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A cytogenetic examination of eight species of Tribolium (Coleoptera: Tenebrionidae)

Lisa Anne Shimeld

A CYTOGENETIC EXAMINATION OF EIGHT SPECIES OF TRIBOLIUM (COLEOPTERA: TENEBRIONIDAE).

A Thesis

Presented to the

Faculty of

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In Partial Fulfillment

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ABSTRACT

A technique was developed to make permanent preparations of Tribolium chromosomes. After dissection testes are hypotonically treated with Simmons citrate, fixed in 3:1 methanol and glacial acetic acid, and are spread along the surface of a slide that has been covered with fixative. Utilizing this technique eight species of Tribolium representing three species-groups were chromosomally examined. In the castaneum species-group T. castaneum and T. freemani have 2N = 20 chromosomes and a 9 + Xy_p meioformula. T. audax and T. madens have 2N = 20 chromosomes and supernumeraries; four are seen in T. audax and ten in T. madens. The meioformula of T. audax is 9 + Xy_p + BII 1 + BI 1, and T. madens is 9 + Xy_p + BII 3 + BI 2. In the confusum species-group T. confusum, T. destructor, and T. anaphe have 2N = 18 chromosomes. T. confusum has an 8 + neo-XY meioformula while T. destructor and T. anaphe have nine bivalents with no heteromorphic sex chromosomes identified. T. brevicornis, of the brevicornis species-group had 2N = 18 and nine bivalents during metaphase I. No heteromorphic sex bivalent was identified. Measurements of meiotic chromosomes revealed significant differences in size intraspecifically and interspecifically.

ACKNOWLEDGEMENTS

This thesis is dedicated to my husband Brad Johnson for his faith in me and his patience throughout this project. I would also like to thank the following individuals. First, to my master professor, Dr. Alexander Sokoloff for his kindness, guidance and vision of the future. Secondly to Lynne Haskell for her assistance in the preparation of the beetles for this study, and finally to everyone else who without their help this project would not have been possible. Additional thanks to the faculty and staff of the Biology Department for their support.

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INTRODUCTION

Tribolium flour beetles are important pests of stored grains and cereal products. Similar beetles have been associated with humans for as long as seeds have been stored to prevent starvation. Records from Shakespeare's day show that on the long voyages of Elizabethan mariners, food stores were liable to be damaged by "stored product" beetles, generally known as weevils (Crowson, 1981).

Not all associations of beetles and man are negative; examples from some of the most primitive recent human tribes suggest that beetle larvae may have been a significant element in the diets of many paleolithic peoples (Crowson, 1981). In addition to this, the use of Tribolium as material for diverse laboratory and experimental investigations is long established. Flour beetles are readily available and culturable, and Tribolium is utilized widely by many researchers today.

The genus Tribolium contains over thirty species, and is a member of the order Coleoptera. This order contains the beetles and weevils and consists of four suborders 1) Adephaga, 2) Archostemata, 3) Myxophaga and 4) Polyphaga. Coleoptera would have to be considered the most sucessful of insect orders if the number of representative species is significant. There have been over 300,000 species of beetles described, making up over 25% of all cataloged insects. Over 1000 new species of Coleoptera are described each year

(Sokoloff, 1972). In regards to the abundance of Coleopteran species, Crowson (1981) quotes T.H. Huxley "that one thing we know about a divine Creator, supposing one to exist, is that he has a particular interest in Coleoptera", this remark is true even today.

The suborder Polyphaga contains 150 - 170 families of beetles. The genus Tribolium belongs to the Tenebrionidae (Crowson, 1981). Members of Tenebrionidae are versatile. Adults and larvae can be found in diverse habitats (with the exception of aquatic ones) including, rotten wood, the undersides of logs or rocks, and even in the arid deserts of Africa and the American southwest. They feed on decaying vegetable matter, animal waste products, seeds, cereals, fungi and living plants. Included in Tenebrionidae is the subfamily, Tenebrioninae, and the tribe Ulomini, which contains the genus Tribolium, and the other tenebrionid flour beetles that constitute the important pests of stored products (Sokoloff, 1972). In 1948 Hinton examined the relationships of these beetles and grouped the thirty species of Tribolium into five species-groups (Figure 1). These species-groups are associated with geographical regions and are included in Table 2.

Chromosomal studies of beetles have been undertaken but are difficult because of the small size of the cells; cytologically their chromosomes are more difficult to work with compared to those of many other insect orders. (Smith,

1952b; Crowson, 1981; Camacho, 1982; Garber, 1972). In the absence of "giant chromosomes", and of true salivary glands, cytogenetic studies in beetles must rely on other tissues as a source of chromosomes. Cell divisions of non-germinal tissues are rare in adult beetles, being limited primarily to regeneration of the mid-gut epithelium, and blood or hypodermal cells in the process of wound healing (Crowson, 1981). This limits the choice of tissues suitable for cytogenetic investigations.

The target tissue chosen for cytogenetic study of tenebrionid beetles must provide a source of rapidly dividing cells to improve the chances of finding sufficient metaphase chromosomes. Metaphase chromosomes are desirable because it is during this stage that the chromatin is most highly condensed and comparisons of chromosomes are easiest to make. The final divisions in gametogenesis in most beetles takes place during, and usually early in, adult life. However, the long adult lives of Tribolium involve more than one period of reproductive activity making these tissues an excellent source of rapidly dividing cells.

Spermatogonial tissues are preferred over oogonial tissues for two reasons. First, by examining spermatogonial tissues at metaphase I it may be possible to determine the condition of the X and Y chromosomes and any pairing associations that have occurred. Secondly, because spermatogenesis continues throughout the reproductive life of

male Tribolium beetles these tissues almost always contain actively dividing cells. Division of nongerminal tissue is rare in Coleopterans, therefore most cytogenetic investigations have focused on the meiotic stages of reproductive tissues. Spermatogenesis is a more rapid process than oogenesis providing more metaphase cells and thus making testes the best choice for chromosomal studies. The choice of spermatogonial tissue determines that most observations will focus on meiotic cells.

An interesting attribute of beetles is that a definite chromosome complement of their ancestral form can be postulated with a high degree of probability. This form persists in a considerable percentage of the recent species (Smith, 1952b; Crowson, 1981; Juan and Petitpierre, 1988). Four of the eight species involved in this study were cytologically examined by S.G. Smith in 1952 (Figure 3). The four species were T. castaneum, T. confusum, T. madens, and T. destructor. In addition to his work on Tribolium beetles, Smith did extensive analyses within the order Coleoptera. Of the approximately 25,000 species of Coleoptera in North America representing about 150 families, Smith has reported on at least 191 species from 66 families. Based on the results of these studies, Smith (1952b) concluded that the primitive number of chromosomes for Coleoptera consists of nine pairs of autosomes, an X approximately the size of the autosomes, and a minute Y. During metaphase I the sex

chromosomes are V-shaped and are connected at two terminal points in a parachute-like formation. This association is denoted, Xy_p and was observed by Smith in most members of the superfamily, Tenebroinoidea. According to Smith (1953) members of the order, Coleoptera usually display metacentric or acrocentric centromeres. His observations are supported by the more recent findings of Crowson (1981), and Juan and Petitpierre (1988). Smith's cytological examinations of the genus Tribolium determined that T. castaneum and T. madens are consistent with the primitive condition. Both species have nine pairs of autosomes and display the Xy_p association of the sex chromosomes. In addition to this T. madens possesses five small supernumeraries whose origins and function are still unknown.

Smith (1952b) denotes the supernumeraries as either BII or BI. "B" refers to B-chromosomes, but he does not elaborate as to the meaning of II or I. It is apparent that the supernumeraries of T. madens are of two distinct morphological types. Three of them are metacentric and consequently have two lobes. The two remaining supernumeraries are telocentric and therefore single-lobed. Bipartite, metacentric supernumeraries will be denoted as BII and single-lobed ones as BI in this study.

White (1954) suggests that supernumeraries, originated not from the disintegration of autosomes but from fusions or fragmentations of them. These supernumeraries are referred to

as B-chromosomes or accessory chromosomes, and are comprised primarily of heterochromatin. When this condition occurs, it is not always consistent throughout the population (White, 1954; Blackwood, 1956; Catcheside, 1956; Swanson, 1967). Many types of supernumeraries have been described in insects and other groups of animals (White, 1976; Ostergren, 1947; Lewis, 1957). Considerable differences exist in the morphology and behavior of these supernumeraries at mitosis and meiosis.

The presence or absence of supernumeraries doesn't seem to affect phenotypic expression of the organism (Catcheside, 1956). The presence of supernumeraries probably has some effect on viability or fertility or in other ways too subtle to notice (Crowson, 1967; Gresson, 1948; Waddington, 1957).

In addition to the supernumeraries described in T. madens, Smith (1952b) observed other exceptions to the usual number of chromosomes observed in most Coleopterans. Some species have fewer chromosomes, a condition he regards as derived from the earlier one. He hypothesized that this situation resulted from the translocation of the sex chromosomes to a pair of autosomes, and refers to this larger complex as the neo-X and neo-Y. In spermatogonial metaphase preparations of T. confusum Smith observed that the neo-X is often J-shaped and larger than any other member of the complement, and the neo-Y is relatively small and telocentric. The location of the centromere in the neo-Y of T. confusum supports Smith's hypothesis as to its origins. It

is generally accepted that acrocentric or telocentric chromosomes are more recent than metacentric chromosomes in the phylogeny of a species indicating the development of the neo-Y occurred rather recently. (White, 1954).

The staining reactions of the neo-X and neo-Y offer further support of Smith's hypothesis. When stained with Feulgen fuchsin the primitive X, as seen in T. castaneum, exhibits both euchromatin and heterochromatin. The neo-X observed in T. confusum and T. destructor show similar staining activity, but both are larger than the primitive X. This indicates that the neo-X consists of more functional genes than does the primitive one.

