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CHROMOSOMAL ABERRATIONS IN A NATURAL POPULATION OF CHIRONOMUS TENTANS EXPOSED TO CHRONIC LOW-LEVEL ENVIRONMENTAL RADIATION

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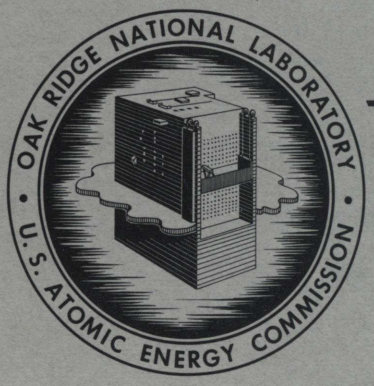

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Radiation Ecology Section

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Submitted as a thesis to the Faculty of the Graduate School of The University of Tennessee in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Zoology

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CHAPTER I

INTRODUCTION

The increase of radioactive fallout from nuclear tests and the potential contamination of environments by radioactive waste have focused attention on the problem of radiation effects on natural populations. Recognition of this problem and its implications has stimulated interest in the general fields of population ecology and population genetics. Although the study of biological material in relation to ionizing radiation has steadily increased since H. J. Muller discovered the mutagenic effect of X-rays, few of these investigations have involved natural populations. The cytogenetic effect of chronic low-level radiation from radioactive waste on a natural population has never been investigated.

Questions which immediately arise in relation to the effects of chronic low-level radiation on natural populations are: (1) At what level of radiation will effects be detectable? (2) How harmful is the effect of radiation on a population? (3) What is the effect of an increased mutation rate on a natural population? (4) At what level of irradiation will the increase of lethal and deleterious genes become too great a burden for the continued survival of the population? (5) Will the flow of new gene combinations into the gene pool accelerate the process of speciation? Since ionizing radiation causes point mutations and chromosomal aberrations, more than the number of spontaneously produced mutations and chromosomal aberrations should occur and be added to the gene pool of a large natural population receiving chronic low-level irradiation. Most of the aberrations would be eliminated rapidly;

however, if some of the newly arising chromosomal arrangements were of high selective value, they would remain in the population or might even replace the standard gene arrangement. Or, the arrangement might have a high selective value only during a certain season and become established in the population at some equilibrium. Also other aberrations could appear, be carried in the population for a few generations and then be lost by genetic drift.

Larvae of Chironomus tentans Fabricius live in the radioactive sediments of White Oak Creek and the Clinch River. The radionuclides sorbed in the silt have resulted in a continuous dose of ionizing radiation, significantly higher than background, since the first release of radioactive waste material to this area in 1943. The population of Chironomus in the contaminated area is receiving 4 to 230 rads/year (20 to 1000 times natural background) of chronic ionizing radiation (Nelson and Blaylock, 1963).

Cytological examinations of the irradiated and some unirradiated populations in the same area were made with the following objectives: (1) To determine whether the total number of chromosomal aberrations or the variety of aberrations from a large natural population receiving chronic low-level irradiation differs from that of a natural population in an uncontaminated area. (2) To determine the amount of chromosomal polymorphism that occurs in the natural populations of C. tentans in this area. (3) To study seasonal and local geographic changes in the frequencies of inversions found in these natural populations.

CHAPTER II

MATERIALS AND PROCEDURES

A. Description of Study Areas

The irradiated natural populations of C. tentans is located in White Oak Creek. The drainage basin of White Oak Creek includes most of the experimental facilities and all of the waste disposal operations of the Oak Ridge National Laboratory. White Oak Creek empties into the Clinch River at mile 20.8, latitude 84° 19'00" west and longitude 35° 54' 00" north. This section of the Clinch River, which is a tributary of the Tennessee River, is located in the middle of the ridge and valley province of East Tennessee.

1. White Oak Creek

Chironomus tentans larvae were collected near the mouth of White Oak Creek where it empties into the Clinch River at mile 20.8 (Figure 1). The depth of White Oak Creek below White Oak Dam and for three-fifths of a mile downstream to where it empties into the Clinch River is controlled primarily by the pool level of Watts Bar Reservoir, a Tennessee Valley Authority flood control reservoir. At the summer pool level White Oak Creek is approximately forty feet wide where it enters the Clinch River and varies in depth from 2 feet at the bank to approximately 12 feet in the stream bed. At minimum pool elevation a decrease in depth of approximately five feet reduces the width of White Oak Creek where it enters the Clinch River to about twenty feet and exposes wide mud banks with occasional large deposits of organic material from the stream side vegetation. Water temperature vary from 6 to 10°C in the winter to 18°C in the summer.

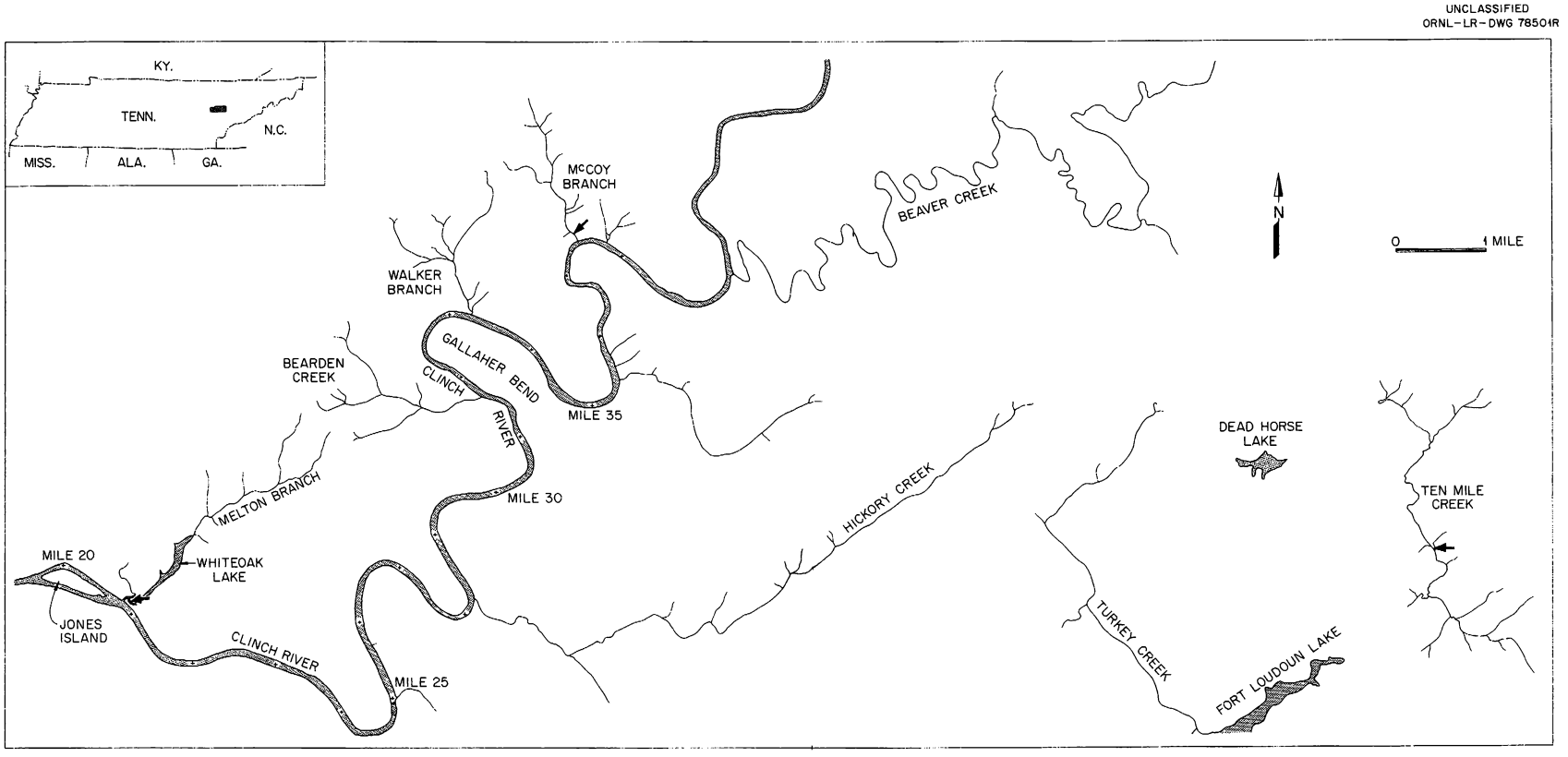

Fig. 1. Location of collecting areas on the Clinch River and Ten-Mile Creek.

White Oak Lake which covered an area of approximately 35 acres was used as an impoundment for the waste released from the Oak Ridge National Laboratories from 1943 to 1955. Eventually, equilibrium was reached in the lake between inflow and outflow of radioactive material and the lake was drained. The gates of the dam were reworked and are maintained at a sufficient elevation above Watts Bar Reservoir so that a standing pool of approximately five acres exists back of the dam. By this means a one-way flow of water is assured even during high elevations of Watts Bar Reservoir. The radioactive wastes now flow into White Oak Creek and through part of the bed of the former waste impoundment to be released from the standing pool to White Oak Creek.

Since the first release of waste from the Laboratory in 1943, some low-level activity has entered White Oak Creek and the Clinch River (Lee and Auerbach, 1960). The major radioactive constituents in the wastes are rare earths, Sr^{89}, Sr^{90}, Cs^{137}, Co^{60}, and Ru^{106}. In addition, White Oak Creek discharges substantial quantities of nonradioactive chemicals, particularly nitrates, into the Clinch River.

Calculations of the absorbed dose rate have been made for the Chironomus larvae inhabiting the radioactive bottom sediments of White Oak Creek and the Clinch River(Nelson and Blaylock, 1963). These calculations were made by assuming that the bottom organisms received a submersion dose of beta radiation and a one-half submersion dose of gamma radiation. These calculations also assumed that there were equal concentrations by weight of radioactivity in the organisms and in the mud. Furthermore, a reciprocity relationship was implied in which the absorbed dose in the mud derived from radioactivity within the organism was equal to the absorbed dose in the organism derived from the radioactivity

in the mud. Since the sediment contains concentrations of radioactivity about 4 orders of magnitude greater than the overlying water (Hart, 1961), the radioactivity in the water was disregarded for the purpose of this calculation.

The Chironomus populations are subject to average doses of radiation as follows:

Background	0.230 rad/year	
Average CRM* 21.5 to 1.1	4.37	19 times background
Average CRM 19.1 to 16.3	8.52	37 times background
White Oak Creek	230	1000 times background

*CRM - Clinch River Mile

2. McCoy Branch

McCoy Branch empties into the Clinch River 16.7 miles upstream from the mouth of White Oak Creek (Figure 1, page 4). It is a small unpolluted stream that drains pasture land and second-growth forest before emptying into the Clinch River. The stream is about 5 feet wide where it rushes over a clay and mud bottom with occasional stretches of rubble. However the formation of many small pools slows the progress of the stream and then its width may reach 10 feet. These pools, which may be one to three feet deep, collect organic material on the mud bottom.

About one-third of a mile from its mouth (arrow, Figure 1, page 4), the stream forms a marshy area about forty feet wide for a distance of approximately 50 yards when it divided a ridge of second-growth forest. During the recent clearing of the area for the construction of Melton Hill Dam, a new TVA flood control dam on the Clinch River, debris from the many alder trees and shrubs collected and apparently changed

the habitat of the stream and marsh areas. Only one or two larvae of C. tentans were found in the area previous to the clearing of the vegetation in the early summer, but by September 1962 a large population of C. tentans was established in the stream and marsh area.

3. Ten-Mile Creek

Ten-Mile Creek, located approximately 15 miles east of White Oak Creek, is not connected to the other water systems (Figure 1, page 4). The stream originates from springs, and drains farm land and pasture land before disappearing into a limestone cave. The stream width varies from 10 to 20 feet; its depth from 1 to 4 feet. Ten-Mile Creek moves along sluggishly over a mud bottom, then increases its velocity over rocky or rubble bottoms to form pools and eddies where large amounts of debris collect. The primary collection site (arrow, Figure 1, page 4) was at the end of an old water-race from a grist mill. This is an area about 6 feet wide and 60 feet long with a constant supply of water that connects with the stream. Water depth is from one to three feet over a deposit of mud and silt one to two feet deep. This silt is inhabited by a large population of Chironomus larvae consisting of many species.

4. Other collecting sites

All other collecting sites which were located on the Clinch River will not be described in detail because of the small number of larvae taken from them. The Clinch River mile numbers of these sites, where collecting boxes were located, are given in Table 1.

B. Life Cycle of Chironomus tentans

Chironomus tentans Fabricius, which is widely distributed over North America and Europe, is found in the larval form in White Oak Creek and the Clinch River. The life cycle of this organism has been described in detail by Sadler (1935). On the second or third day after hatching, the larvae begin to construct minute tubes in which they live. The tubes are composed of silt and very small particles of other available materials. These tubes are open at each end, and are enlarged from time to time to accommodate the increasing size of the larvae. The larval stage lasts from 22 to 48 days; during this period the larvae molt three times. Four blood gills appear simultaneously with the first molt. After the first molt, the larvae are a pinkish color which gradually darkens into blood red. The duration of the fourth instar varies far more than does that of either of the three preceding stages. It may vary from 4 days to 3 weeks under identical rearing conditions. At the end of the fourth instar stage, the thorax becomes greatly enlarged, indicating that transformation is about to take place. Larvae pupate in their tubes with the pupal period lasting about three days. After emerging, the adults live for three to five days during which mating and egg deposition take place. Mating usually occurs during a mating flight; however, experimental matings in cages have been successful.

