	9/6/66	Х				6	
	8/7/66				Х	22	
	1/8/66	X	X			27	_
	5/8/66			Х		41	168
	23/ 8 /66				X	32	
	16/10 /66	X				12	
	3/ 5 /67	Х	Х			28	
	9/6/66	X	X			51]
	1/8/66	X	X			5	
	23/8/66				X	4	88
	16/10 /66	Х				4	
	3/ 5 /67	X	X			24	
VII	9/6/66	X	X			6	4
	1/8/66	X				44	4
	5/8/66			Х		14	99
	16/10 /66	Х				19	1 ·
	3/5/67	X	Х			16	

Table 2

Table 3 : Some Morphological Features of the Seven Larval Species

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ABBREVIATIONS

Abd. Gills	= Abdominal Gills (see Fig. 10) = Accessory Tooth (mandible)
Access: 1000H	
Ant. Ratio	= Antennal Ratio
Col.	= Colour
Dist. Laut. Or.	= Distinct Lauterborn Organ
Dk. Red	=Dark Red
Lat.	= Lateral Teeth (labial plate)
Lat. Append.	= Lateral Appendages (see Fig. 10)
Mand. Teeth	= Mandible Teeth
Med.	= Median Tooth (labial plate)
Minute	= Small Teeth on Median (labial plate)
mm.	= Millimeters
pr.	= Pair

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Species	Segments	Length mm.	Head Size mm.	Ant. Ratio	Dist. Laut.Or.	Labial Teeth	Mand. Teeth	Access. Tooth	Abd. Gills	Lat. Append.	Col.
1	12	8-10	.45x.59	14:4:2	No	2 Med. 12 Lat.	3 Light	No Yes	No	No	Red
11	12	18-20	.69x.95	37:12:8: 5:2	No	1 Med. 12 Lat.	3 Dark 1 Light	Yes	l pr.	No	Dk. Red
- 111	13	22 - 24	.75x1.17	27:10:10: 2	Yes	l Lt.Med. 10 Lat.	3 Dark	No	No	No	Med. Red.
١V	12 -	15-17	•73 x 83	30:11:4: 3	No	l Lt.Med. 2 Minute 12 Lat.	3 Dark	Yes	2 pr.	Yes	Red
V	12	15-17	.72x.82	30:11:4: 3	No	l Lt.Med. 2 Minute 12 Lat.	3 Dark	Yes	2 pr.	Yes	Red
VI	12	15-17	.65x.73	27:8:3:3: 1	No	1 Lt.Med. 10 Lat.	4 Dark	Yes	No	No	Red
VII	· .12	24-26	.85x.95	35:14:7: 7:3	No	l Dk.Med. 2 Minute 12 Lat.	3 Dark	Yes	2 pr.	Yes	Red

Table 3.

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Table 4 : Tentative Identification of the Seven Species *

Sp. 1	Τ	= '	Polypedilum sp.
Sp. 3	II	=	Glyptotendipes barbipes (Stagger)
Sp. 3	III	¢	Procladius sp.
Sp. 3	IV	= '	Chironomus sp. (plumosus group)
Sp. V	V	=	Chironomus sp. (plumosus group)
Sp. V	νī	=	Macropelopia sp.
Sp. 7	VII	=	Chironomus tentans Fabricius

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* all reared material with associated larvae and pupal exuviae has been turned over to the University of British Columbia, Entomology Museum for complete and final identification. . 36

Figures 1 to 7 : Salivary Gland Polytene Chromosome Maps

Symbols :

L	=	Left Arm
R	=	Right Arm
В	=	Balbiani Ring
С	=	Centromere
N	=	Nucleolar Organizer



C С Э.Э α 27 5.2 5.6 Э.Э 5 α SF -6.1 6.1 7.4 <u>o</u> 5.6 ¢ 7.4 9.3 4 5.2 5.6 2.2 7.2 5.6 32 $\overline{\mathbb{N}}$ С. С.







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Symbols:

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Sp. I
 Sp. II
 Sp. III
 Sp. IV
 Sp. V
 Sp. VI
 Sp. VII



Figure_9 : Head Capsule Pigmentation Patterns

i = general ventral aspect ii = general dorsal aspect ia = light extreme of ventral aspect ib = dark extreme of ventral aspect (see Table 1 for abbreviations used)

A. Sp. I
B. Sp. II
C. Sp. III
D. Sp. IV
E. Sp. V
F. Sp. VI
G. Sp. VII











Figure 10 : Terminal Abdominal Segments of the Larvae (4 th. Instar)

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(see Table 1 for abbreviations used)

A.	Sp.	I	(x100)
Β.	Sp.	II	(x 70)
C.	Sp.	III	(x. 70)
D.	Sp.	IV	(x. 60)
Ε.	Sp.	v	(x 60)
F.	Sp.	VI	(x 80)
G.	Sp.	VII	(x 60)



Figure 11 : Some Morphological Features of the Head Capsules in the Seven Larvae (4 th. Instar)

i = eyespots
ii = antenna
iii = mandible, labial plate, paralabial plate

(see Table 1 for abbreviations used)