During metaphase I, the neo-X and neo-Y form a heteromorphic pair, the neo-XY. When stained with Feulgen fuchsin, Smith observed that the XY bivalent in T. confusum had three major components; the differential arm of the X that was positively heteropycnotic at pachytene but could not be distinguished from the autosomes at metaphase; the pairing arm of the X that was euchromatic at pachytene and metaphase; and the Y chromosome which was indistinguishable from euchromatin at pachytene but was negatively heteropycnotic at metaphase.

Smith feels that the lack of heteropycnosis in T. confusum's neo-Y indicates that the chromosome is genetically inert. He hypothesizes that T. confusum represents an intermediate form and that this situation occurred at the

expense of the euchromatin of the autosome involved in the translocation. The neo-Y observed in T. destructor lacks this heteropycnotic section so Smith suggests that T. destructor must have evolved from T. confusum, and that the genes that were lost were probably inert at that time.

The neo-XY or a similar condition was also observed in T. brevicornis and T. anaphe by Moore and Sokoloff (1982). They observed a diploid number of 18 chromosomes in both species. These chromosomes were of similar size during spermatogonial metaphase, but a structure resembling the neo-XY observed in T. confusum by Smith (1952b) was seen in a metaphase I preparation in T. brevicornis (Figure 4).

After studying over 24 American species of Coleoptera, Crowson (1981) also concluded that the basic complement consists of nine pairs of autosomes, a fairly large X and a small y which associates with the X in meiosis to form the Xy_p bivalent. This type of X-y association occurs in some other primitive insect types, and is suspected to have been a feature of the ancestors of Coleoptera at the beginning of the Permian period.

Given this basic number of nine pairs of autosomes and a sex bivalent, Crowson feels that the chromosomes of beetles demonstrate the principle that with a reasonably small starting number of chromosomes, increases in number are considerably more frequent than decreases throughout the course of evolutionary history. Some exceptions to this

theory are demonstrated by the findings of Juan and Petitpierre (1988) and within the genus Tribolium.

Juan and Petitpierre (1988) studied twenty species of Mediterranean tenebrionids and reported diploid numbers of 18 and 20 chromosomes. Although most of the species that were examined had 9 + Xy_p meioformulas, some with 8 + Xy_p and one species with a 9 + Xy meioformula were reported. The reduction in chromosome number in the species with an 8 + Xy_p meioformulas is due to the loss of a pair of autosomes, and is similar to the situation observed in some Tribolium species.

Virkki (1974) postulates that the Xy_p condition displayed by many Coleopteran species may have arisen from Xy or XY ancestors. In Coleoptera the Xy_p association is believed to have involved a nucleolus. According to Virkki, this association was responsible for the formation of the Xy_p complex. In Xy or XY types the persistence of this nucleolar association makes possible a reversion to the Xy_p association.

Crowson (1981) hypothesizes that prior to the Xy_p condition the original sex-determining pair consisted of an X the size of an autosome and a small y, but no nucleolus. He feels that the most significant difference in the development of this trait was the absence or presence of a nucleolus in association with the y-chromosome in meiosis.In situations where the nucleolus was lost, the only possible kind of sex

bivalent which could develop was an XY situation which was observed and described by Smith (1952b) as the neo-X and neo-Y. This new sex bivalent relies on a pairing segment of the autosomes to which they have translocated to, for the association of the sex chromosomes.

The present study involved the development of a technique to karyotype Tribolium beetles. Once established, the procedure was applied to the eight species of Tribolium beetles available at the California State University Tribolium Stock Center. Utilizing this technique, the chromosomes of each of the eight species of Tribolium was chromosomally examined. The diploid number of chromosomes for each species, the number of autosomes, and the condition of the sex chromosomes during metaphase I was determined. These results were then compared to those obtained by Smith (1952b), and Moore and Sokoloff (1982).

In her studies of Blattella, Dr. Mary H.Ross (1986) noted the difficulty in determining the centromere location utilizing current cytogenetic techniques. She did however note pronounced differences in chromosome length when comparing the autosomes of Blatella to each other. Juan and Petitpierre (1988) also utilized the measurement and analysis of chromosome length in their study of tenebrionid beetles. They determined that the differences in chromosome

length were significant enough to distinguish one species from another based on that characteristic. The length of Tribolium chromosomes will be examined in this study.

MATERIALS AND METHODS

The following technique provides a simple method of producing permanent preparations of *Tribolium* chromosomes. The process requires less than two hours to complete, and after staining, the slides are ready for immediate observation. The tissue is completely fixed and dried and the slides last for an indefinite period of time allowing for examination at a later date.

The insects used in this study were the eight species of *Tribolium* available at the *Tribolium* Stock Center at California State University, San Bernardino. These beetles represent 3 species-groups and are listed in Table 1.

Table 1 The eight species of *Tribolium* included in this study.

Species-group	Species included in this study
1. Brevicornis	*Tribolium brevicornis*
2. Confusum	*Tribolium confusum* *Tribolium anaphe* *Tribolium destructor*
3. Castaneum	*Tribolium castaneum* *Tribolium madens* *Tribolium audax* *Tribolium freemani*

Testes were removed from adult males by microdissection in a drop of Insect Ringers solution. The tissue was transfered to a hypotonic solution of sodium citrate where it should remain for 20 minutes. This process results in swelling of the chromosomes, making them easier to observe.

The tissue was fixed for a minimum of one hour in a mixture of 3 parts absolute methanol and 1 part glacial acetic acid (Baragaño, 1978; Brown, 1972; Jones, 1962). Before using, microscope slides were thoroughly cleaned with methanol. After cleaning, several drops of fixative were applied to one end of the slide, and it was tilted so that the solution spread evenly across the surface.

Two or three testes were placed at one end of the slide (Figure 1-a). A second slide was aligned over the first at a 90 degree angle (Figure 1-b), and gentle pressure applied to spread the tissue. The top slide held at at a 45 degree angle above the bottom slide was turned and lifted (Figure 1-c). The top slide was then dragged over the bottom one to spread the material evenly across the surface (Figure 1-d). The preparation was allowed to air dry and it was then stained with 10% Giemsa for 20 minutes. The slide was then rinsed, air dried and observed under oil immersion.

A Nikon phase-contrast microscope was used to study the chromosomes. Photographs were taken using a green filter with a Nikon 35mm camera. Technical Pan 2415 film was employed with printing on high contrast paper.

Chromosome measurements were made with an occular micrometer. For each of the eight species included in this study the chromosomes from six metaphase I cells were measured. The results of these measurements were analyzed with the Tukey test. Significance was determined at the 5% level.

Using this method I was able to examine the chromosomes of eight species of Tribolium flour beetles. Although no other insect groups have been examined using this technique it should provide a suitable means for other cytogenetic investigations.

REAGENTS

1. Sodium citrate hypotonic solution - 1% solution in distilled H_2O.
2. Insect Ringers solution - To 100 ml distilled H_2O add: 0.65g NaCl, 0.042g KCl, and 0.025g $CaCl_2$.
3. Fixative - 3 parts absolute methanol to 1 part glacial acetic acid. Prepare fresh daily.
4. Giemsa stain - 10% Harleco Giemsa.

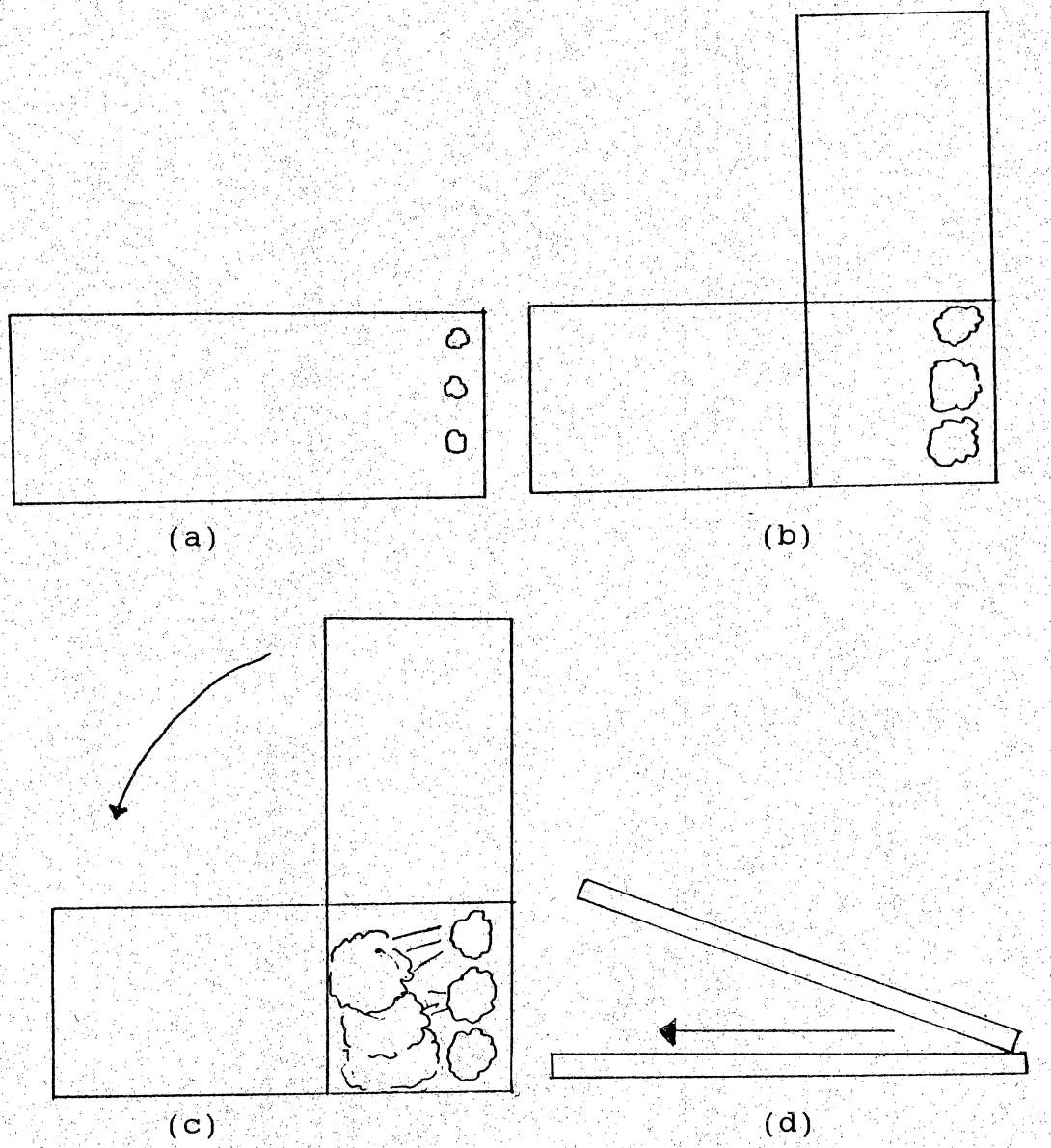


Figure 1. Preparation of slides. (a) Place several testes at one end of the slide. (b) Place a second slide over the first at a 90 degree angle, apply gentle pressure to spread the tissue. (c) Turn and lift the top slide. (d) Drag the top slide across the bottom one to spread the tissue evenly across the surface.