Females usually oviposit on the second or third day after emerging, each female laying from 1,400 to 3,000 eggs in a single mass. The time required for the eggs to hatch depends upon water temperature,

varying from 3 days at 22.1°C to 17.5 at 8.8°C. Four or five generations appear annually around Ithaca, New York. In the Oak Ridge vicinity, Chironomus adults have been observed emerging every month in the year. The conclusion based on climate and observation of adults is that at least eight generations appear annually in the Oak Ridge area.

The C. tentans larvae in this area are most abundant in the early summer and fall. This species appears to be an opportunistic species which takes advantage of the new habitats created by the spring moisture and the autumn leaf fall. The number of C. tentans decreases in the summer but one or two other species become more abundant. For this reason larvae of some species of Chironomus usually can be found in abundance most of the year.

Since adults live for only three to five days, migration in this form is restricted. Also, the larvae usually remain in the vicinity of egg deposition; however strong currents and floods can transport them a great distance downstream. In general, migration of a distance more than a mile would be unusual (Acton, 1957). The population of C. tentans in the Clinch River system is probably one large panmictic population with limited gene flow between areas separated by a distance of a few miles.

C. Collecting Methods

1. Bottom sampling

Bottom samples were taken at a depth of 6 feet from the stream with a long handled scoop, and in greater depths with an Eckman Dredge.

Each sample was strained and larvae separated from the organic material with forceps. An alternate method, which seemed to be suitable for collecting the fourth instar larvae, was to agitate the diluted sample for a short time to break up the tubes built by the larvae. Within one to five minutes, the larvae which floated to the top of the sample were removed with a tea strainer or forceps. Slide preparations were usually made from the larvae on the day following collections. However, the larvae can be maintained from one to three weeks in 1500 ml containers filled with water and a little bottom debris at 15 to 20°C.

2. Collecting boxes

Wooden collecting boxes were used where it was difficult to obtain a sufficient number of larvae by conventional bottom sampling techniques. Three boxes were used at a collecting site to insure a constant supply of larvae, and up to nine boxes were used at White Oak Creek to insure a random sample of the population. These boxes were 3 feet wide, 6 feet long, and 1 1/4 feet deep. Leaves and twigs in various stages of decomposition were collected from the surrounding area and used to cover the bottom of the boxes. The material provided food and shelter for Chironomus larvae and also temporary protection from fish and other predators. The boxes were anchored in the water in a shaded area near the edge of the stream. Since the boxes leaked at the seams, water entered, but the buoyancy of the boxes maintained water at a depth of 6 to 12 inches inside. After approximately six weeks, the boxes were heavily populated with Chironomus larvae and

provided a constant and easily obtainable supply for the next four to six months. After this time, because of a build-up of various predator populations in the boxes or changes in the organic material, the populations of Chironomus larvae decreased. Boxes were then removed from the water for a few days and returned with a fresh supply of organic material.

D. Slide Preparation Technique

The methods used for slide preparation in this study were standard techniques or variations of previously described standard techniques. The method described for Drosophila (Nicoletti, 1959) was satisfactory for salivary gland preparation for Chironomus. This method was used for preparing about one-half of the slides. Salivary glands were removed from the 4th instar larvae in an isotonic salt solution, fixed in 45 per cent acetic acid for one minute, and then transferred to a drop of lactic-acetic orcein for ten minutes. This is a good technique for preliminary work with the chromosomes. Finer bands, however, were not distinguishable and the nucleolar organizer could not be identified in the majority of the slides. If larvae other than the 4th instar were used, the chromosomes did not spread readily and the bands were indistinguishable. The greatest advantage to this method was that the temporary slides were good for as long as six months at room temperature.

Since the technique used for preparing the rest of the slides was a combination of the method used by Beermann (1952) and Keyl and

Keyl (1959) with some variation, it will be described in detail. Larvae were placed on a siliconized slide. The siliconized slide, being hydrorepellent, retained water as discrete droplets. Water adhering to the larva was absorbed by bringing bibulous paper in contact with it. Forceps were used to grasp the larva at about the fifth body segment. Another pair of forceps was used to grasp the head and pull the head capsule just far enough away from the body to keep from breaking the gut, about five millimeters. Usually the salivary glands were attached to the head capsule and were in the body fluid that flowed from the larvae. While one forceps held the larvae at the fifth segment, the other forceps were used to grasp the thoracic segments and force more body fluid and the salivary glands out, in case this was not accomplished in the first movement. A dissecting needle was used to separate the salivary glands and move them away from the other material. The glands were fixed in a drop of alcohol and acetic acid (3 parts 95 per cent alcohol : 1 part acetic acid) for 30 seconds. They were then transferred with clean forceps to a drop of stain (4 parts acetic orcein : 1 part acetic carmine, Keyl and Keyl, 1959) on a siliconized cover slip and stained from four to six minutes. This staining was for phase contrast observation, but the staining time should be extended to ten minutes for bright field observation. A drop of 72 per cent lactic acid, approximately the size of the drop of stain, was placed on the cover slip near the stain. The two drops were thoroughly mixed with a clean dissecting needle. Smearing was accomplished by inverting the cover slip on an unsiliconized slide. By use of this

method, small bands can be distinguished and the nucleolar organizer can be identified in most of the preparations. The temporary slides are good for at least four weeks at 5°C. Permanent slides were made by the dry ice technique (Baker, 1952). One difficulty which may be encountered is the sticking of the salivary glands to the cover slip when slides are made permanent.

CHAPTER III

LARVAE COLLECTED

Sample collections of C. tentans larvae are summarized in Table I according to location and month of collection. An asterisk by a collection site in Table I indicates that the larvae were obtained from this location only by the collection box method. Numbers that appear under each location, except Ten-Mile Creek, are the Clinch River Mile (CRM) number, which is the distance of each collection site from the mouth of the river. The largest number of larvae, 365, was collected from White Oak Creek, the area that is contaminated with radioactive waste. Approximately the same number, 356, was collected from six control populations. Larvae from all control locations were used in the irradiated versus nonirradiated population study; however, because of the small numbers from some control locations, only the samples from White Oak Creek, McCoy Branch, and Ten-Mile Creek were used in chromosomal polymorphism studies.

Chironomus tentans larvae were collected from boxes at Gallahar Bend in 1961 and 1962. The water depth at this location fluctuates rapidly because of the alternate release and impoundment of the Clinch River by Norris Dam, one of the Tennessee Valley Authority dams. Variable water levels made maintenance of collection boxes at this location difficult, and after the loss of three sets of boxes, the site was abandoned. Intensive bottom sampling of the surrounding area produced several Chironomus larvae but only one or two were C. tentans. The

TABLE I

Collections of *Chironomus tentans* Larvae

Location	Year	Month												Totals
		Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	
White Oak Creek CRM 20.8	1960							31						31
	1961					5	49	19	37	76	30	19	19	254
	1962					9	15	31	14			11		80
Total Collection														365
McCoy Branch CRM 37.5	1962									9	73	48	62	192
Total Collection														192
Ten-Mile Creek	1962									13	10	22	26	71
	1963	29												29
Total Collection														100
Gallahar Bend CRM 32.5	1961*											22	7	29
	1962*		4				10							14
Total Collection														43
Norris Dam CRM 79.8	1962*						7							7
Total Collection														7
Beaver Creek CRM 39.5	1962*						2							2
Total Collection														2
Grassy Creek CRM 14.7	1962*					4	7	1						12
Total Collection														12
Total Larvae Collected														721

extension of the search for C. tentans larvae, however, led to the discovery of the control population at McCoy Branch.

At least seven other species of Chironomus were taken from the collecting site at Ten-Mile Creek. Three of these species were more abundant than C. tentans; the large larvae of Chironomus commutatus were the most abundant. Only about one out of every twenty larvae collected was a C. tentans, but there has been a constant supply of larvae since the population was discovered.

CHAPTER IV

CHROMOSOME MAPS

Since banding pattern of the salivary gland chromosomes of C. tentans from East Tennessee is different from the banding pattern of Europe and Canada, chromosomal maps were constructed of the salivary chromosomes from this area. These maps were used as a reference in scoring the chromosomal aberrations, and as an aid in learning the standard banding pattern. The banding pattern that was found most frequently was considered the standard banding pattern.

The chromosome maps are photographic composites (Figures 2, 3, and 4). Sections of the chromosomes in an acetic orcein preparation were photographed. The photographs were enlarged and an entire chromosome (2000 x) assembled from these sections. The chromosomes were divided into sections of approximately the same lengths. Bands that usually can be identified in most preparations were used to designate section divisions. The numbers of the sections are not intended to correspond with the numbers of Beermann's (1952) cytological maps, but were used for scoring aberrations.

Because the maps were constructed during the early part of the study, an acetic orcein preparation was used instead of an acetic orcein : acetic carmine preparation. The amount the chromosome is stretched during the smearing of the preparation can determine whether certain bands are distinguishable. In the construction of the chromosome maps, an attempt was made to maintain a uniform amount of

stretching; in certain instances it was impossible to maintain this uniformity.

Fig. 2. The salivary gland chromosomes of Chironomus tentans Fabricius from East Tennessee. Chromosome 1.

Fig. 3. The salivary gland chromosomes of Chironomus tentans Fabricius from East Tennessee. Chromosome 2.

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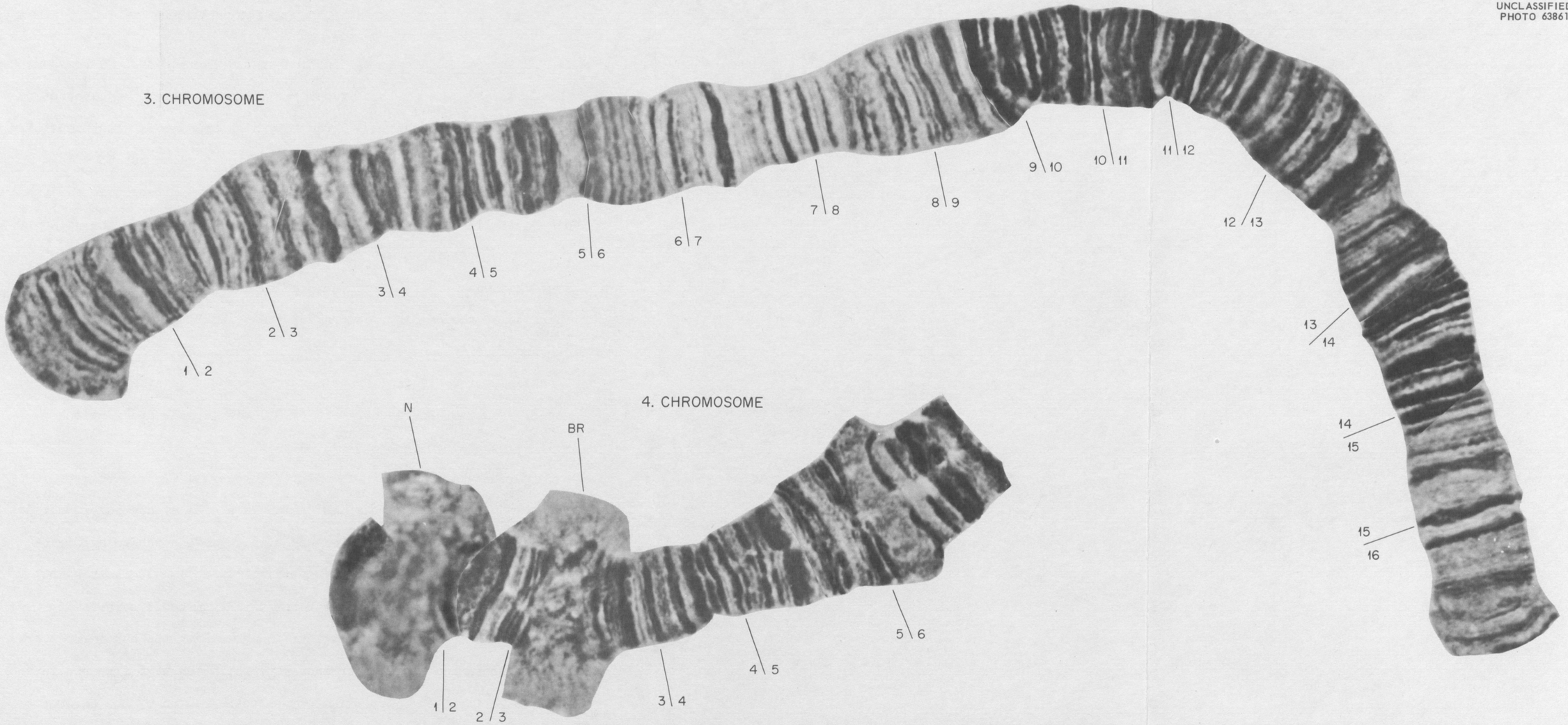


Fig. 4. The salivary gland chromosomes of Chironomus tentans Fabricius from East Tennessee. Chromosomes 3 and 4.

CHAPTER V

STANDARD CHROMOSOMES

Chironomus tentans has four rod-shaped chromosomes. Chromosome 1 and chromosome 2 are almost identical in length; also they are noticeably longer than chromosome 3. The fourth chromosome is about one-third the length of the longest two and contains the structures known as Balbiani rings. The three longest chromosomes are metacentric and the short fourth is acrocentric. Beerman (1952) has described the salivary gland chromosomes in detail and has published cytological maps. Acton (1959) crossed C. tentans from Europe and Canada and made a detailed comparison of the banding patterns of the salivary gland chromosomes. Since the banding patterns of C. tentans from Europe and Canada were not studied, it would be impractical to attempt a detailed comparison. However, by use of Beermann's maps (1952) and Acton's descriptions (1959), a general comparison will be made.