Α.	=	Sp.	I
в.	=	Sp.	II
с.	=	Sp.	III
D.	=	Sp.	IV
E.	=	Sp.	V
F.	=	Sp.	VI
G.	=	Sp.	VII





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Plates 1 to 7 : Composite Photographs of the Fundamental

Patterns of the Salivary Gland Chromosomes

in the Seven Species Studied

Sp. I
 Sp. II
 Sp. III
 Sp. IV
 Sp. V
 Sp. VI
 Sp. VII















LITERATURE CITED

- Acton, A.B., 1956. The Identification and Distribution of the Larvae of Some Species of Chironomus (Diptera). Proc. R. Ent. Soc. London (A), 31:161-164
- Acton, A.B., 1959. A Study of differences between widely separated Populations of <u>Chironomus</u> (=<u>Tendipes</u>) <u>tentans</u> (Diptera). Proc. Roy. Soc., B, 151:277-296
- Basrur, P.K., 1959. The Salivary Gland Chromosomes of Seven Segregates of Prosimulium (Diptera:Simuliidae) with Transformed Centromere. Can J. Zool., 37:527-570
- Basrur, P.K., 1962. The Salivary Gland Chromosomes of Seven Species of Prosimulium (Diptera:Simuliidae) from Alaska and British Columbia. Can.J. Zool., 40:1019-1033
- Basrur, V.R. 1957. Inversion Polymorphism in the Midge <u>Glyptotendipes</u> barbipes (Staeger). Chromosoma, 8:597-608
- Bates, M., 1940. The Nomenclature and taxonomic Status of the Mosquitoes of <u>Anopheles maculipennis</u> complex. Ann. Ent. Soc. Amer., <u>33</u>:343-356
- Bauer, H., 1936. Beitrage zur vergleicheuden Morphologie der Speicheldrusen-chromosomen (untersuchungen an den Riesenchromosomen der Dipteren II) Zool. Jb. Abt. allg. Zool., 56:239=276
- Bauer, H., 1945. Chromosomen und Systematik bei Chironomiden . Arch-Hydrobiol., 40:994-1008
- Bauer, H., 1953. Chromosomen und Systematik bei Chironomiden. Der Aufbau der Chromosomen aus den Speicheldrusen von <u>Chironomus</u> thummi Kiefer. Z. Zellforsch., <u>23</u>:280-313
- Beermann, W., 1952. Chromomerenkonstanz und Spezifische Modifikationen der Chromosomenstruktur in der Entwicklung und Organdifferenzierung von Chironomus tentans. Chromosoma, <u>5</u>:139-198

Beermann, W., 1953. Chromosomenpolymorphismus und Bastardierungzweier Chironomus - Arten. Zool. Anz. (suppl.), <u>17</u>:290-295

Beermann, W., 1956. Chromosoma, 8:1-11

Beermann, W., and V. Clever, 1964. "Chromosome Puffs", Sci. Amer.(April)

Bridges, C.B., 1935. Salivary Gland Chromosome Maps. Hered., 26:60-64

- Bridges, C.B., E. Skoog, and J. Li, 1936. Genetical and Cytological Studies on a Deficiency (Notopleural) in the Second Chromosome of Drosophila melanogaster. Genet., 21:788-795
- Bryce, D., 1960. Studies on the Larvae of the British Chironomidae Diptera with Keys to the Chironomidae and Tanypodinae. Trans. Soc. Brit. Entom., 14:19-62
- Chernovskii, A. A., 1949. Identification of Larvae of the Midge Family Tendipedidae. Nat. Lend. Lib. Sci. Tech. <u>62-13587</u> (Trans. of Russian Book) 302 p.
- Conger, A.D., and L.M. Fairchild, 1963. A quick Freeze Method for making Smear Slides Permantent. Stain. Tech., 28:281-283
- Dobzhansky, T., 1939. Experimental Studies on Genetics of Freeliving Populations of Drosophila. Biol. Rev., 14:339-368
- Dobzhansky, T., 1947. Adaptive Changes Induced by Natural Selection in Wild Populations of <u>Drosophila</u>. Evol., <u>1</u>:1-16
- Dobzhansky, T., 1951. "Genetics and the Origin of Species", New York, Columbia, 364 p.
- Dobzhansky, T., and A.H. Sturtevant, 1938. Inversions in the Chromosomes of Drosophila pseudoobscura. Genet., 23:28-64
- Dunbar, R.W., 1959. The Salivary Gland Chromosomes of Seven Forms of Black Flies included in <u>Eusimulium Aureum</u> Fries. Can. J. Zool., 37:495-525
- Farris, J.S., 1966. Estimation of Conservatism of Characters by Constancy within Biological Populations. Evol., 20:587-591
- Gordon, I., 1955. Importance of Larval Characters in Classification Nat., 4489:911-912
- Hamilton, A.L., 1965. An Analysis of a Freshwater Benthic Community with Special Reference to the Chironomidae. Appendix II, Taxonomy of the Chironomidae of Marion Lake, British Columbia Ph. D. Thesie
- Johannsen, O.A., 1937a. Aquatic Diptera. Part III Chironomidae: Subfamilies Tanypodinae, Diamesinae, and Orthocladinae. Mem. Cornell. Univ. Agric. Exp. Stn., 205:1-84
- Johannsen, O.A., 1937b. Aquatic Diptera. Part IV Chironomidae: Subfamily Chironominae. Mem. Corn. Univ. Agric. Exp. Stn., 210:1-56