RESULTS

Chromosomes were observed at spermatogonial metaphase and metaphase I (Figures 5-20). Table 3 lists the species surveyed and the number of mitotic and meiotic cells that were examined. The chromosomes of six metaphase I cells were measured for each of the eight species involved in this study and are recorded in Tables 4-11. The chromosomes of the eight species were compared intraspecifically and interspecifically using Tukey's analysis. These results are recorded in Tables 12-27.

CASTANEUM SPECIES-GROUP

Four members of the castaneum species-group were examined, T. castaneum, T. freemani, T. madens, and T. audax. All four species have 2N = 20 chromosomes and Xy_p meioformulas, but the karyotypes of T. audax and T. madens contain additional supernumerary members. The bivalents of all four species are rather uniform in size, and metacentric centromeres are predominant.

T. castaneum and T. freemani both have 2N = 20 chromosomes. The chromosomes of T. castaneum at spermatogonial metaphase are all bipartite, and most are metacentrics. The most conspicuous exception is the small y-chromosome (Figure 5). T. freemani's mitotic metaphase chromosomes are also primarily metacentric, but several quadripartite members were identified. The y-chromosomes is

again the smallest member of the complement (Figure 7).

T. audax and T. madens also have 2N = 20 chromosomes but their karyotypes contain additional supernumerary chromosomes. During mitotic metaphase the karyotype of T. audax contained four supernumerary elements. Two of these are bipartite metacentrics while the other two elements are telocentric (Figure 9). The supernumeraries are somewhat smaller than the autosomes but not obviously so, this makes it difficult to distinguish them from each other during mitotic metaphase. Ten supernumeraries are present in spermatogonial metaphase karyotypes of T. madens, six bipartite metacentrics, two acrocentrics and two telocentrics (Figure 11). As was the case with T. audax, there was a small difference in size between the autosomes and the supernumeraries.

Meiotic preparations of T. castaneum and T. freemani were similar (Figures 6 and 8). Both species have pre- dominately metacentric autosomes and the sex chromosomes associate in the parachute formation. T. castaneum metaphase I cells exhibited a 9 + Xy_p meioformula with metacentric autosomes averaging from 2.0 - 3.83 microns in length. The Xy_p sex bivalent averaged 1.42 microns (table 8). Measurements of T. freemani metaphase I autosomes average from 1.04 - 3.0 microns in length, most of these having metacentric centromeres. The sex chromosomes are associated

in an Xy_p bivalent and averaged slightly less than one micron long (Table 5).

Metaphase I preparations of T. audax contain two supernumeraries in addition to the 9 + Xy_p chromosomes (Figure 10). One of these is bipartite and metacentric, and the other is small and telocentric. Autosomes average 1.92 - 3.58 microns in length, the supernumeraries average 1.00 and 1.08, and the Xy_p was 1.0 micron long (Table 10). The supernumeraries are easier to distinguish from the autosomes in meiotic metaphase than they are in mitotic metaphase.

T. madens displayed five supernumeraries during meiotic metaphase (Figure 12). Three are metacentric and the remaining two are small and probably telocentric, all are conspicuously smaller than the autosomes. The autosomes average from 1.5 - 4.25 microns in length, the supernumeraries ranged from 0.875 - 1.13, and the Xy_p bivalent averaged 0.79 microns (Table 11).

CONFUSUM SPECIES-GROUP

The three members of the confusum species-group that were examined include T. confusum, T. destructor, and T. anaphe. All three species have 2N = 18 chromosomes but some variation in their meioformulas was observed.

During spermatogonial metaphase T. confusum karyotypes consist of 16 autosomes, most of these are metacentric and all are bipartite. The X is metacentric, bipartite and is

larger than the autosomes. The y is a large telocentric chromosome, approximately the same size as some of the autosomes (Figure 13).

Karyotypes of T. destructor contain 16 metacentric, bipartite chromosomes, a large submetacentric X, and a y that is slightly smaller than the large arm of the X (Figure 15). T. anaphe karyotypes (Figure 17) are similar to these except that the X-chromosome is quadripartite and the y-chromosome is not as large as the one observed in T. confusum.

Metaphase I preparations of T. destructor often display a conspicuously large, submetacentric autosome (Figure 16). The remaining chromosomes are metacentric or acrocentric, and all of them are bipartite. It was not possible to distinguish the Xy complex from the other bivalents based on morphology or differences in staining characteristics.

T. anaphe's meiotic cells lack the large autosome that was evident in T. destructor. Metacentric, submetacentric and telocentric, bipartite chromosomes make up the complement (Figure 18). Some metaphase I karyotypes contain a bivalent that resembled the Xy_p association. This structure is not apparent in all of the cells that were examined.

BREVICORNIS SPECIES-GROUP

Only one member, T. brevicornis of this species-group was available for this study. Spermatogonial metaphase karyotypes contain 18 members (figure 19). Most of the chromosomes are metacentric and bipartite although some quadripartite

bivalents were identified. It is difficult to distinguish the X-chromosome from the autosomes due to the similarities in morphology and staining. The y-chromosome is large, approximately the size of the autosomes.

During metaphase I, nine metacentric, bipartite chromosomes were observed (Figure 20). The chromosomes are uniform in size and it is not possible to distinguish a heteromorphic sex bivalent. These results agree with those reported by Moore and Sokoloff (1982).

DISCUSSION

The eight species of Tribolium investigated in this study are consistent with the other tenebrionids that have been previously examined (Smith, 1952b; Moore and Sokoloff, 1982; and Juan and Petitpierre, 1988). Diploid numbers of 18 and 20 chromosomes and three meioformulas, 9 + Xy_p, 8 + neo-XY, and 9 autosomes with no heteromorphic sex pair identified.

CASTANEUM SPECIES-GROUP

Tribolium castaneum

Examination of Tribolium castaneum spermatogonial metaphase chromosomes revealed metacentric centromeres in most of the autosomes, with some acrocentric and telocentric members identified. Almost all of the autosomes are bipartite. The X is metacentric, bipartite, and approximately the size of the autosomes, while the y is small and telocentric. These results are the same as those obtained by Smith (1952b) in his study of the cytogenetic characteristics of T. castaneum (Figure 3).

Metaphase I chromosomes of T. castaneum are predominantly metacentric. When comparing the chromosomes to each other significant differences in length were observed between all members except 4 and 5, and 7 and 8. The Xy_p bivalent is significantly smaller than autosome number 9. The X stains darkly and the y appears as a thin loop attached to the X at terminal ends.

Tribolium freemani

Although the mitotic autosomes of T. freemani are similar in size, some morphological differences were noted. Variations include the position of the centromere and the number of arms per chromosome. Most of the autosomes are metacentric, although submetacentric and acrocentric members were observed. Both bipartite and quadripartite chromosomes were identified. The X-chromosome is blocky, bipartite, and has a submetacentric centromere, while the the y is small and acrocentric. This is the first time that T. freemani has been cytologically examined.

Most of the meiotic metaphase chromosomes are metacentric and difficult to distinguish based on morphology. Tukey's analysis of chromosome length showed that there are no significant differences between most of the autosomes (Table 21). There was not a significant difference in length between the Xy_p bivalent and chromosome number 9 but differences in morphology allow a distinction to be made. The Xy_p consists of a relatively large, dark staining X and a small, closely associated y.

Tribolium audax

Diploid cells of T. audax contain 2N = 20 chromosomes and four supernumeraries. Most members of the complement are metacentric, except for the small, acrocentric y. Two of the supernumeraries are metacentric, and are denoted BII 1 and BII2, and the two others are telocentric and are denoted BI 1

and BI 2. The supernumeraries are not much smaller than the autosomes and therefore are difficult to distinguish from them by size during spermatogonial metaphase. T. audax has been previously examined by Shaw (Sokoloff, 1972) who observed nine autosome pairs, plus three pairs of supernumeraries, plus Xy_p.

T. audax's meioformula consists of nine autosomes, the Xy_p bivalent and two supernumeraries, denoted BII 1 and BI 1. One of the supernumeraries is metacentric and the other is telocentric. There are no significant differences between the autosomes when comparing one member to the adjacent autosomes. This was also true when comparing the Xy_p bivalent to the autosomes and the supernumeraries. The supernumeraries are significantly different in size from the autosomes. However, it is not possible to distinguish between supernumeraries by size.

Tribolium madens

Spermatogonial metaphase karyotypes consist of 19 + y and ten supernumerary chromosomes. The autosomes are similar in size, and most are bipartite with metacentric centro- meres. Six of the supernumeraries are metacentric, and are denoted BII 1, BII 2 and BII 3, while the other four are telocentric and are denoted, BI 1 and BI 2.