Chromosome 1 is shown in Figure 2, page 19. This chromosome agrees with Beermann's maps in not having a nucleolar organizer, but it contrasts with the Canadian chromosomes which have a nucleolar organizer. A doublet in the proximal one-third of section 11 in this area is usually puffed and the bands are not distinguishable. The first three sections of the left arm appear to agree with the banding pattern of the European and Canadian chromosomes, while the next five sections appear to be inverted. Approximately the same region of the chromosome is inverted in the European and Canadian chromosomes. In the

right arm, only sections 19 and 20 correspond to the European and Canadian banding pattern.

Chromosome 2 is shown in Figure 3, page 20. Acton (1959) describes the left arm of the Canadian banding pattern as being entirely different from the European banding pattern. The banding pattern of this area is like neither the European nor Canadian banding pattern. A puffed region in section 7 is found in approximately the same position in the chromosomes from the British Isles (Acton, 1957). The right arm of the Canadian banding pattern is essentially like the European banding pattern, but in East Tennessee, inversions involving sections 15 through 19 are apparent.

A study of the photographic map of chromosome 3 (Figure 4, page 21) shows that chromosome 3 from this area is like the Canadian chromosome in that the nucleolar organizer, which is found near the middle of the chromosome in Europe, is missing. Furthermore, the banding pattern of the left arm is different from the European and Canadian banding pattern. The dark triplet that is found in section 5B of the standard European arrangement has been moved by a series of inversions to section 7 (Figure 4, page 21). The banding pattern of sections 15 and 16 of the right arm appears identical to the standard European banding pattern. The Canadian banding pattern is the same as the European pattern or it may differ by an inversion which would include a part of section 16 from this area; hence the banding pattern from East Tennessee is unlike both the European and Canadian.

A photographic map of chromosome 4 is shown in Figure 4, page

21. Chromosome 4 differs from the European and Canadian chromosomes by having a nucleolar organizer at the site of the first Balbiani ring.

Section 6 contains a second Balbiani ring and puff, which are in the same position in the European and Canadian chromosomes. The banding pattern is essentially the same.

In C. tentans from Britain, Sweden, Germany, and Austria, the banding pattern of the salivary chromosomes is fundamentally identical. In general, the inversions found in the populations differ only in frequency from one area to another. When Acton (1959) studied the widely separated populations of C. tentans from Europe and Canada, he found the banding pattern of Canadian C. tentans differed from the European much more than the European or Canadian larvae differed among themselves. The morphological differences between the Canadian and European chromosomes consisted chiefly of inverted regions. Some of the banding patterns observed in the second and third chromosomes differed so much from Beermann's maps that Acton identified them in reference to a standard Canadian banding pattern.

Canadian larvae of C. tentans would have been considered identical to European larvae except for the variability in the chromosome banding. The chromosomes of C. tentans and Chironomus pallidivittatus, closely related species, were compared by Beermann (1955). Using this comparison as a basis for judging the amount of divergence that existed between C. tentans from Europe and Canada, Acton concluded that the population of the two continents may even now belong to different races or perhaps incipient species.

The study of incipient taxonomic divergence in C. tentans has been continued by Acton (1962). Populations of C. tentans from western Canada and one population from Alaska have been studied. Banding patterns of the chromosomes in eastern and western Canadian C. tentans were different only in the frequency of inversions. Alaskan and Canadian populations of C. tentans differed by fixed inversions. Alaskan forms were more like European C. tentans than were Canadian C. tentans; however, Alaskan larvae contained some inversions found only in the Canadian populations.

A general comparison of the chromosomes from East Tennessee with those of Canada discloses several differences. The difference between C. tentans of this area and Canada is close to the level of the differences between Canadian and European C. tentans.

CHAPTER VI

CHROMOSOMAL ABERRATIONS

Fourteen different inversions and one deletion were observed in the salivary gland chromosomes of C. tentans from the irradiated and control populations. The inversions found in both the irradiated and control populations were considered inversions endemic to this area. These inversions were scored using the chromosome maps (Figures 2, page 19; 3, page 20; 4, page 21).

The notations used for scoring the aberrations follow. The first number denotes the chromosome in which the aberration is located and the letter L or R refers to the particular arm of the chromosome, left or right. The lower case letter a, b, etc., refers to the particular aberration. When an inversion extends into both arms of the chromosome, it is scored as 2RLd with the letters R and L referring to both arms. A complex arrangement where two inversions are found together is referred to as 2Lab. This is the notation used for inversions a and b occurring in the left arm of chromosome 2. The letter D following R or L refers to a deletion.

The breakpoint of an inversion that has been found several times will be located more accurately than an inversion found only one time. Figures 5 through 12 are photographs of the aberrations. All the photographs are of acetic orcein preparation except 5a and 10a which are acetic orcein : acetic carmine preparations. General comparisons of inversions are made with the European and Canadian inversions when the

banding pattern of the different areas appears to be the same. A description of the inversions and their breakpoints follows:

Inversion 1Ra

The inverted region includes the distal 1/3 of section 17 through section 19 (Figure 5a). This inversion is found to be the most frequent in both the irradiated and control populations (Figure 15, page 64). The homozygote of this inversion was found (Figure 5b). No corresponding inversion has been reported in the Canadian population.

Inversion 1Rb

The inverted region includes the last bands in the distal part of section 10 and the proximal two-thirds of section 11 (Figure 6a). This inversion is near the site of the nucleolar organizer in the Canadian population and includes the puffed section of the chromosome in this region. This inversion was found five times in both the irradiated and control populations (Figure 15, page 64).

Inversion 1Lc

The inverted region includes all of section 2 except the first four bands and extends over the proximal two-thirds of section 3 (Figure 6b). This inversion was found three times in the irradiated area and once in the control population at McCoy Branch. No corresponding inversion has been reported in the Canadian population.

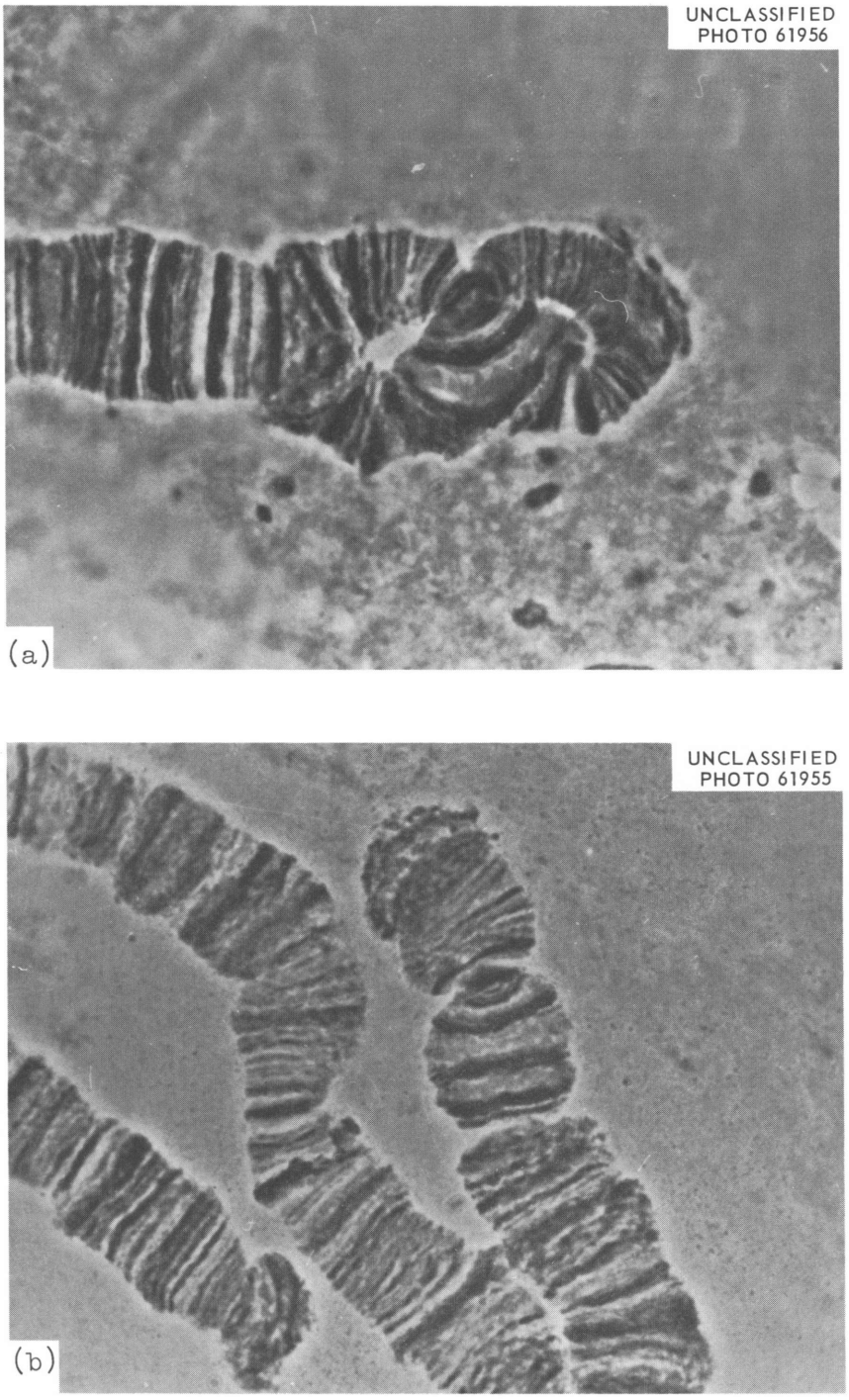
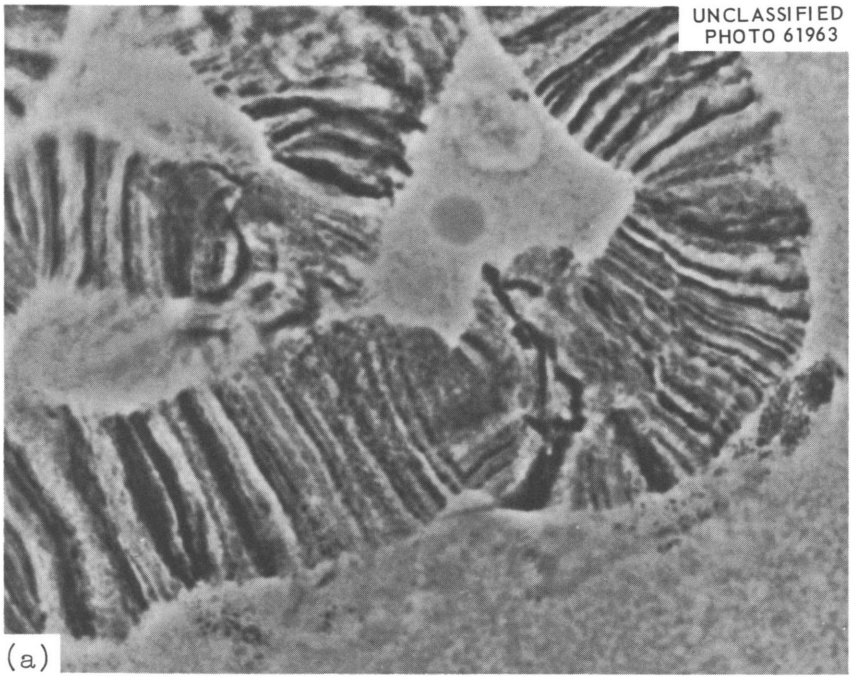


Fig. 5. (a) Inversion lRa; (b) Homozygous inversion of lRa.



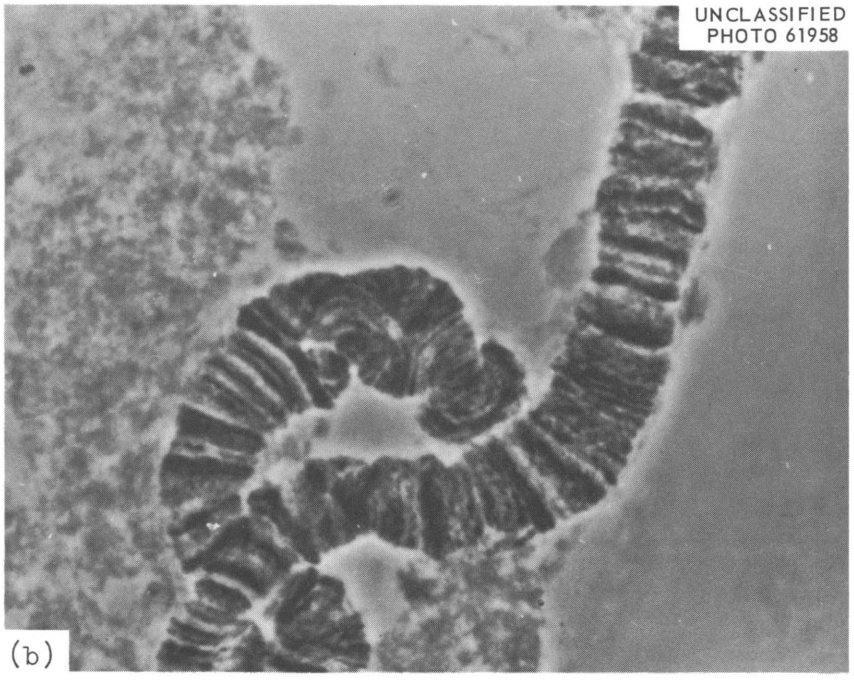


Fig. 6. (a) Inversion lRb; (b) Inversion lLc.