- Johannsen, O.A., and H.K. Townes, 1959. Guide to the Insects of Connecticut. Part IV. The Diptera or true flies. Fifth Fascicle: Midges and Gnats. Bull. Conn. St. Geol. Nat. Hist., Survey 80:1-170
- Keyl, H.G., and I. Keyl, 1959. Die Cytologische Diagnostik der Chironomiden. Arch. f. Hydriöbiol., 56:43-57
- Lancefield, D. G., 1929. A Genetic Study of Two Races or Physiological Species of Drosophila obscura. Z.I.A.V., 52:278-317
- Li, C.C., 1955. "Population Genetics" Chicago, U. of Chic. Press., pp. 1-28
- Lipina, N.N., 1928. "Chironomid Larvae and Pupae. Their Ecology and Systematics.", Moscow, Izd. Nauchm. Inst. Rybn. Khoz
- MacInnes, J.W., and R.B. Uretz, 1966. Spectral Analysis of the Binding of Acridine Orange to Polytene Chromosomes. Proc. Nat. Acad. Sci., 55:1109-1113
- Northcote, D., and P. Larkin, 1963. "Western Canada" Chapt. 16 Limnology of N.A., Publ. Am. Soc. Limnol. and Ocean. pp. 451-485
- Painter, T.S., 1934. A New Method for the Study of Chromosome Aberrations and the Plotting of Chromosome Maps in <u>D</u>. melanogaster. Genet., <u>1</u>9:175-188
- Patterson, J.T., and W.S. Stone, 1940. Evolution of the virilis Group in Drosophila. U. Texas P., 4032:218-250
- Pavan, C., and M.E. Breuer, 1952. Polytene Chromosomes in Different Tissues of Rhychosciara. J. Hered., 53:149-157
- Rizki, M.T., 1951. Morphological Differences between Two Sibling Species <u>Drosophila pseudoobscura</u> and <u>Drosophila persimilis</u>. P.N.A.S., 156-159
- Roback, S.S., 1957. The Immature Tendipedids of the Philadelphia Area. Monogr. Acad. Nat. Sci. Philad., 9:1-152
- Rothfels, K.H., and T.W. Fairlie, 1957. The Non-Random Distribution of Inversion Breaks in the Midge <u>Tendipes decorus</u>. Can. J. Zool., 35:221-263
- Schlee, D., 1966. Praparation und Ermittlung von Mebwerten an Chironomidae (Diptera) Int. Sym. Chironomiden Limnologische Schriftenreihe, 41/42:169-194

Scudder, G.G.E., 1967. Unpublished Observations.

Stemmens, C., and P.N. Bhaduri, 1941. Staining the Nucleolus. Stain. Tech., <u>16</u>:119-120

- Sublette, J.E., 1960. Chironomid Midges of California. I. Chironominae exclusive of Tanytarsini (=Calopsectrini). Proc. U.S. Natn. Mus., 112:197-226
- Sublette, J.E., 1964a. Chironomidae (Diptera) of Louisiana. I. Systematics and Immature Stages of Some Lentic Chironomids of West-Central Louisiana. Tulone. Stud. Zool., 11(4):109-150
- Sublette, J.E., 1964b, Chironomid Midges of California. II. Tanypodinae Diamesinae and Podonominae. Proc. U.S. Natn. Mus., 115:85-135
- Thienemann, A., 1924. Ueber die Chironomidengattung Lundstroemia nebst einen Bestimmungstabelle fur die Loruen und Puppen der Sectio Tanytarsus genuinus. Zool. Anz. 58
- Thienemann, A., 1929. Chironomiden-Metamorphosen II. Die Sectio Tanytarsus genuinus. Arch. Hydriobiol. XX:93-123
- Townes, H.K., 1945. The Neartic Species of Tendipedini (Diptera, Tendipedidae (=Chironomidae). Am. Midl. Nat., 34:1-206
- Wallace, B., 1966."Chromosomes, Giant Molecules, and Evolution", New York, Norton, 171p.
- Wharton, L.T., 1942. Analysis of the <u>repleta</u> group of <u>Drosophila</u> Univ. Tex. Pub., 4228:23-52