Metaphase I preparations of Tribolium madens consisted of the 9 + Xy_p meioformula and five supernumerary members in eleven of the cells examined. Three of these supernumeraries

are metacentric and the other two are acrocentric. The remaining cells each contained three supernumeraries.

It is not possible to discriminate between autosomal members based on their lengths. In addition to this, neither the Xy_p nor the supernumeraries can be distinguished from the autosomes by size. Individual supernumeraries can however be identified by the position of their centromere.

When comparing the chromosomes of these four members of the the castaneum species-group it is apparent that some differences between them do exist. During spermatogonial metaphase the chromosomes of both T. castaneum and T. freemani are primarily metacentrics. Tukey's analysis determined that there are sufficient differences in chromosome lengths to distinguish between the two species. They can also be distinguished by morphological differences. Quadripartite chromosomes are seen in T. freemani but only bipartite chromosomes were identified in T. castaneum.

The presence of supernumerary chromosomes in T. audax and T. madens makes their distinction from each other and the other species in this study quite simple. Tukey's analysis of the autosomes of these two species did not show significant differences to distinguish between based on the size of their chromosomes.

CONFUSUM SPECIES-GROUP

Tribolium confusum

T. confusum has a diploid number of 18 chromosomes, 16 + X + Y. Most of the autosomes are metacentric but some acrocentrics were identified. During spermatogonial metaphase a neo-X and a neo-Y were observed. The neo-X is metacentric and larger than the autosomes and the neo-Y is acrocentric and similar to the autosomes in size. These results correspond with those obtained by Smith in his 1952 study of Tribolium.

During metaphase I, eight autosomal chromosomes and a large neo-XY sex bivalent were observed (Figure 14). The autosomes average 2.25 - 3.75 microns in length and the neo-XY averages 4.08 microns long. Most of the autosomes are metacentric and bipartite, the remainder being acrocentric or telocentric. The differences in chromosome length that were observed are not sufficient to distinguish between adjacent members. Although the neo-XY is the largest member of the complement it is not significantly larger than the autosomes.

Tribolium destructor

The T. destructor diploid compliment includes 16 autosomes, an X and a Y chromosome. Although not as large as the neo-X and neo-Y of T. confusum, the sex chromosomes of T. destructor are larger than those observed in the castaneum species-group. The X-chromosome is slightly larger than the

autosomes but the Y is similar to them in size. Most of the autosomes are metacentric and bipartite, although some submetacentric and telocentric members were observed. This makes it difficult to distinguish the sex bivalents from the other chromosomes during mitosis. This situation was also observed in T. destructor by Smith.

Metaphase I cells of T. destructor have eight autosomal members and a neo-XY sex bivalent. T. destructor's neo-XY is smaller than the complex observed in T. confusum. The autosomes average from 1.17 - 3.0 micron in length and the neo-XY averages 3.5 microns. It is not possible to distinguish between most of the autosomes by size.

Tribolium anaphe

Diploid preparations of T. anaphe were consistent with the other members of the confusum species-group examined, having 18 chromosomes. T. anaphe's sex chromosomes and autosomes are similar in size, indicating perhaps, that a loss of heteropycnotic material has occurred.

Metaphase I cells contain 9 chromosomes. The XY is slightly larger than the autosomes and is composed of two, unequal portions: the autosome and translocated X, and the relatively large Y. The autosomes average from 1.04 - 2.42 microns in length and the neo-XY averages 2.75 microns long. These differences are not sufficient to differentiate between adjacent autosomes or between the sex bivalent and the autosomes.

When comparing chromosome lengths of these three species to each other Tukey's analysis showed sufficient differences to distinguish between T. confusum and T. anaphe or T. destructor. It is not possible to differentiate between T. anaphe and T. destructor by the size of their chromosomes. Significant differences exist between the neo-XY in T. confusum and the XY sex bivalents of T. anaphe and T. destructor. This supports Smith's theory (1952b) concerning loss of heterochromatin in T. destructor's sex complex, a structure that he feels was derived from the older neo-XY. A similar situation has obviously occurred in T. anaphe, as indicated by the lack of a heteromorphic sex bivalent.

BREVICORNIS SPECIES-GROUP

One member of the brevicornis species-group was examined in this project, Tribolium brevicornis (Figures 19 and 20). Spermatogonial metaphase preparations contained a diploid number of 18 chromosomes. These chromosomes are similar in size, and a heteromorphic sex pair was not observed.

Metaphase I cells contain nine chromosomes, most displaying metacentric centromeres. The chromosomes are similar in size with their lengths ranging from 2.17 - 3.58 microns. The differences in chromosome length is not sufficient to distinguish the autosomes from each other or the sex chromosome complex from the autosomes.

When chromosomes 1-9 were compared among the eight species

a significant difference was seen in all but chromosome number four. For this reason Tukey's analysis was applied to all of the chromosomes with the exception of number four. This characteristic of Tribolium allows the eight species involved in this study to be distinguished from each other based on the size of their chromosomes.

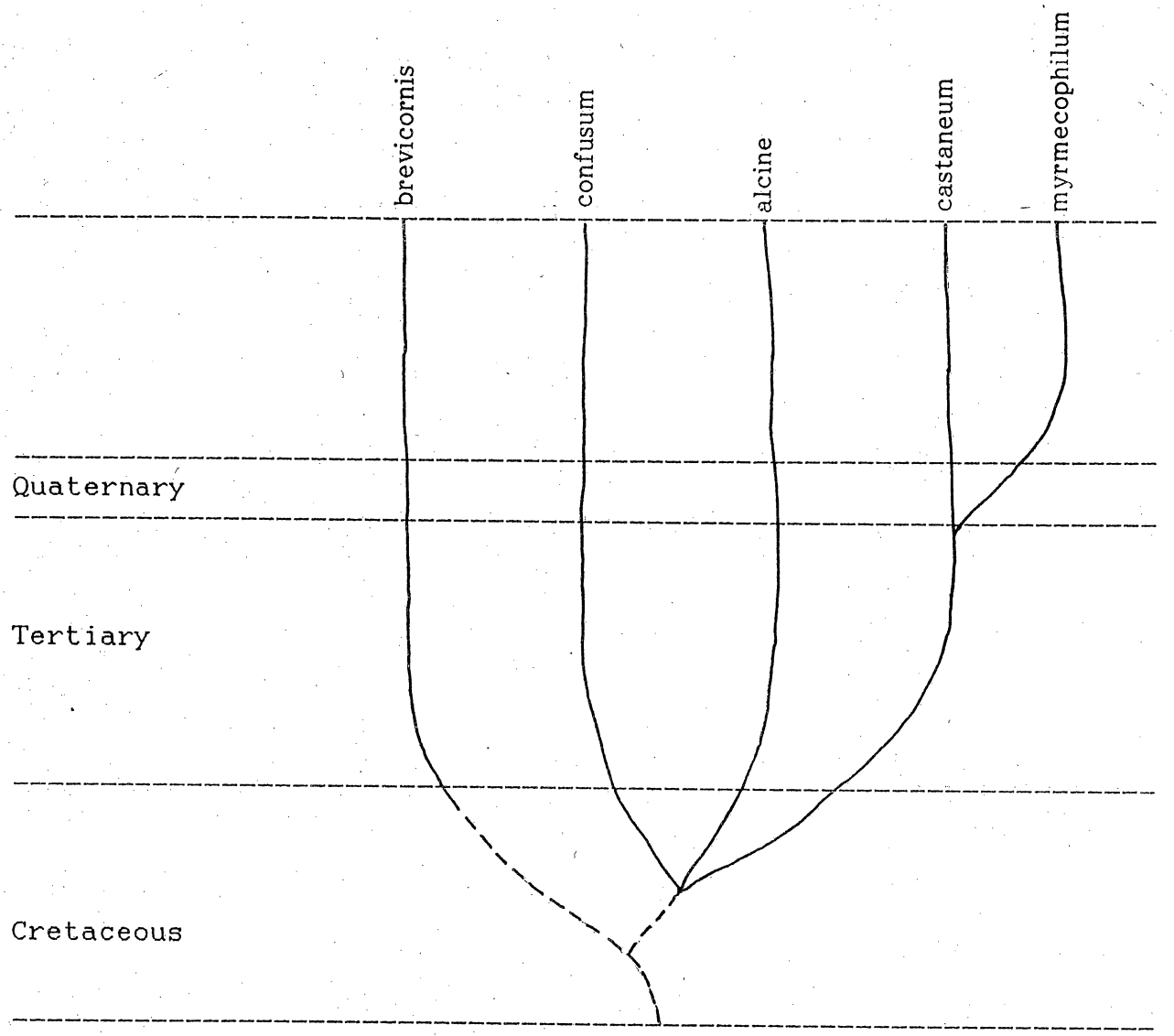


Figure 2 Phylogenetic diagram illustrating relations of species-groups of Tribolium to each other. (adapted from Sokoloff 1972).

Figure 3

Chromosomes of Tribolium at metaphase. (After Sokoloff, 1972).

(a) T. castaneum, spermatogonial metaphase (19 + y).

(b) T. castaneum, metaphase I (9AA + Xy_p).

(c) T. confusum, spermatogonial metaphase (16 + X + Y).

(d) T. confusum, oogonial metaphase (16 + X + X).

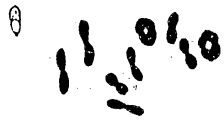
(e) T. confusum, the eight autosomal bivalents at pachytene showing centric blocks of heterochromatin and neo-XY attached to the nucleolus.

(f) T. destructor, spermatogonial metaphase (16 + X + Y).

(g) T. destructor, metaphase I (8AA + neo-XY).

(h) T. madens, metaphase I (9AA + Xy_p + 3BII + 2BI).