Inversion 1Rd

The inverted region includes the distal two-thirds of section 12 and extends through the proximal two-thirds of section 15 (Figure 7a), and is the longest inversion in chromosome 1. It was found five times in the irradiated population only, and was always found with inversion 1Ra.

Inversion 1Re

This short inversion includes the distal two-thirds of section 13 and includes two bands of section 14 (Figure 7b). The inversion was found one time in the irradiated area only.

Inversion 2Lab

Inversions a and b were found together consistently. Inversion a includes the distal three-fourths of section 3 and extends through the proximal one-fifth of section 6. Inversion b includes the last dark band of section 7 and the proximal three-fourths of section 8 (Figure 8a). Many of the chromosomes are not synapsed over this entire period, and the two inverted regions can be seen in one chromosome. This inversion is found at a relatively high frequency in all populations (Figure 15, page 64). Beermann (1955) and Acton (1957) have reported inversions that occur only in the males of C. tentans. Since inversion 2Lab occurs in the same arm as the inversions reported in Acton, they probably have the same type of inheritance. Unfortunately, the larvae were not sexed when the slides were prepared. Since the two inversions

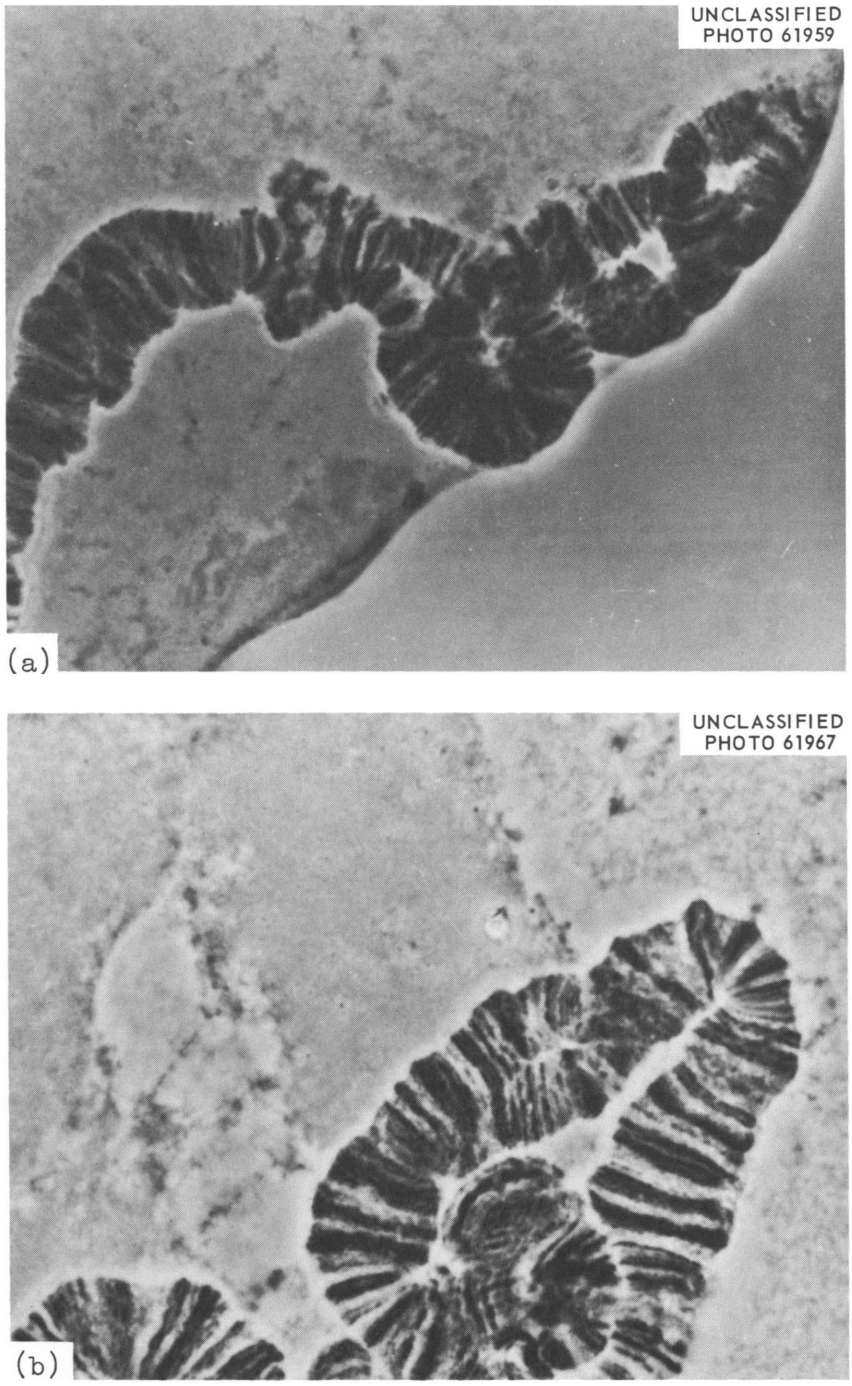


Fig. 7. (a) Inversion 1Rd and Inversion 1Ra; (b) Inversion 1Re.

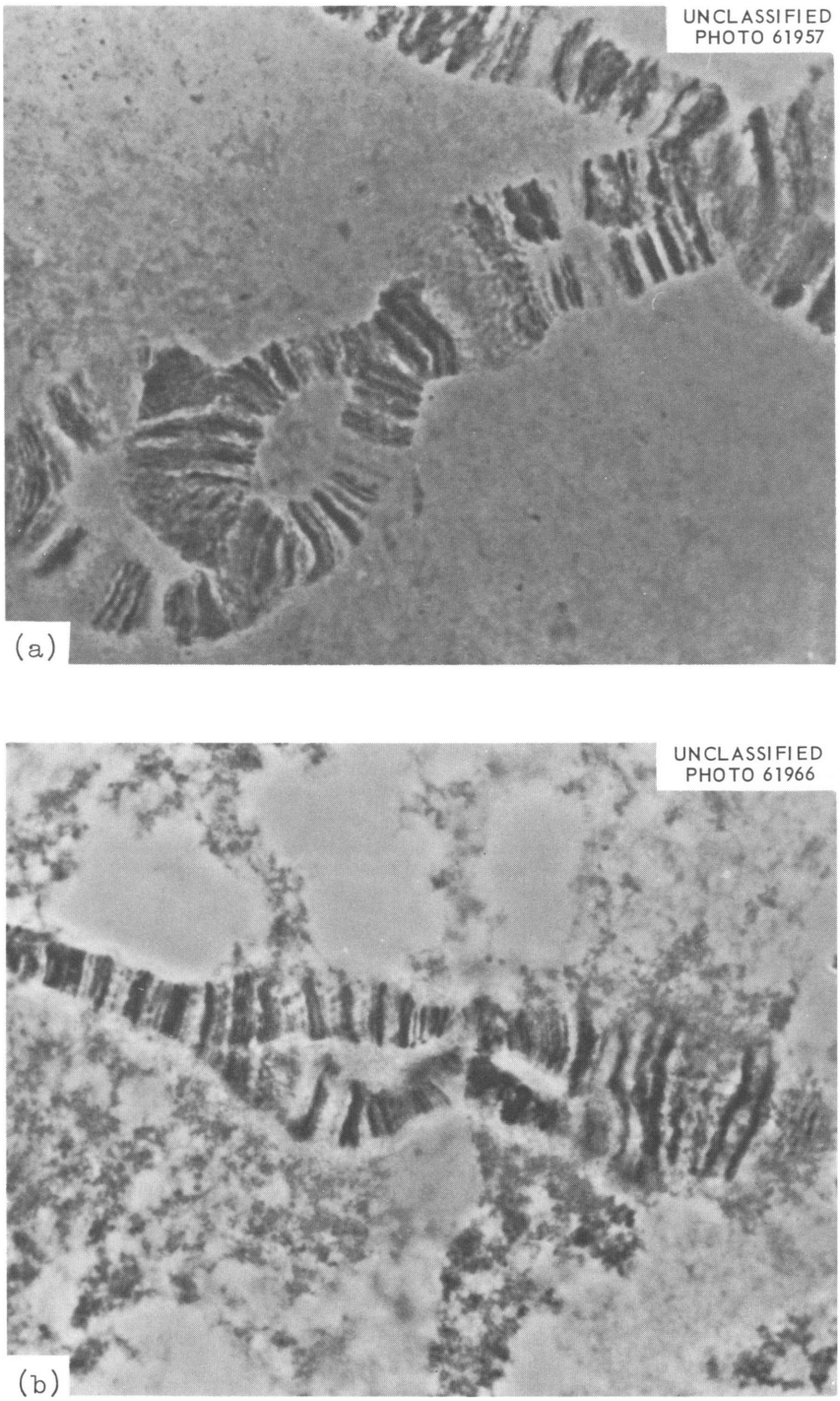


Fig. 8. (a) Inversion 2Lab; (b) Inversion 2Rc.

were found together, they were treated in the analyses as one inversion.

Inversion 2Rc

The inverted region includes the last two dark bands of section 12 and extends through the proximal half of section 14 (Figure 8b). This inversion was found one time in the irradiated area only.

Inversion 2RLd

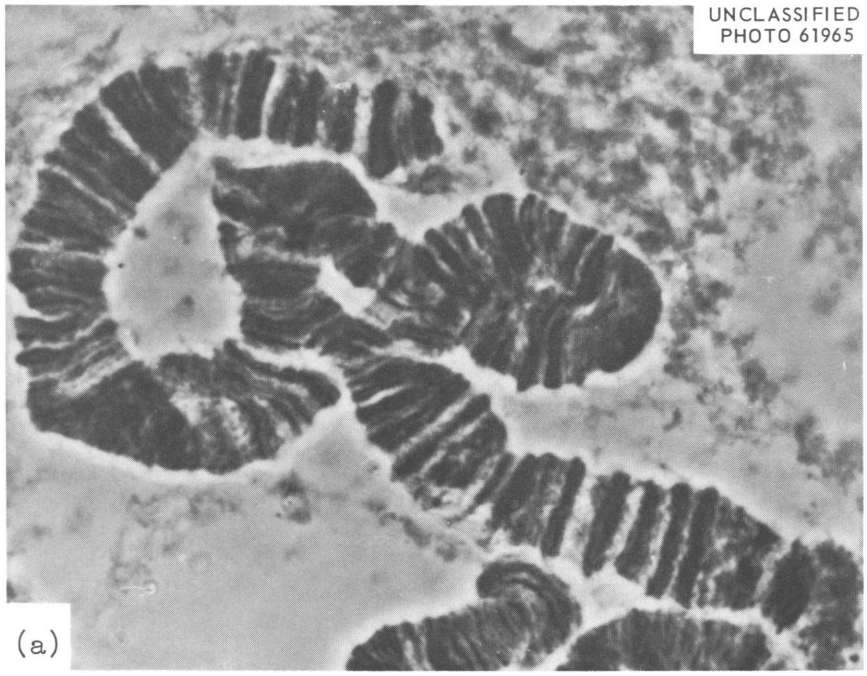
This is the longest inversion observed in the chromosomes of this area. The inverted region extends from the distal half of section 8 through the proximal two-thirds of section 16 (Figure 9a). Chromosome 2 is a metacentric chromosome, and since inversion 2RLd overlaps inversion 2Lab and extends through part of section 16, it is a pericentric inversion. This inversion was found one time in the irradiated population.

Inversion 2Re

Inversion 2Re extends from the distal one-half of section 20 to the end of the chromosome (Figure 9b). This inversion was found one time in the irradiated population. No corresponding inversion has been reported in the Canadian population.

Inversion 3Ra

The inverted region includes section 10 through 14 except for the last group of dark bands (Figure 10a). This is found at a high frequency in



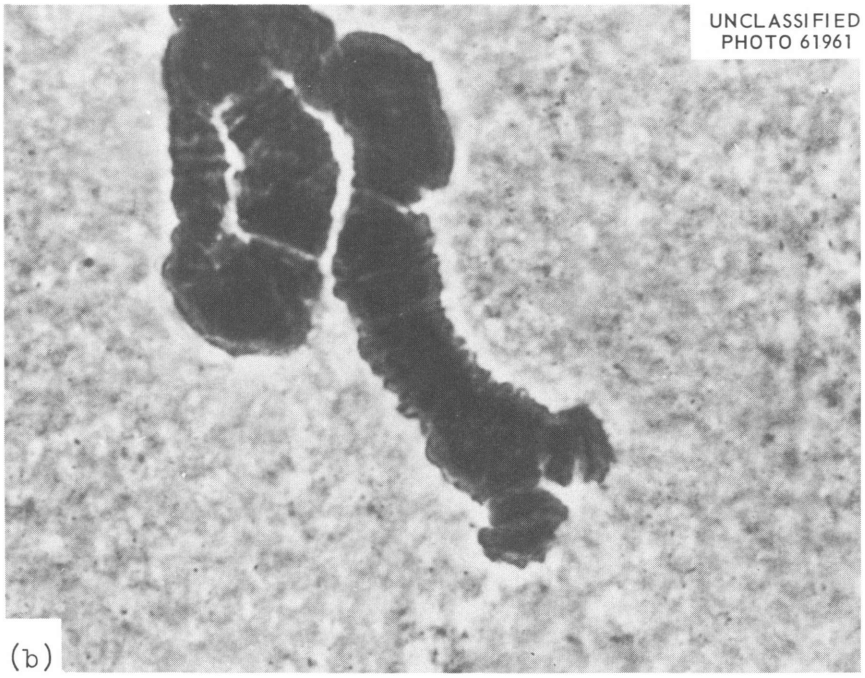


Fig. 9. (a) Inversion 2RLd; (b) Inversion 2Re.