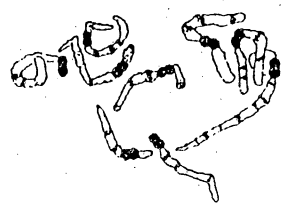
(a)



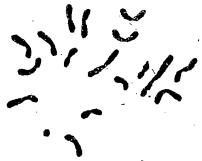
(b)

(c)

(d)

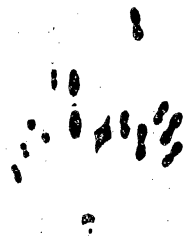


(e)



(f)

(g)



(h)

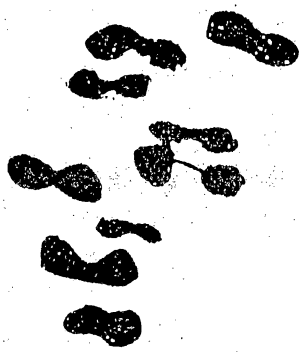
Figure 4

Chromosomes of Tribolium brevicornis and Tribolium anaphe After Moore and Sokoloff, 1982.

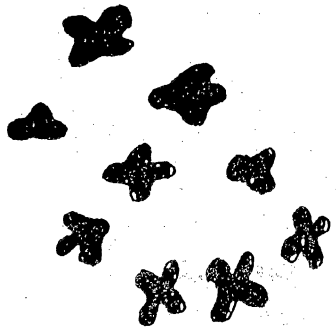
(a) Side view of first meiotic metaphase in a cell from the testes of T. brevicornis showing bipartite chromosomes.

(b) Side view of first meiotic metaphase in a cell from the testes of T. brevicornis showing quadripartite chromosomes.

(c) Polar view of spermatogonial mitosis in the testes of T. anaphe.



(a)

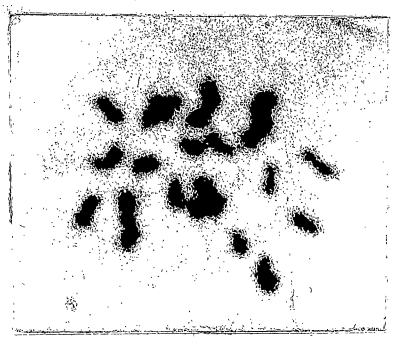


(b)

(c)

Figure 5

Spermatogonial metaphase and karyotype of Tribolium castaneum, 19 + y. 2,100X.



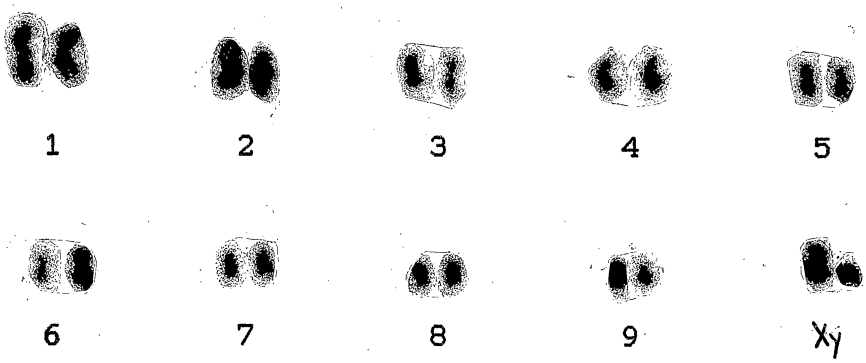
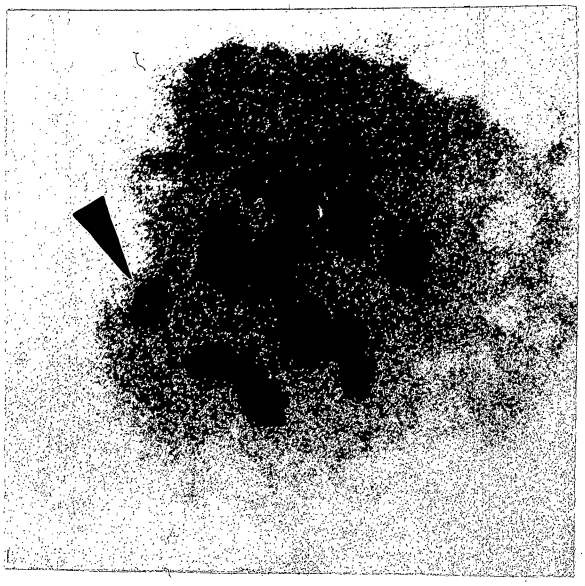
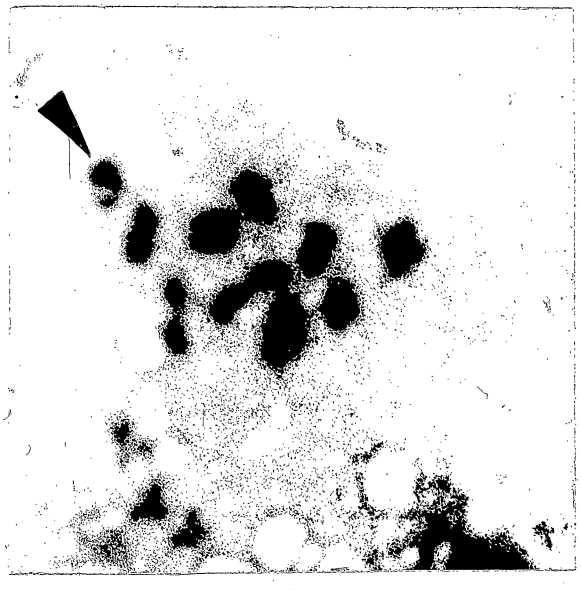

1
2
3
4
5
6
7
8
9
Xy

Figure 6

(a) - (b). Metaphase I of Tribolium castaneum with 9 + Xy_p, showing the Xy_p arrowed. 3,500X.



(a)



(b)

Figure 7

Spermatogonial metaphase and karyotype of *Tribolium freemani*, 19 + y. 3,650X.

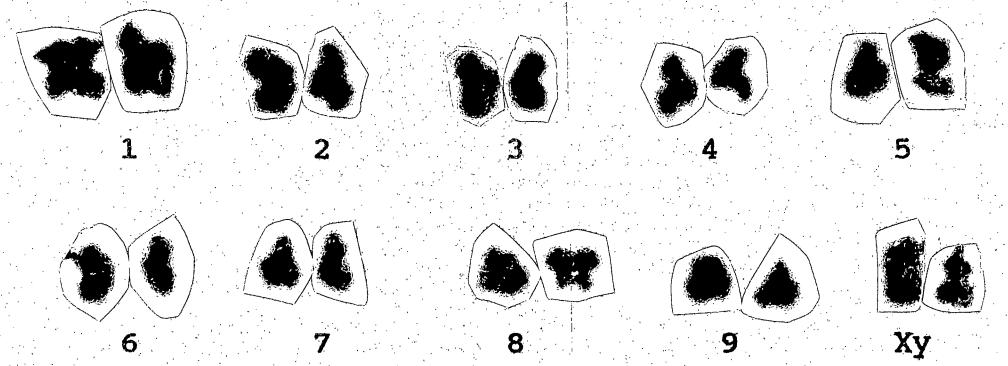
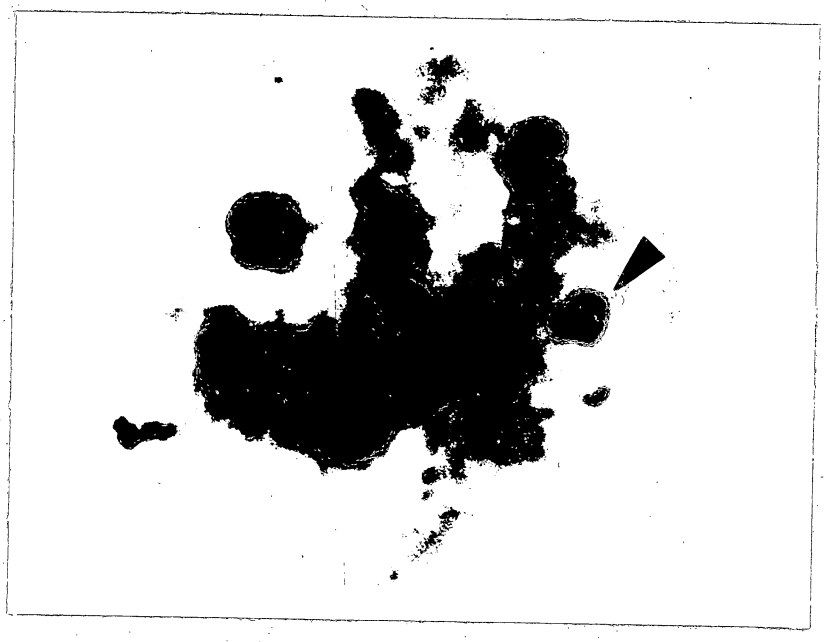
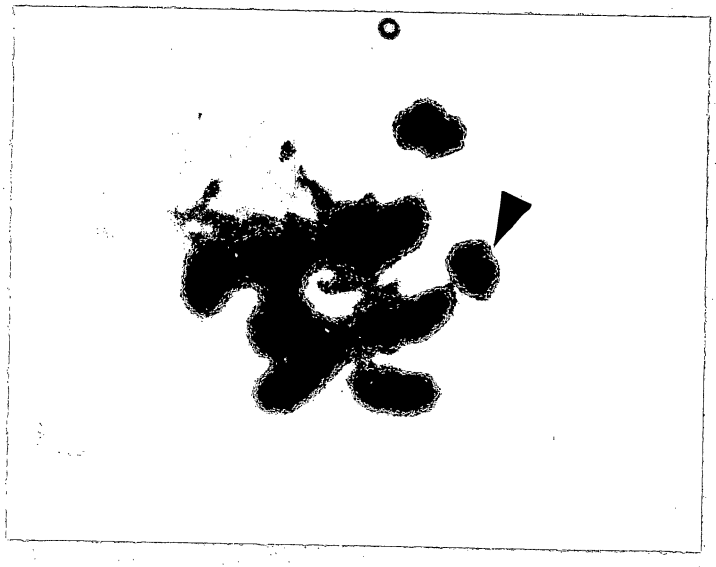

1
2
3
4
5
6
7
8
9
Xy

Figure 8

(a) - (b). Metaphase I of *Tribolium freemani* with 9 + Xy_p, showing the Xy_p arrowed. 4,000X.



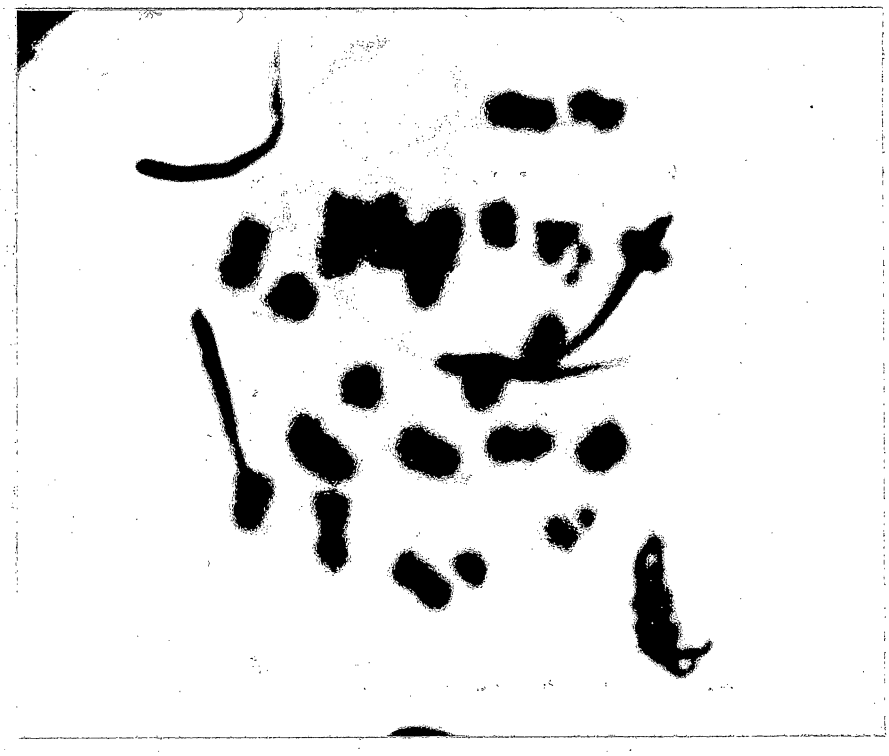
(a)



(b)

Figure 9

Spermatogonial metaphase and karyotype of *Tribolium audax*, 19 + y + BII 2 + BI 2. 3,050X.



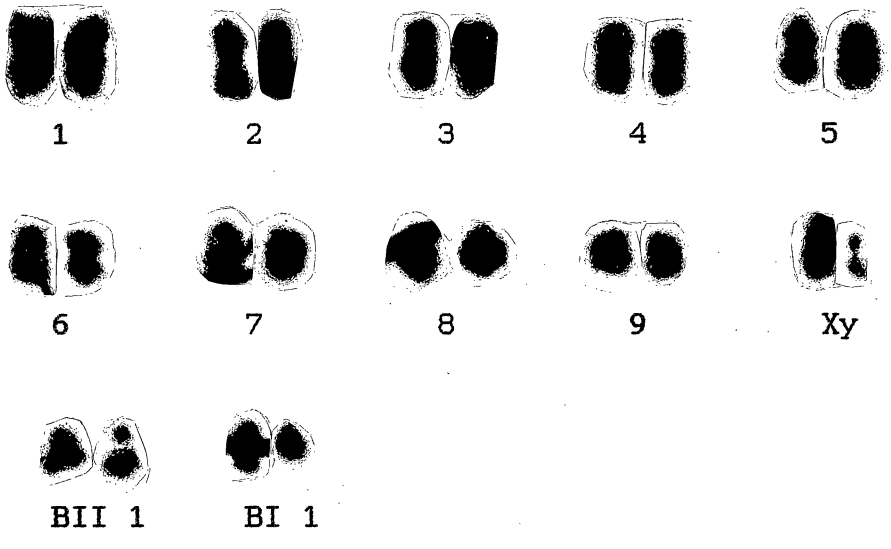
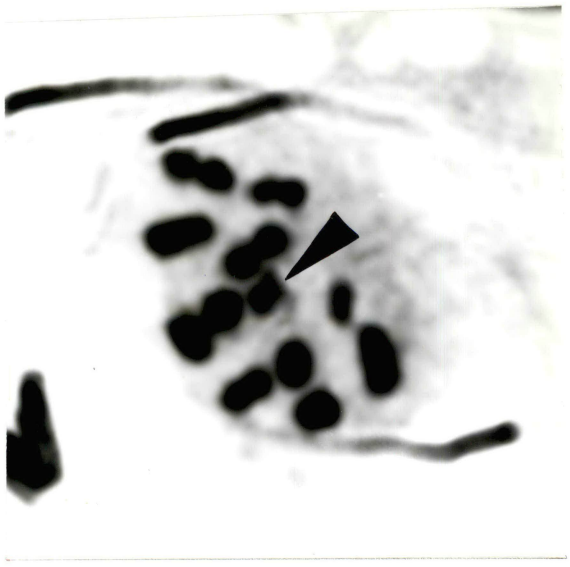
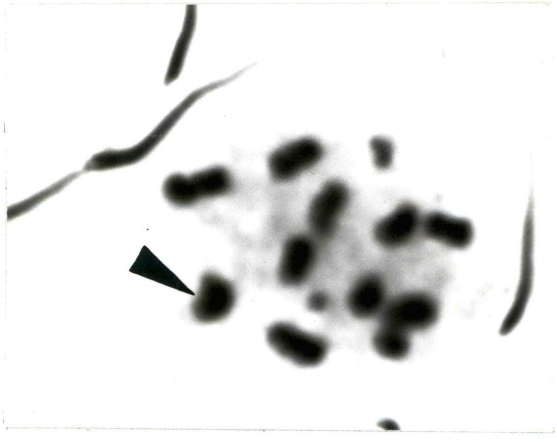

1
2
3
4
5
6
7
8
9
Xy
BII 1
BI 1

Figure 10

(a) - (b). Metaphase I of Tribolium audax with 9 + Xy_p + BII 1 + BI 1, showing the Xy_p arrowed. 2,800X.



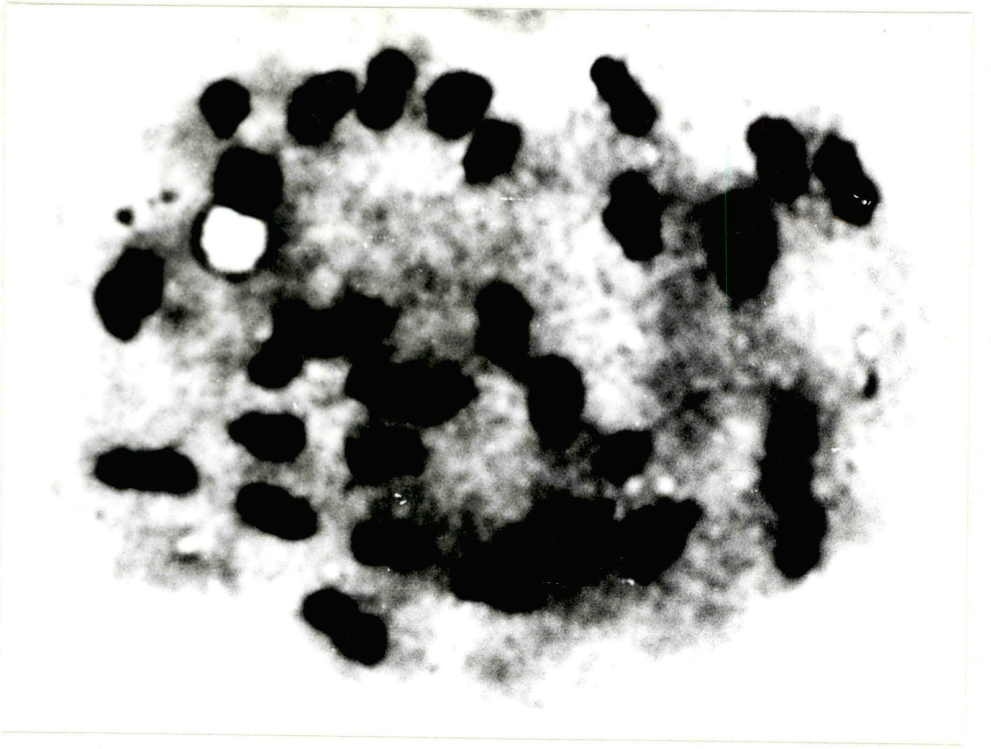
(a)



(b)

Figure 11

Spermatogonial metaphase and karyotype of *Tribolium madens*, 19 + y + BII 3 + BI 2. 3,050X.