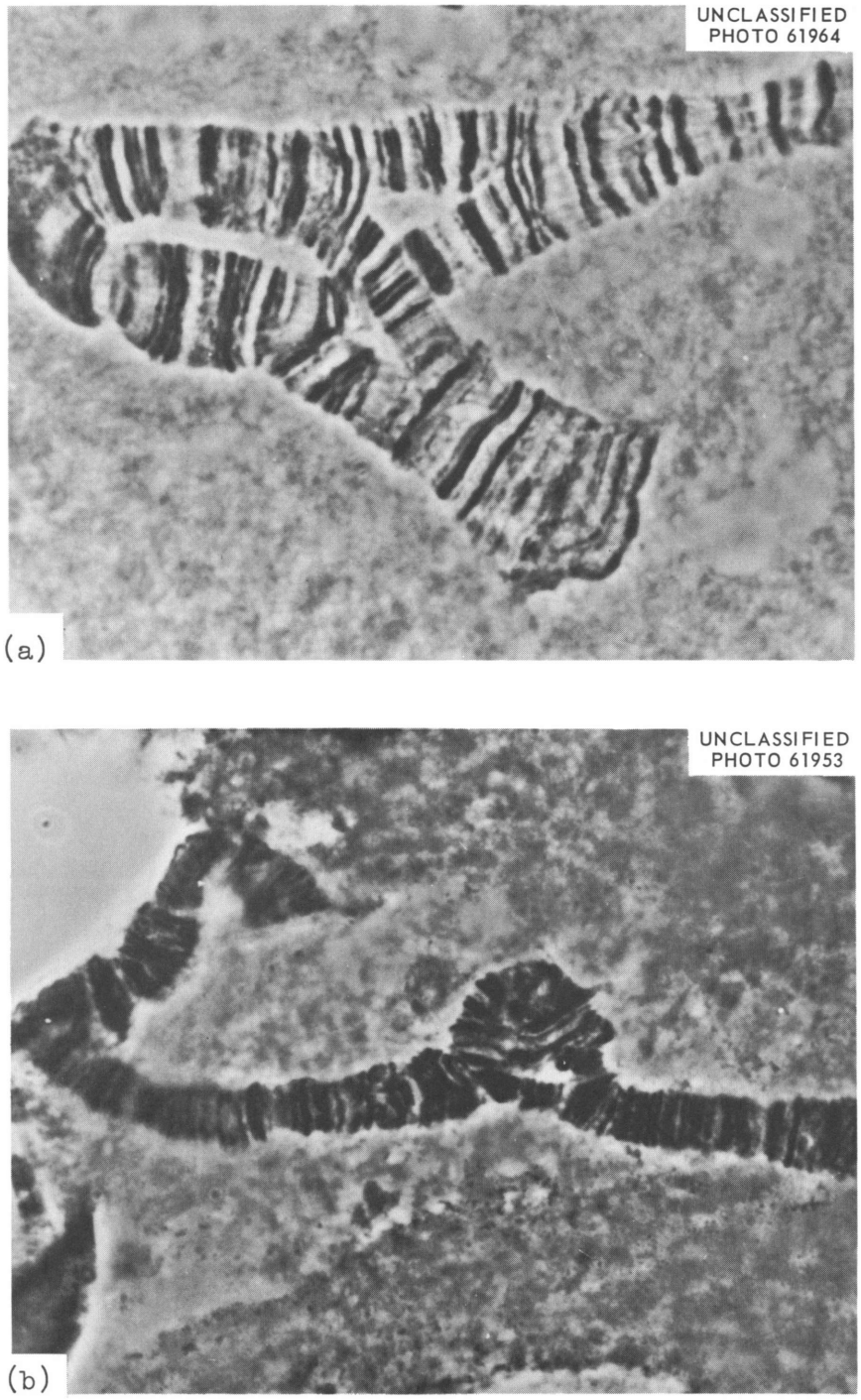


Fig. 10. (a) Inversion 3Ra; (b) Inversion 3Rb.

both the irradiated and control populations (Figure 15, page 64). No homozygous inversions were found. There is an inversion in the Canadian population that covers approximately the same region, but since the standard banding patterns are considered different, a comparison was not made.

Inversion 3Rb

The inverted region includes the distal one-fourth of section 9 through the proximal one-third of section 13 (Figure 10b). This inversion, found one time in the irradiated population, overlaps one breakpoint of inversion 3Ra.

Inversion 3Lc

This is a short inversion in section 2, with the proximal breakpoint after the first three dark bands and the distal breakpoint just in front of the two dark bands ending the proximal half of the section (Figure 11a). It was found one time in the irradiated population.

Inversion 4La

The inverted region is section 2 (Figure 11b). This inversion was found in the irradiated area. The nucleolar organizer and Balbiani ring make it difficult to distinguish the exact breakpoint of this inversion. An inversion in the Canadian population covers the same section, but it appears to be larger.

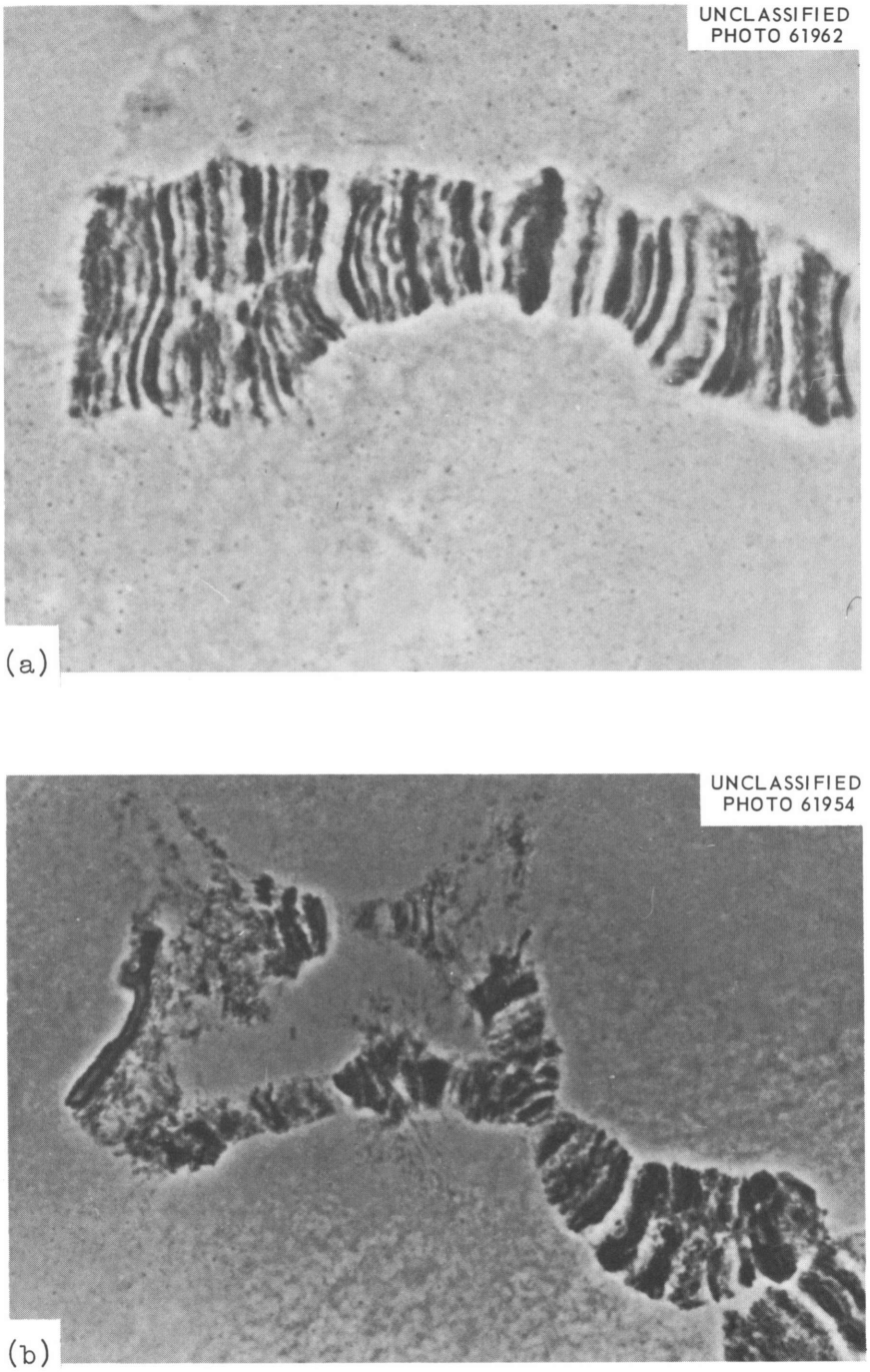


Fig. 11. (a) Inversion 3Lc; (b) Inversion 4La.

Inversion 4Rb

The inverted region includes the distal one-fifth of section 5 and the proximal one-half of section 6. A camera lucida drawing was made of the inversion instead of a photograph, but it is not shown. This inversion was found one time in the irradiated population. No corresponding inversion has been reported in the Canadian population.

Deletion 4RDa

In the proximal half of section 6 one dark band and part of the puffed region were deleted (Figure 12). This was found one time in the irradiated area; no corresponding deletion has been reported in the Canadian population.

The relative position and approximate lengths of the chromosomal aberrations in C. tentans illustrated in Figures 5a through 12 are summarized in Figure 13.

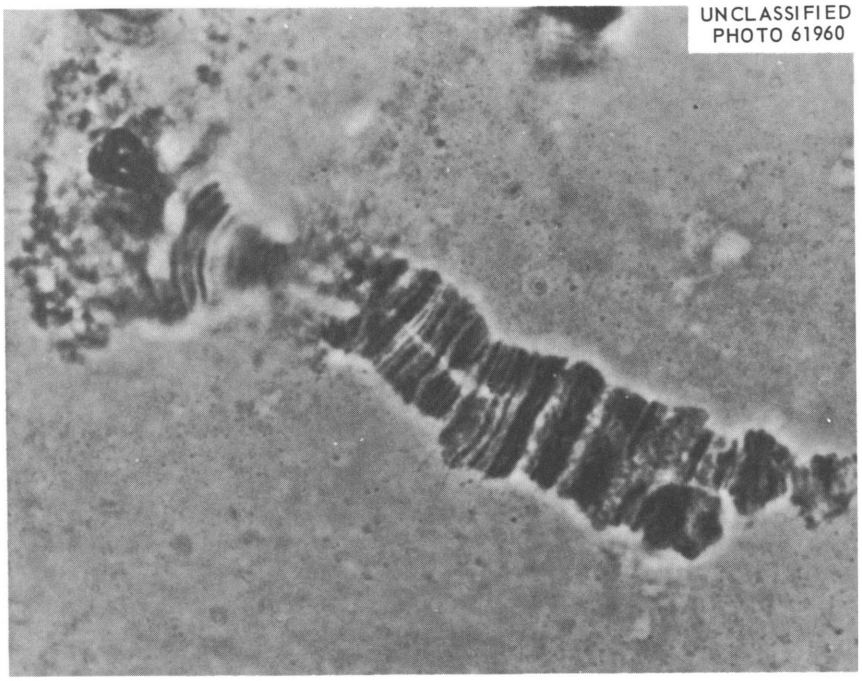


Fig. 12. Deletion 4RDa.

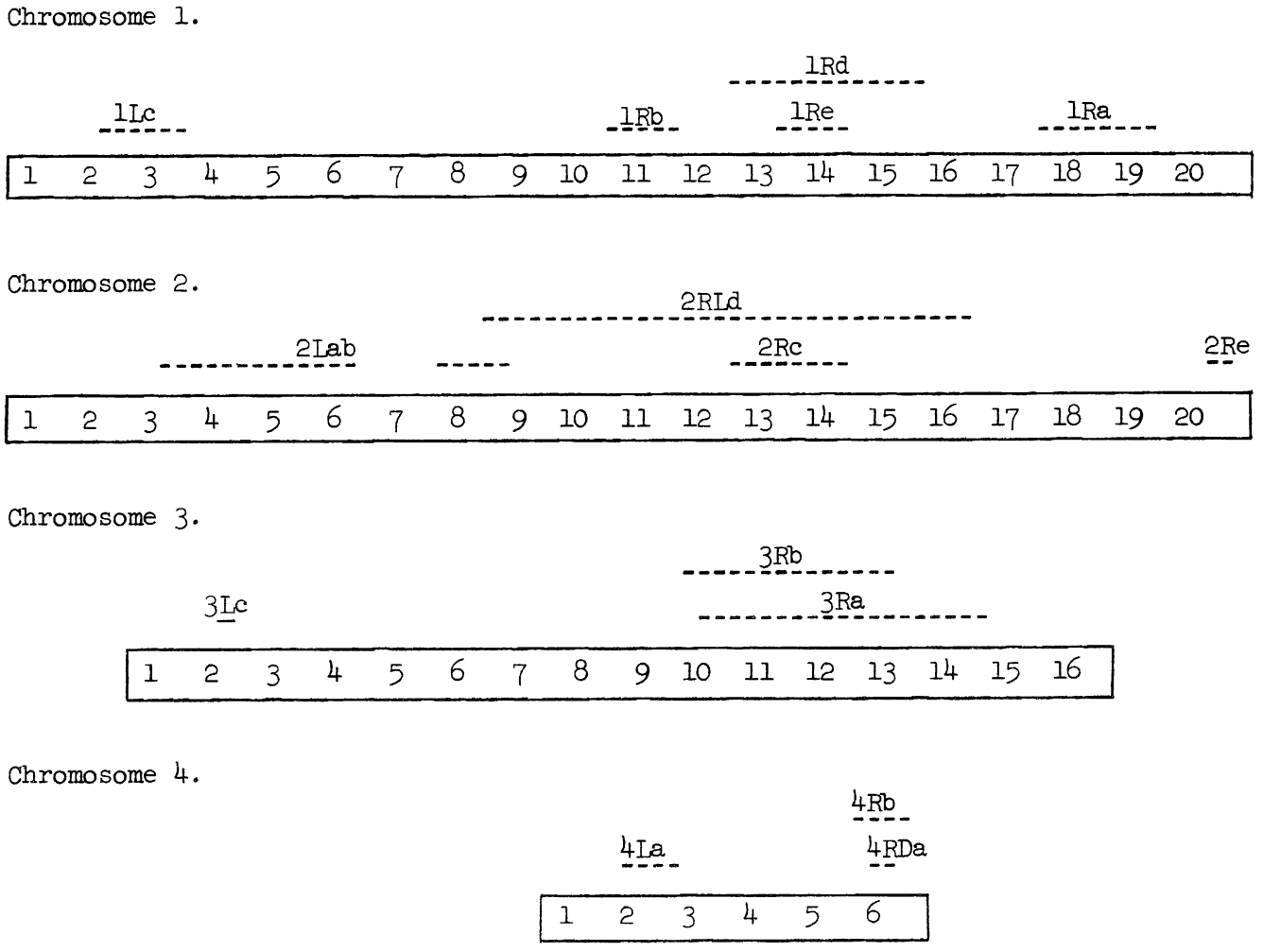


Fig. 13. The approximate location and relative size of chromosomal aberrations in C. tentans.