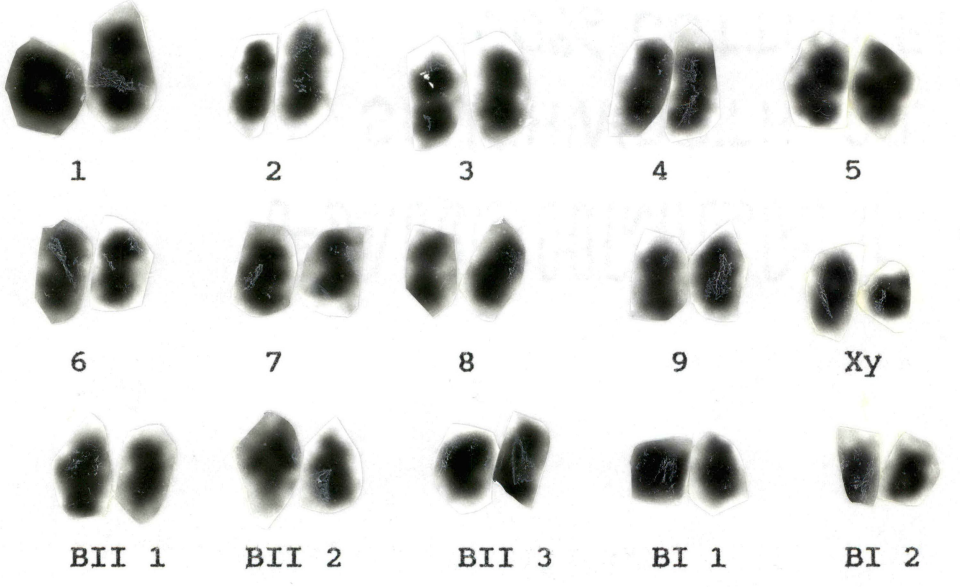

1
2
3
4
5
6
7
8
9
Xy
BII 1
BII 2
BII 3
BI 1
BI 2

Figure 12

(a) Metaphase I of Tribolium madens with 9 + Xy_p + BII 3 + BI 2, showing the Xy_p arrowed. 3,075X.

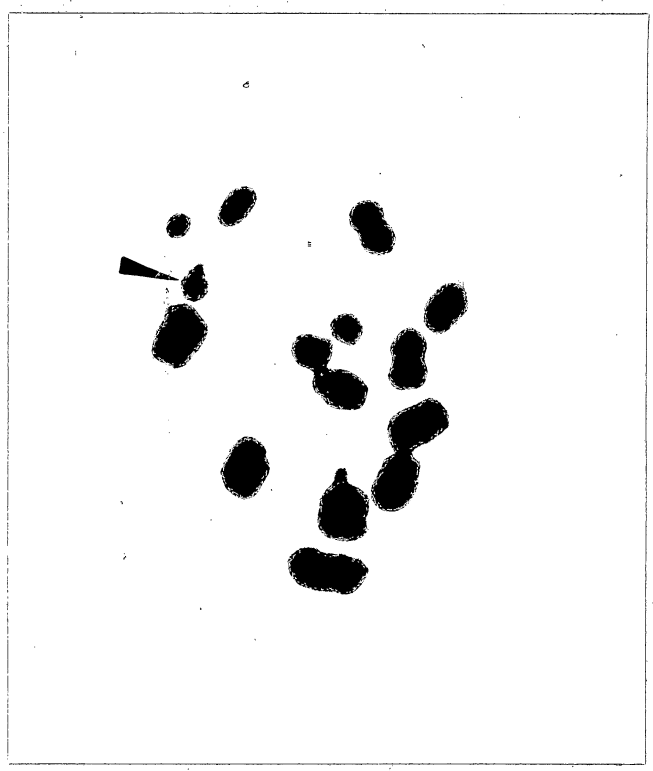


Figure 13

Spermatogonial metaphase and karyotype of *Tribolium confusum*, 16 + X + Y. 2,950X.

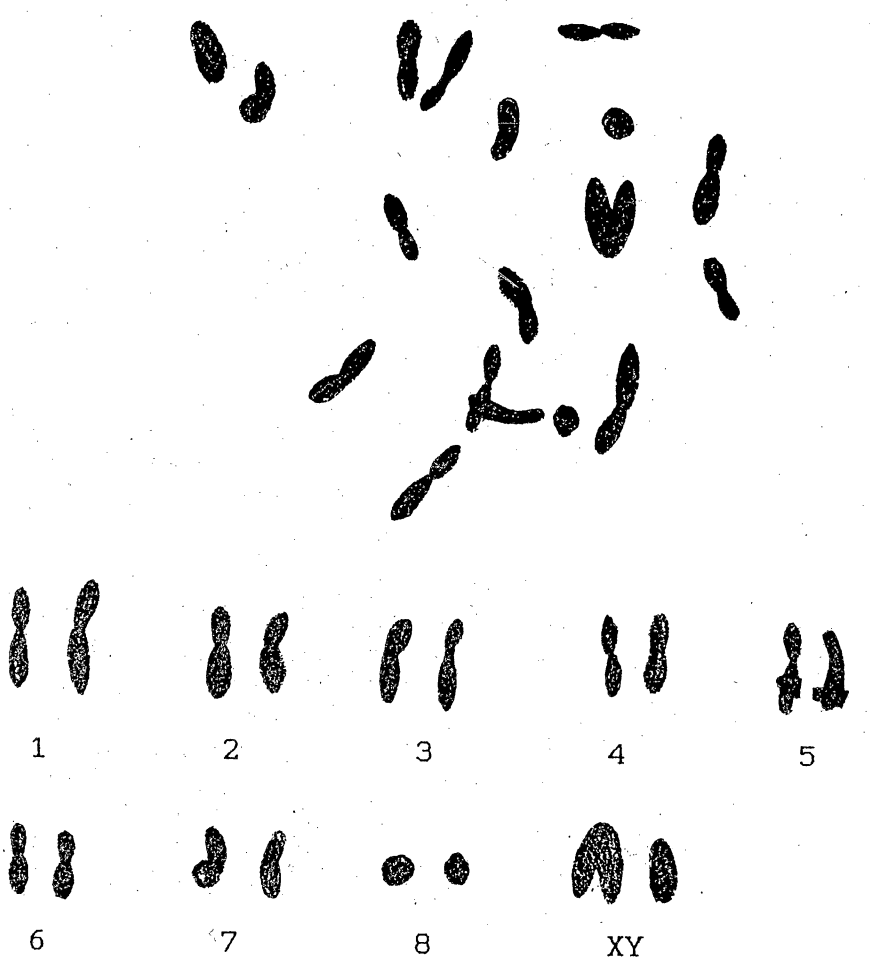
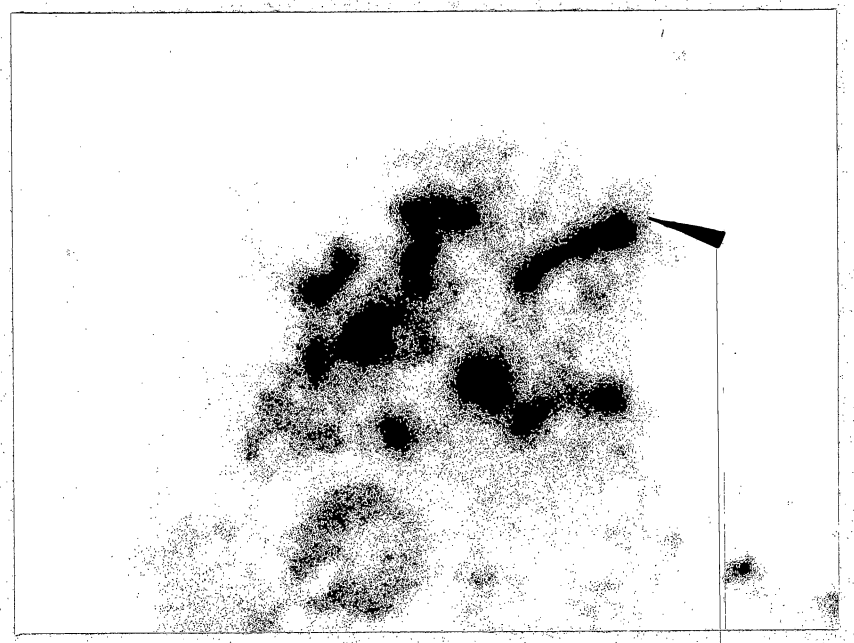

1
2
3
4
5
6
7
8
XY

Figure 14

(a) - (b). Metaphase I of Tribolium confusum with 8 + neo-XY, showing the neo-XY arrowed. 3,350X.

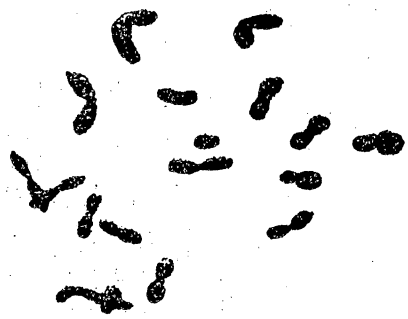


(a)

(b)

Figure 15

Spermatogonial metaphase and karyotype of Tribolium destructor, 16 + X + Y. 2,850X.