CHAPTER VII

SEASONAL DISTRIBUTION OF INVERSIONS

Samples were taken from the White Oak Creek population of C. tentans during July of 1960, 1961, and 1962. These samples were used in the construction of contingency tables to test whether the frequency of larvae containing the heterozygous inversions 1Ra, 2Lab, and 3Ra varied from year to year. The observed and expected numbers are given in Table II along with the calculated Chi-square values. No changes were detected in the annual frequencies of the heterozygous inversions 1Ra, 2Lab, and 3Ra. The Chi-square values were not significant at the five per cent level. In a similar manner the seasonal frequency of these heterozygous inversions was tested from June through December 1961 in the White Oak Creek population, Table III. In the McCoy Branch and Ten-Mile Creek populations, the frequencies of the heterozygous inversions were tested for September through December 1962 and September 1962 through January 1963 respectively (Table IV).

The frequency of inversion 1Ra in each population was tested by construction of a contingency table. The frequency of the inversion considers the homozygous inversion as well as the heterozygous inversion; the former is scored as two and the latter as one when determining the frequency of an inversion in a population. There was no annual change in the frequency of inversion 1Ra from 1960 to 1962 (Table II) in the White Oak Creek population. Although $P = 0.06$ the observed frequency of 1Ra for the years 1960 and 1962 was exactly the same.

TABLE II

Three Year Comparison of the Endemic Inversions Found in the White Oak Creek Population of *Chironomus tentans*

Dates Collected		7-8-60	6-23-61 7-23-61	7-12-62 7-30-62
No. Larvae Collected		31	51	31
Frequency of the Heterozygous Inversion 1Ra	obs.	8	12	3
	exp.	6.3	10.4	6.3
			$X^2 = 3.01$	$P = 0.23$
Frequency of the Heterozygous Inversion 2Lab	obs.	7	6	4
	exp.	4.7	7.6	4.7
			$X^2 = 1.90$	$P = 0.41$
Frequency of the Heterozygous Inversion 3Ra	obs.	3	5	1
	exp.	2.5	4.1	2.5
			$X^2 = 1.30$	$P = 0.41$
Frequency of Inversion 1Ra	obs.	8	26	8
	exp.	11.5	19.0	11.5
			$X^2 = 5.78$	$P = 0.06$

TABLE III

Seasonal Distribution of Heterozygous Inversions in White Oak Creek

	June	July	Aug	Sept	Oct	Nov	Dec	Total
No. Larvae Examined	49	19	37	76	30	19	19	249
				Inversion 1Ra				
Obs.	9	4	5	17	9	3	5	
Exp.	10.2	4.0	7.7	15.7	6.2	4.0	4.0	
				$X^2 = 3.74$ P = 0.61				
				Inversion 2Lab				
Obs.	4	3	4	12	5	5	4	
Exp.	7.3	2.8	5.5	11.3	4.5	2.8	2.8	
				$X^2 = 6.91$ P = 0.34				
				Inversion 3Ra				
Obs.	4	2	3	10	8	0	3	
Exp.	5.9	2.3	4.5	9.2	3.6	2.3	2.3	
				$X^2 = 10.29$ P = 0.12				

TABLE IV

Seasonal Distribution of Heterozygous Inversion in McCoy Branch and Ten-Mile Creek

Inversion 1Ra

McCoy Branch

	Sept	Oct	Nov	Dec	No. Larvae Examined
Obs.	4	28	15	8	192
Exp.	2.6	20.9	13.8	17.8	

$X^2 = 12.15$ P = 0.007

Inversion 2Lab

McCoy Branch

	Sept	Oct	Nov	Dec	No. Larvae Examined
Obs.	2	17	10	18	192
Exp.	2.2	17.9	11.8	15.2	

$X^2 = 1.12$ P = 0.77

Inversion 3Ra

McCoy Branch

	Oct	Nov	Dec	No. Larvae Examined
Obs.	10	3	8	183
Exp.	8.4	5.5	7.1	

$X^2 = 1.77$ P = 0.43

Inversion 1Ra

Ten-Mile Creek

	Sept	Oct	Nov	Dec	Jan	No. Larvae Examined
Obs.	3	3	7	4	8	100
Exp.	3.3	2.5	5.5	6.5	7.3	

$X^2 = 2.09$ P = 0.72

TABLE V

Seasonal Distribution of the Inversion 1Ra

White Oak Creek, 1961

No. Larvae Examined	49	19	37	76	30	19	19	Total
	June	July	Aug	Sept	Oct	Nov	Dec	
Obs.	21	6	17	19	11	3	5	249
Exp.	16.1	6.3	12.2	25.0	9.9	6.3	6.3	

$X^2 = 8.27$ P = 0.22

McCoy Branch, 1962

No. Larvae Examined	9	73	48	62	Total
	Sept	Oct	Nov	Dec	
Obs.	10	76	35	20	192
Exp.	6.6	53.6	35.3	45.5	

$X^2 = 40.15$ P < .005

Ten-Mile Creek, 1962

No. Larvae Examined	13	10	22	26	29	Total
	Sept	Oct	Nov	Dec	Jan	
Obs.	3	3	7	6	8	100
Exp.	3.5	2.7	5.9	7.0	7.8	

$X^2 = 0.52$ P = 0.94

distribution of the frequency of inversion 1Ra in populations from different locations. The inversion frequencies of 2Lab and 3Ra were not determined because no homozygous inversion was recognized.

The Chi-square values in Table III, page 43, gave no indication of any seasonal change in the frequencies of the heterozygous inversions 1Ra, 2Lab, and 3Ra from June through December of 1961 in the White Oak Creek population. Similar results were obtained for these inversions in the other populations with the exception of inversion 1Ra in the McCoy Branch population. In Table IV, page 44, the Chi-square value for the heterozygous inversion 1Ra in the McCoy Branch population was significant at the one per cent level. There was also a very significant change in the inversion frequency in the same samples (Table V, page 45). The change in the frequency of the inversion 1Ra in the McCoy Branch population from September to December 1962 is shown in Figure 14, page 47. A very rapid drop occurs in the frequency of the inversion from October to December. Fortunately, the samples from McCoy Branch during this period were of sufficient size to establish the reality of this decrease in the frequency of the inversion. The frequency of the inversion 1Ra in the White Oak Creek and Ten-Mile Creek populations did not show a similar decrease for the corresponding period. As can be observed in Figure 14, the frequencies of 1Ra from White Oak Creek agree with the frequencies of 1Ra from Ten-Mile Creek, although the White Oak Creek samples were collected in 1961 and the samples from McCoy Branch and Ten-Mile Creek were collected in 1962. Since no rapid decrease was observed in the Ten-Mile Creek population or White Oak Creek population,

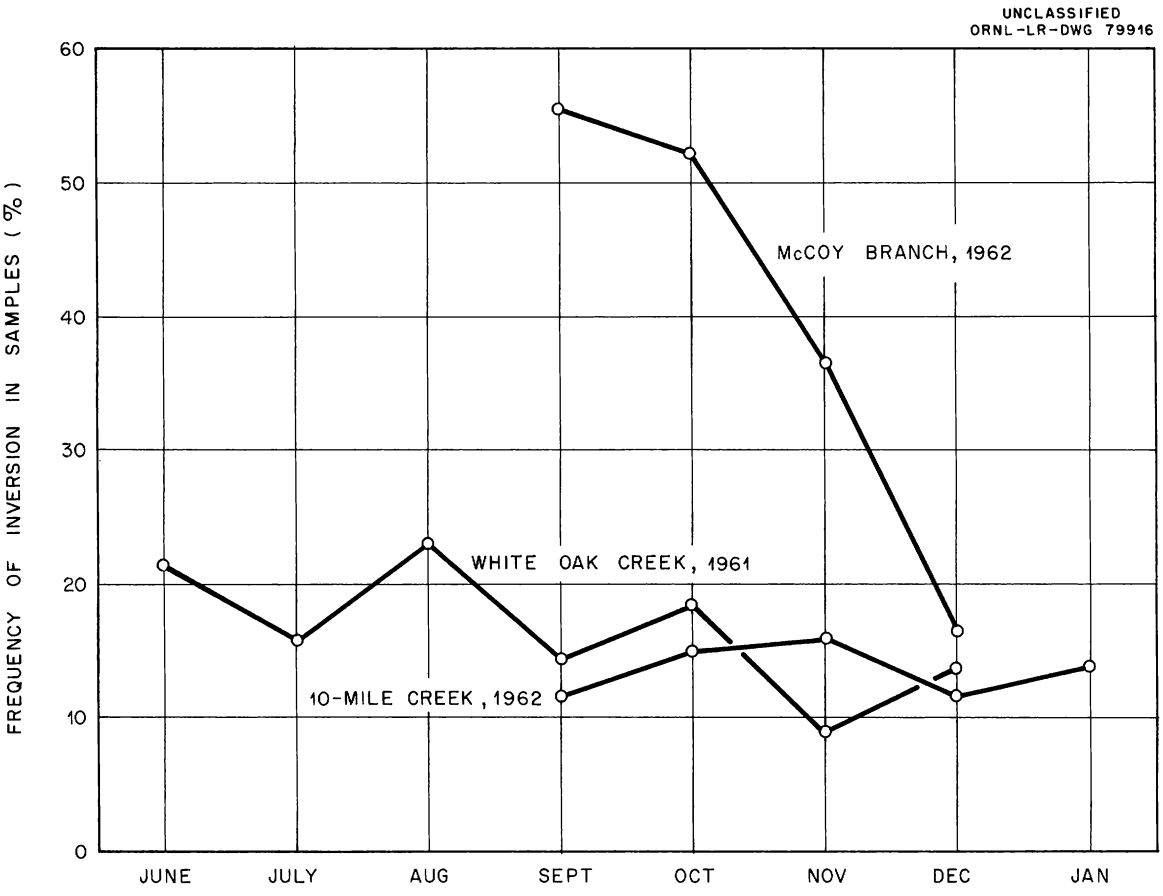


Fig. 14. Seasonal distribution of Inversion 1Ra in three populations of Chironomus tentans.

this decrease cannot be attributed to a climatic factor, such as temperature, which changes seasonally.

The seasonal fluctuation of chromosomal rearrangements in Drosophila pseudoobscura has been established by Dobzhansky (1948). To test this phenomenon in Chironomus, Acton (1957) compared the frequencies of ten inversions found in a population of C. tentans in the British Isles for a period of one year. One of these inversions showed a statistically significant variation; however, Acton was doubtful about the reality of the fluctuation. He concluded that any change in the frequency of these inversions was a gradual one.

The frequency of the heterozygous inversion and the frequency of the inversion 1Ra in the McCoy Branch population showed a statistically significant variation from September to December. Since the variation was not observed in the other two populations tested over the same season, it was not considered a seasonal change. This change in the frequency of the inversion will be discussed later.

There was no indication of a seasonal change in the frequencies of the heterozygous inversions 2Lab and 3Ra in the White Oak Creek and the McCoy Branch populations. These inversions were not tested in the Ten-Mile Creek population because of sample sizes. The frequencies of the inversions 2Lab and 3Ra were not determined because the homozygotes were not scored, but since the frequency of the heterozygotes of 1Ra agreed with the frequency of the inversion 1Ra, it can be assumed that the frequencies of the inversions 2Lab and 3Ra are not influenced by seasonal change.

CHAPTER VIII

GEOGRAPHIC DISTRIBUTION OF INVERSION FREQUENCIES

The frequency of the heterozygous inversion of 1Ra, 2Lab, and 3Ra was tested in contingency tables to determine whether there was a difference in the frequencies between the geographic locations. The expected and observed numbers with the Chi-square values are given in Table VI. Since it was shown that the frequencies of these heterozygous inversions have not changed for three years in the White Oak Creek population, the collection from September to December of 1961 was compared with the collections from the other two areas in 1962. The samples from each location were accumulated and the totals tested. This accumulating of samples was justified since no seasonal variation in the frequency of heterozygous inversions was detected, inversion 1Ra at McCoy Branch being the exception. Also, since the samples were collected during the same period or during a corresponding period, any change caused by climatic factors should have had an equal effect on all three populations.

There was no significant difference in the frequencies of these heterozygous inversions between geographic locations. The frequency of the heterozygous inversion 1Ra from McCoy Branch was not significantly different from that in the other areas, although there was a rapid decrease in the inversion frequency from October to December in this population. It is possible to explain this decrease by the great decrease of homozygous inversions that was found in this population.

TABLE VI

Comparison of the Frequency of Heterozygous Inversions in Populations of *Chironomus tentans* from Different Locations

Locations	White Oak Creek	McCoy Branch	Ten-Mile Creek
Dates	Sept.-Dec., 1961	Sept.-Dec., 1962	Sept.-Dec., 1962
No. Larvae Examined	144	192	71
	Inversion 1Ra		
Obs.	34	55	17
Exp.	37.5	50.0	18.5
		$X^2 = 1.28$ P = 0.53	
	Inversion 2Lab		
Obs.	26	47	14
Exp.	30.8	41.0	15.2
		$X^2 = 2.19$ P = 0.40	
	Inversion 3Ra		
Obs.	21	21	8
Exp.	17.7	23.6	8.7
		$X^2 = 1.09$ P = 0.60	

The frequency of inversion 1Ra in these three populations was tested in a manner similar to that used to test the inversion heterozygotes. The results are given in Table VII. There is a significant difference between the frequency of the inversion at McCoy Branch and at the other two locations. The probability of a greater Chi-square was less than 0.005. Figure 14, page 47, shows that the frequency of inversion 1Ra decreases to approximately the same frequency as that in the other two populations in December: P = 0.72. Table VII shows the results.

It has been observed by other investigators that the frequency of inversions found in Chironomus may vary in populations that are separated by a distance of one mile. Philip (1942) found a significant variation in the frequencies of inversions from different locations in C. dorsalis and C. riparis. Acton (1957) compared the frequencies of ten inversions from seven populations of C. tentans found in the British Isles. The greatest distance between any of these populations was five miles. Statistically significant differences were found in the frequency of some of these inversions between all locations, except two which were located about three-fourths of a mile apart. Acton suggested that the difference in frequencies was caused by the selective value of the inversion in the location, or because of a seasonal fluctuation.

Rothfels and Fairlie (1957) observed that the frequencies of inversions in Tendipes decorus (= Chironomus attenuatus) taken from sites one mile apart on the Don River in Canada were the same; therefore the samples from these sites were considered samples from one

TABLE VII

Comparison of the Frequency of Inversion 1Ra in Populations of *Chironomus tentans* from Different Locations

Location	White Oak Creek	McCoy Branch	Ten-Mile Creek
Dates	Sept.-Dec., 1961	Sept.-Dec., 1962	Sept.-Dec., 1962
No. Larvae Examined	144	192	71
Obs.	38	141	19
Exp.	70.1	93.4	34.5
		$X^2 = 60.7$	$P < .005$

Location	White Oak Creek	Ten-Mile Creek
Dates	Sept.-Dec., 1961	Sept.-Dec., 1962
No. Larvae Examined	144	71
Obs.	38	19
Exp.	38.2	18.8
		$X^2 = 0.06$ $P = 0.47$

Location	White Oak Creek	McCoy Branch	Ten-Mile Creek
Dates	Dec., 1961	Dec., 1962	Dec., 1962
No. Larvae Examined	19	62	26
Obs.	5	20	6
Exp.	5.5	18.0	7.5
		$X^2 = 0.69$	$P = 0.72$

panmictic population. This observation differs from the findings of Acton and Philip, but the environmental conditions probably account for the differences. In the T. decorus populations there were no land barriers as there were between the populations of C. tentans from isolated ponds. Some of the populations from isolated ponds were temporary, since only one sample was obtained before the larvae disappeared. In other instances, analyses were made using one small sample from rain barrels; however, because of the fecundity of the Chironomus female, the entire sample could have been the progeny of one mating. The frequency of the inversions could easily differ because of the sampling method.

In the present study, frequencies of the heterozygous inversions of 1Ra, 2Lab, and 3Ra in the populations from the three geographic areas tested were similar as shown by the Chi-square tests. The distance and land barriers between the Ten-Mile Creek population and the other populations assured that it was a relatively isolated population. The frequencies of the inversions in the collected samples should agree with the frequencies in the populations from these areas, because of the sample sizes and length of the period over which the samples were collected. These findings indicate that 1Ra, 2Lab, and 3Ra are inversions that have been established in these populations for a long time and are endemic to the populations of C. tentans from this area.

CHAPTER IX

ADAPTIVENESS OF INVERSION 1Ra

Since the homozygous inversion of 1Ra could be recognized, the larvae collected from the different populations were scored for the standard arrangement, the heterozygous inversion and homozygous inversion. The expected number of each arrangement was determined by applying the Hardy-Weinberg law. Observed and expected numbers are given in Table VIII along with the calculated Chi-square values. A Chi-square was not calculated for all three classes in the sample from Ten-Mile Creek because the expected number of homozygous inversions was small. If the homozygous inversions are grouped with the heterozygous inversions, and the two classes tested, the Chi-square is not significant. It may be seen by inspection that the observed number of the three classes closely agrees with the expected number (Table VIII).

The inversion 1Ra is at equilibrium in the population at Ten-Mile Creek, but it is not in the other two populations. An inspection of the numbers of the different classes in the White Oak Creek population and McCoy Branch population shows a deficiency of heterozygous inversions. This deficiency was suggested previously when the frequency of the heterozygous inversions between geographic locations was not different but the inversion frequency in the population was. A very rapid decline was observed in the inversion frequency of 1Ra in the McCoy Branch population from October to December 1962 (Figure 14, page 47). This decline was attributed to a significant decrease in the

TABLE VIII

Distribution of Inversion 1Ra Compared to the Expected Frequencies as Determined by the Hardy-Weinberg Law

	Standard Arrangement	Inversion Heterozygote	Inversion Homozygote	No. Larvae Examined
		White Oak Creek June-Dec., 1961		
Obs.	182	52	15	249
Exp.	173.6	68.7	6.7	
		$X^2 = 14.66$	$P < .005$	
		McCoy Branch Sept.-Dec., 1962		
Obs.	94	55	43	192
Exp.	76.8	89.3	25.9	
		$X^2 = 28.30$	$P < .005$	
		Ten-Mile Creek Sept., 1962-Jan., 1963		
Obs.	74	24	1	100
Exp.	74.8	23.4	1.8	

homozygous inversions during this same period (Table IX).

In the theory of balanced chromosomal polymorphism the inversion heterozygote has a higher adaptive value than either homozygote. The previously accepted studies show that the heterozygous inversion is equal to or exceeds the expected number as predicted on the basis of the Hardy-Weinberg Law. The studies on Chironomus dorsalis, Chironomus riparius (Philip, 1942), C. tentans (Beermann, 1952; Acton, 1957) and Tendipes decorus (Rothfels and Fairlie, 1958) agree with the general theory of balanced chromosomal polymorphism.

In the present study the distribution of the chromosomal rearrangement, 1Ra was significantly different from the expected distribution when tested from June through December 1961 in the White Oak Creek population. The distribution of the rearrangement differed even more from the expected distribution in the population at McCoy Branch from September to December 1962. Data in Table V, page 45, show that the inversion frequency of 1Ra is changing from month to month in the McCoy Branch population, but does not change in the White Oak Creek or Ten-Mile Creek population during the same season. No change in the inversion frequency for the chromosomal rearrangement 1Ra in the White Oak Creek population from year to year eliminates the probability that this is a new gene arrangement with a high selective value which is rapidly replacing the standard arrangement. This conclusion is further supported by the finding of the same chromosomal rearrangement distributed according to the Hardy-Weinberg frequencies in the isolated population at Ten-Mile Creek.

TABLE IX

Seasonal Distribution of Homozygous Inversions of 1Ra in the *Chironomus tentans* Population at McCoy Branch

	Sept	Oct	Nov	Dec	No. Larvae Examined
Obs.	3	24	10	6	192
Exp.	2	16.3	10.8	13.9	
			$X^2 = 11.05$	P = 0.012	

Da Cunha (1949) observed a similar situation in abdominal coloration of Drosophila polymorpha found in tropical America. Three types of body coloration are inherited as if caused by two alleles of a single gene. When reared under Laboratory conditions, the frequency of the heterozygote exceeds the expected; but, when observed in natural populations, it is much less than the expected. The proposed explanation of this condition was that the heterozygotes are less viable than the homozygotes in the natural environment, but that the deficient viability was offset in the adult stage by some advantage, such as greater fecundity.

A model has been proposed by Levene (1953), where under certain assumptions genetic equilibrium can be obtained without the heterozygotes being superior to both homozygotes in any single niche. The assumptions are that there be more than one ecological niche with one allele favored in one niche and the other allele favored in the other niche. Gene frequencies are dependent upon the adaptive values of the homozygotes in the different niches. The requirements of the model stipulate that mating is at random in the entire population so that the initial zygotic frequencies are q^2AA, 2q(1-q)AA', and $(1-q)^2$A'A' where A and A' are alleles with gene frequencies of q and 1-q respectively. The zygotes are deposited at random in large numbers in each of the niches, and the resulting individuals cannot move preferentially to niches for which they are better fitted. There is differential mortality ending with a fixed number of individuals in each niche with the frequencies of the allele in each niche dependent upon its adaptive

value in the niche. At the time of reproduction, survivors leave the niche, and mating is at random in the entire population. The assumptions must be made that the individuals cannot select the niche for which they are best fitted, and that there is no tendency for mating to occur within a niche rather than at random over the whole population.

The natural population of C. tentans at McCoy Branch appears to fulfill the conditions of Levene's model. Figure 14, page 47, shows the frequency of inversion 1Ra highest during September and October with the frequency dropping rapidly through December. As is shown in Table IX, page 57, the rapid decrease in the frequency of inversion 1Ra is apparently caused by selection against the homozygous inversion.

As previously described, McCoy Branch drains pasture land before forming a marsh in second growth forest. When the forest was removed, heavy earth moving equipment was brought in with the result that many shallow depressions were made. During the clearing process foliage and debris, primarily from alder trees, were deposited in this area making the stream and shallow water-filled depressions excellent habitats for C. tentans. One or two C. tentans larvae along with many of another species were collected from this location previous to the clearing of the forest. About two or three months after the new habitats were established, a large population of C. tentans larvae was discovered in the stream and the shallow depressions.

Larvae resulting from egg deposition in these shallow depressions were essentially isolated from the rest of the population and restricted to a small area. If the inversion homozygote of 1Ra was better adapted

to the shallow depressions, and the standard arrangement better adapted to the stream, one assumption of Levene's model would be satisfied. There would be at least two kinds of niches with the larvae unable to select the niche for which they were most suited. A factor which might be selective under these conditions could be related to the depth of the water. In the shallow depressions the homozygous inversions would have the selective advantage, and the standard arrangements would be eliminated because the larvae would be restricted to the shallow depth. In the stream where the depth varies, the standard arrangement would have the higher adpative value because of the availability of different kinds of niches.

The mating habits of Chironomus are ideal for Levene's model. Males swarm in the early morning or late evening and females leave the surrounding vegetation to join the swarm for a short time. Mating occurs during the brief time the females are in the swarm. The proximity of these niches suggests that the swarm of males would be composed of individuals from all kinds of niches and that the females from all niches would mate with these males.

In this study an equilibrium of the inversion frequencies was not reached, but the frequency of the homozygote decreased rapidly until December. The frequency of the inversion was the highest when these shallow niches were the most abundant, but the frequency decreased as they were eliminated by cold weather or drying conditions. In December the distribution of the inversion 1Ra did not agree with the Hardy-Weinberg frequencies, but the inversion frequency was the same as the

frequency of 1Ra at White Oak Creek and Ten-Mile Creek (Table VII, page 52). The frequency of the standard arrangement, the heterozygous inversion and the homozygous inversions of 1Ra were significantly different from the expected frequencies as predicted by the Hardy-Weinberg Law. Studies showed a deficiency of inversion heterozygotes, which is contrary to the theory of balanced chromosomal polymorphism. Results from the present study show that chromosomal polymorphism can exist in a natural population without the heterozygotes being superior.

Such an adaptation of a gene arrangement would be highly advantageous to the organism. In most streams, if the selection factor was dependent upon depth as suggested, there would always be niches available to maintain the different chromosomal rearrangements in the population. The inversion would then reach an equilibrium, as did the population at Ten-Mile Creek (Table VIII, page 55). This type of adaptation would be an advantage to organisms like Chironomus, and could explain in part the population peaks in the late spring and fall. The increase in moisture in spring creates many shallow niches which are available for ovaposition. The preadapted gene arrangements in the form of the homozygous inversion are available to take advantage of these niches. The population size would increase as these niches contributed to the adult population, but would decrease as the shallow niches are eliminated by the decrease in moisture in the summer. Likewise, the frequency of the homozygous inversions in the population would increase and decrease accordingly. The availability of these shallow niches could explain the excess of homozygous inversions that was

observed in the White Oak population.

This type of adaptation would also be an advantage to populations of organisms inhabiting a river system that has many small feeder streams. The gene pool of the population would contain a preadapted gene arrangement available to take advantage of the niches provided by the shallow streams.

CHAPTER X

CHROMOSOMAL ABERRATIONS IN IRRADIATED AND CONTROL POPULATIONS

Fifteen different aberrations were found in the irradiated population as compared with five different aberrations in the control populations, but the mean number of aberrations per larva did not differ in any of the populations. The 365 C. tentans larvae from the irradiated population at White Oak Creek contained 194 aberrations or 0.53 aberration per larva, while the 356 larvae from six control areas contained 196 aberrations for an average of 0.55 aberration per larva. Quantitatively, the heterozygosity in the populations was essentially the same, but the variety of aberrations in the irradiated population was three times that of the controls. Three of the endemic inversions accounted for the majority of the aberrations in both the irradiated and control populations (Figure 15). Inversion 1Ra and 2Lab occurred more frequently in the control populations, but there was not a significant difference at the five per cent level when tested by a Chi-square: P = 0.47 and 0.44 respectively. Also, 2Ra, which occurred more frequently in the irradiated population than in the control population, was not significantly different: P = 0.68. The frequencies of the different chromosomal aberrations in the irradiated and control populations are given in Figure 15.

Five different inversions were found in the irradiated and control populations of C. tentans and were considered endemic inversions in the natural populations of East Tennessee. A higher degree of

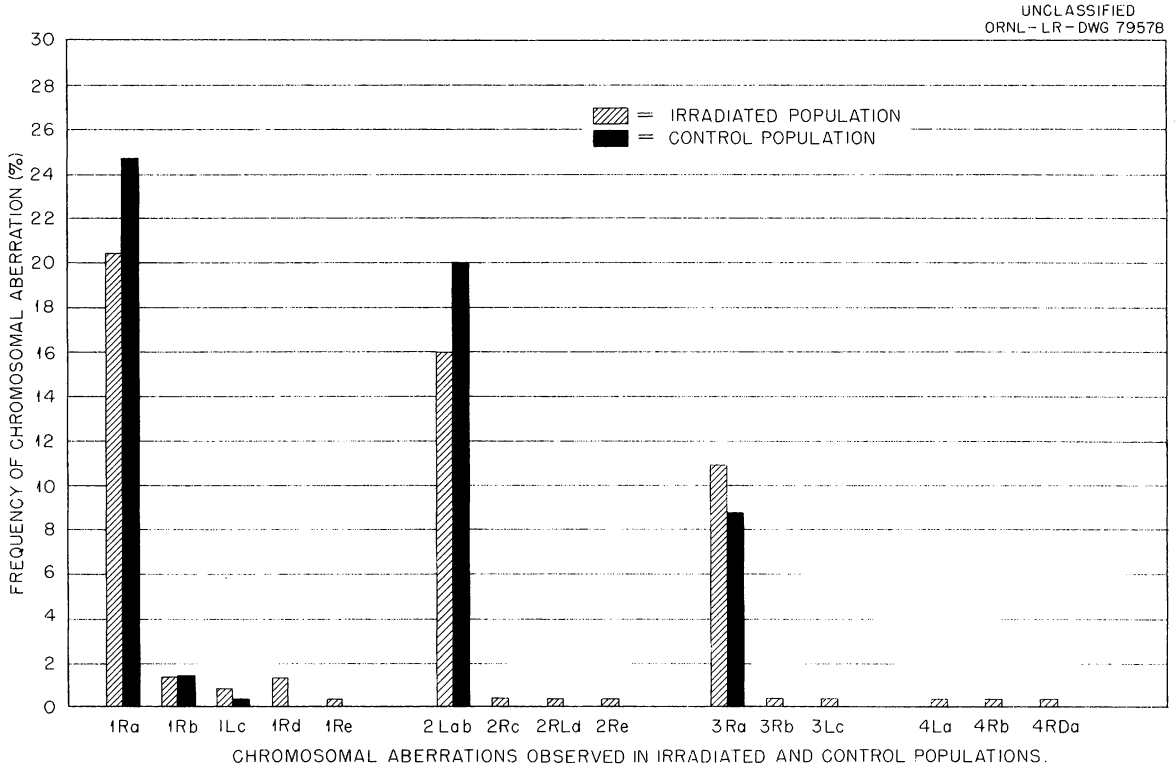


Fig. 15. Chromosomal Aberrations and the Frequencies at which they appeared in the Irradiated and Control Populations of Chironomus tentans.

chromosomal polymorphism occurs in the natural populations of C. tentans found in the British Isles (Acton, 1957). However, the difference in the amount of chromosomal polymorphism in a species may vary from one geographic location to another (Townsend, 1952; Dobzhansky, 1957).

In any sample of larvae one expects to find a few unique aberrations. Generally one expects to find in two samples of approximately the same size equal numbers of unique rearrangements. The finding of nine inversions and one deletion in the irradiated population that were not found in the control population, in samples of the same size, is highly improbable unless chromosomal aberrations were occurring more frequently in the irradiated population. It is reasonable to assume that some of these ten aberrations occur normally in the population of this area at a very low frequency, and would be found in the control populations, if sample sizes were increased. Since the control populations were from six locations and the irradiated population from only one, a larger number of ecological niches was sampled to obtain the control population; hence the probability was greater that the control populations would have retained different highly adapted aberrations. More kinds of aberrations being found in one irradiated population than were found in all six other populations strengthens the conclusion that new chromosomal aberrations are occurring more frequently in the irradiated area.

The presence of ten unique aberrations in the irradiated population adds to the evidence that radiation is affecting the natural population. Of these ten aberrations, nine were observed only once. The low frequency of the majority of these unique aberrations suggests that they

are being eliminated by selection. However, inversion 1Rd was found five times; its unusual frequency will be discussed later. Also the finding of 2RLd strengthens the conclusion that more kinds of chromosomal aberrations are occurring in the irradiated population since it is a pericentric inversion. Pericentric inversions are rare in natural populations, because they give rise to grossly duplicated and deficient crossover products that are zygote lethal. Acton (1957) reported the finding of one larva possessing a pericentric inversion. It is very unlikely that the pericentric inversion 2RLd is endemic to this area; more probably it has just occurred in the irradiated population.

This study preserts the first cytological analysis of a natural population that has been exposed to chronic radiation from radioactive waste, although some related experiments have been conducted in the laboratory and on natural populations. Wallace (1956) reported on the genetic analysis of laboratory populations of Drosophila melanogaster which were exposed to chronic gamma radiation. Under the direction of Stone et al. (1962) the effects of direct and fall-out radiation on natural populations of Drosophila ananassae were studied on the islands in the Atomic Energy Commission Pacific Proving Grounds. Winge et al. (1961) and Corderio (1961) made genetical and cytological analyses of isolated populations of Drosophila willistoni which had received large releases of progeny from heavily irradiated flies. All of these populations that were analyzed cytologically had received large acute doses of radiation or relatively high chronic doses. Seecof (in Stone et al., 1957) detected no increase of new chromosomal aberrations in the heavily

irradiated natural populations of D. ananassae. Paget (1954), however, observed some new aberrations at a high frequency (14.5 per cent) in Wallace's populations that had been exposed to 5.1 r per hour of chronic irradiation. Corderio (1961) found two new inversions restricted to the irradiated natural populations as well as a decrease in chromosomal polymorphism.

An increase of new chromosomal aberrations was not detected in the irradiated natural populations of D. ananassae. The larvae examined were from crosses of collected wild males mated with standard females or F_1 males from collected wild fertile females. However, from laboratory experiments Stone et al. (1957) found that new aberrations could be introduced into these natural populations by ionizing radiation, but concluded that most of the aberrations were eliminated rapidly and would not have been detected by their method of examination. In the present study, C. tentans larvae taken directly from the radioactive bottom sediments were examined cytologically. In this respect Chironomus is an excellent study organism because good salivary squashes can be obtained from larvae collected from their natural environment. This gives the advantage of being able to observe newly occurring aberrations viable only in the larva stage.

In the laboratory populations of Drosophila melanogaster that had received 5.1 r per hour of gamma radiation, Paget (1954) observed a new inversion that occurred at a high frequency (9 per cent). The occurrence of this inversion was enhanced by radiation and the frequency maintained in the population by selection. In the White Oak Creek area

no newly arisen chromosomal aberration occurring at a high frequency was detected in the irradiated population of C. tentans. The inversion 1Lc that was found three times in the contaminated area and only one time in the control area at McCoy Branch could have occurred in the irradiated population and then spread to the control population. But it is more reasonable to assume that this is an inversion endemic in this area because 1Lc was found at a very low frequency in the irradiated population, and migration from White Oak Creek to McCoy Branch is unlikely because of distance.

Inversion 1Rd, which was found only five times during a six week period and not found in the control population, probably arose in the irradiated population. The inversion could have occurred in a gonial cell of one individual and then been eliminated by selection or genetic drift. This supposition is further supported by the fact that 1Rd was found each time with inversion 1Ra. Since more kinds of aberrations are occurring in the irradiated population, all other factors being equal, the probability of an arrangement with a higher adaptive value occurring is greater in the irradiated population than in the control populations.

In comparing the results found in C. tentans from White Oak Creek with the results from related experiments, the life cycle of the different organisms and the dose rate of radiation must be considered. In most of the previous experiments the populations were exposed to large external doses (5.1 r per hr. - 1-2 Kr). However, in White Oak Creek the Chironomus larvae are exposed to environmental radiation

approximately 1000 times background (230 rads per year) from egg deposition to the emergence of adults. Not only are the larvae living in the radioactive bottom sediments, but they are also ingesting radionuclides with their food. Therefore the absorbed dose rate may be higher than that calculated because no consideration was given the fact that the larvae may accumulate some of the radionuclides in their tissues.

More kinds of chromosomal aberrations may be found in Chironomus larvae exposed to chronic irradiation than in other organisms, because mature germ cells are found in the fourth instar larva (Abule-Nasr, 1950). The postmeiotic chromosomes are the most sensitive to radiation induced breaks (Oster, 1955). Restitution of breaks that occur in the postmeiotic chromosomes of a spermatozoon does not take place until fertilization. Therefore, a chromosome break occurring in the spermatozoa of a Chironomus larva could remain open for a relatively long period of time. The probability of two breaks occurring in a spermatozoon of a larva exposed to chronic irradiation increases with exposure time. Since larva in White Oak Creek can remain in the fourth instar stage for a long time and accumulate a relatively large dose of radiation, the probability of the occurrence of chromosomal aberrations is increased.

The natural population of C. tentans inhabiting White Oak Creek has been exposed to chronic low-level irradiation since 1943. Since there is an apparent increase in chromosomal aberrations, it follows that there is a greater increase of one-hit mutations, because one-hit mutations show a linear relationship to dose while the frequency of two-hit aberrations is nearly proportional to the square of the dose at high intensities. The survival of this population is not unexpected

since Wallace (1956) successfully maintained populations of another species of Diptera, Drosophila melanogaster, exposed to enormously higher doses administered continuously at the rate of 5.1 r per hour for over a hundred generations.

Populations of Drosophila and Chironomus have maintained themselves for over a hundred generations when exposed to a high irradiation background. However, before a general hypothesis can be established on the effects of chronic low-level radiation on a natural population, information on other organisms is needed. The need for this type of information is further emphasized by the finding of a difference in mutation rates between mice and Drosophila in response to irradiation (Russell, 1956; Russell and Russell, 1958). The life cycle and other aspects of the organisms must also be considered, especially the fecundity of the individuals. Organisms that produce a large number of progeny can more easily compensate for the loss of individuals from lethal genes and benefit from the elimination of deleterious genes from their gene pool by natural selection. However, in humans (Muller, 1959), where natural selection against many traits has been relaxed and many of the deleterious genes are protected by technology, a small increase in mutation rate may produce a great burden on our society. Therefore, the need for information regarding the effect of chronic low-level radiation on natural populations is immediately recognized.

In the present study, observation of the salivary-gland chromosomes of C. tentans for aberrations has permitted the detection of an effect of chronic low-level irradiation from radioactive waste on a natural population.

CHAPTER XI

SUMMARY AND CONCLUSIONS

The salivary gland chromosomes of Chironomus tentans larvae collected from White Oak Creek, an area contaminated by radioactive waste from the Oak Ridge National Laboratory, and from six uncontaminated areas were examined for chromosomal aberrations. White Oak Creek populations were exposed to absorbed doses as high as 230 rads per year or about 1000 times background. Chromosomal maps were constructed to make a general comparison of the banding pattern of the salivary chromosomes of the C. tentans in the East Tennessee area with those of Canada and Europe. These maps were used as a reference in scoring aberrations.

Fifteen different chromosomal aberrations were found in 365 larvae taken from the irradiated population as compared with five different aberrations observed in 356 larvae from six control populations, but the mean number of aberrations per larva did not differ in any of the populations. The quantitative amount of heterozygosity was essentially the same in the irradiated and the control population, but there were three times the variety of chromosomal aberrations found in the irradiated area. From this evidence it was concluded that chronic low-level irradiation from radioactive waste was increasing the variability of chromosomal aberrations without significantly increasing the frequency.

Five different inversions were found in both the irradiated and control populations; three inversions--1 Ra, 2Lab, and 3Ra--were found at a relatively high frequency and were used in testing the seasonal

and geographic distribution. In the White Oak Creek population the frequencies of the heterozygous inversions did not change significantly for a three year period from 1960 to 1962. Also, no changes that could be attributed to seasonal change were detected in the frequencies of these inversions from June through December of 1961 and September through December of 1962. There was no significant difference in the frequencies of these same heterozygous inversions between three local geographic populations of C. tentans. It was concluded that the three inversions which occurred at the highest frequencies are endemic to the population of East Tennessee and have been established in the population for a long time.

The frequencies of the standard arrangement, the heterozygous inversion and the homozygous inversion of 1Ra were significantly different from the expected frequencies as predicted by the Hardy-Weinberg Law. A deficiency of inversion heterozygotes was found which is contrary to the theory of balanced chromosomal polymorphism, but is consistent with a model proposed by Levene, where under certain assumptions genetic equilibrium can be obtained without the heterozygote being superior. The results show that chromosomal polymorphism can be maintained in a natural population without superiority of the heterozygous individuals.

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