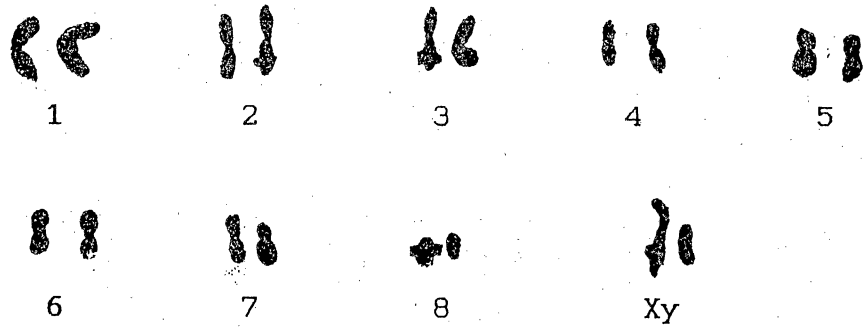
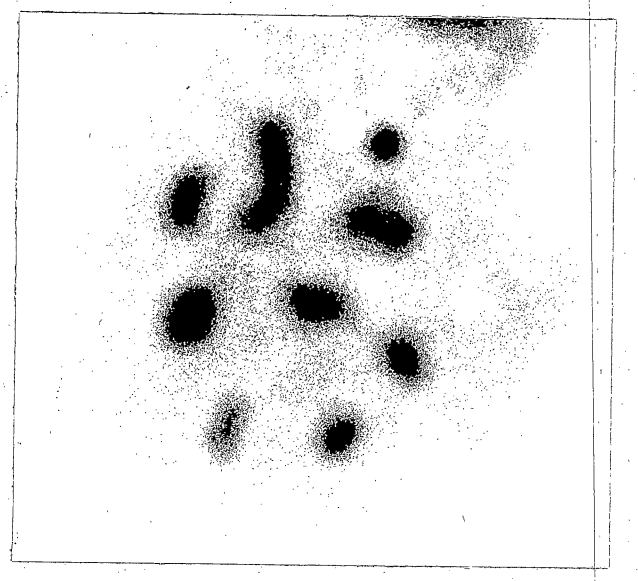
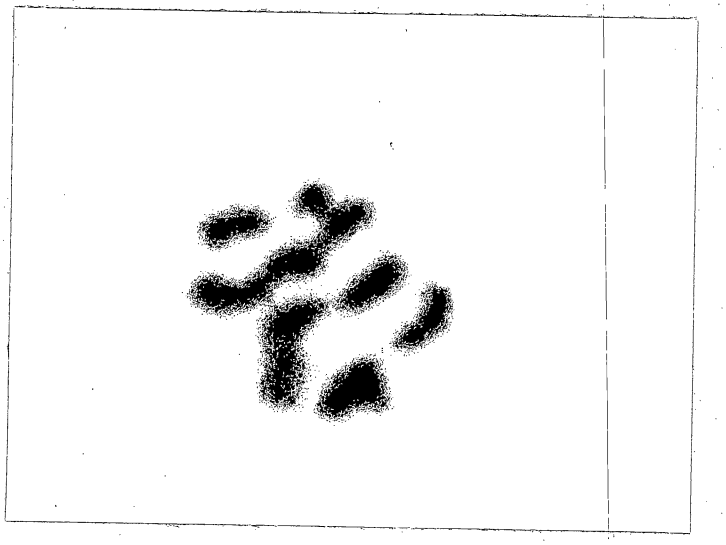

1
2
3
4
5
6
7
8
Xy

Figure 16

(a) - (b). Metaphase I of Tribolium destructor with 9 bivalents, no heteromorphic sex chromosome identified. 3,400X.



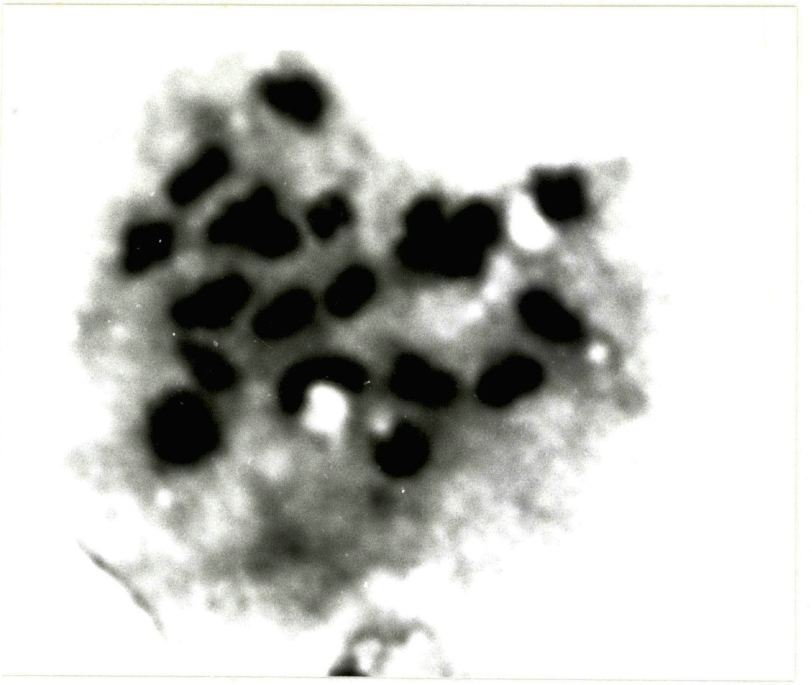
(a)



(b)

Figure 17

Spermatogonial metaphase and karyotype of *Tribolium anaphe*, 16 + X + Y. 4,375X.



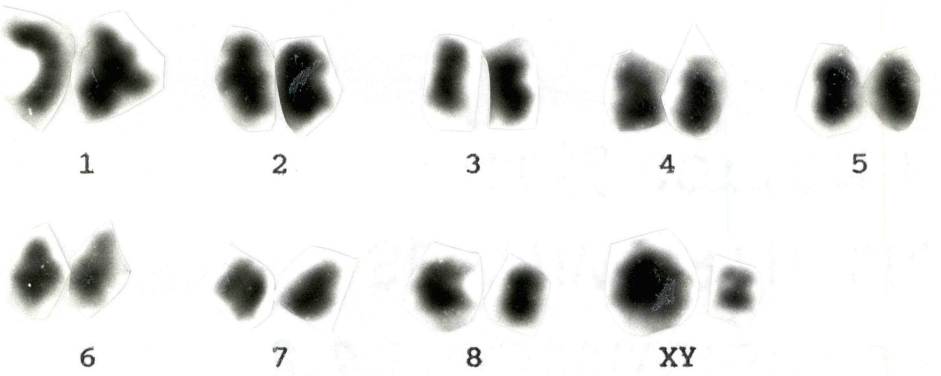
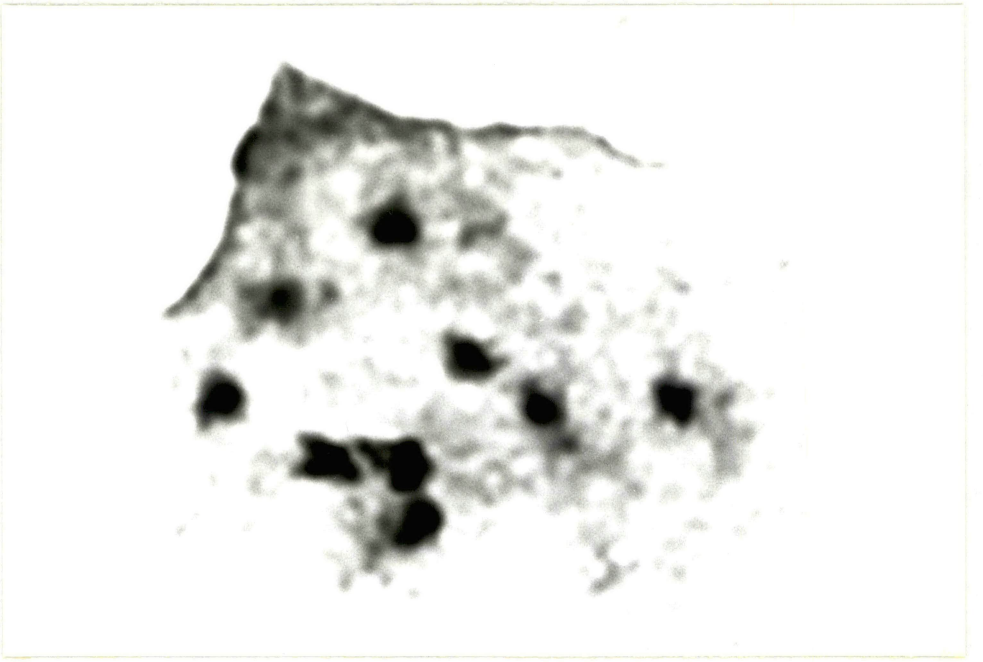
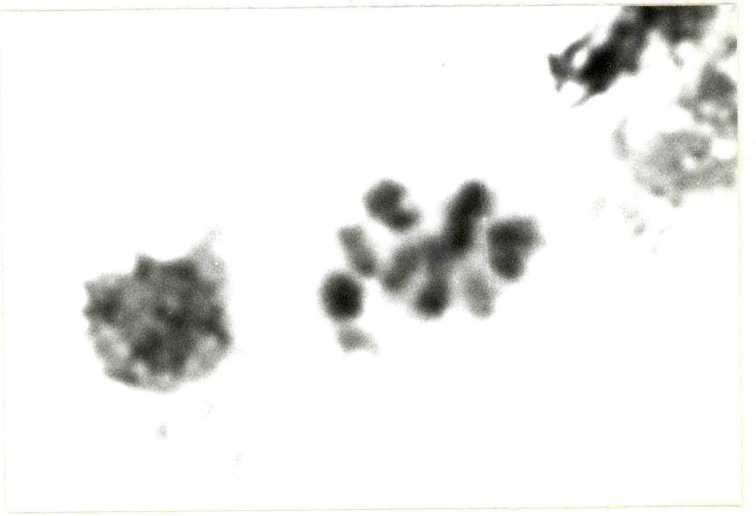

1
2
3
4
5
6
7
8
XY

Figure 18

(a) - (b). Metaphase I of Tribolium anaphe with 9 bivalents, no heteromorphic sex chromosome identified. 4,200X.



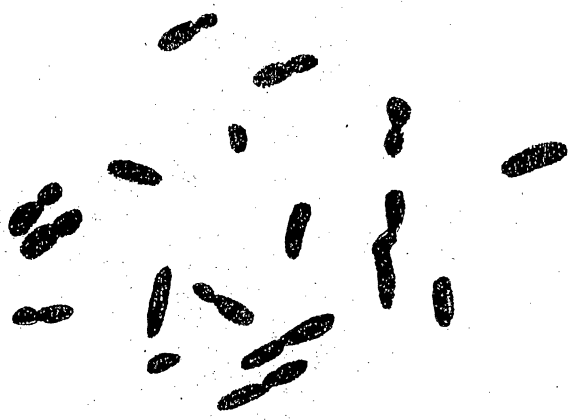
(a)



(b)

Figure 19

Spermatogonial metaphase and karyotype of *Tribolium brevicornis*, 17 + y. 3,200X.



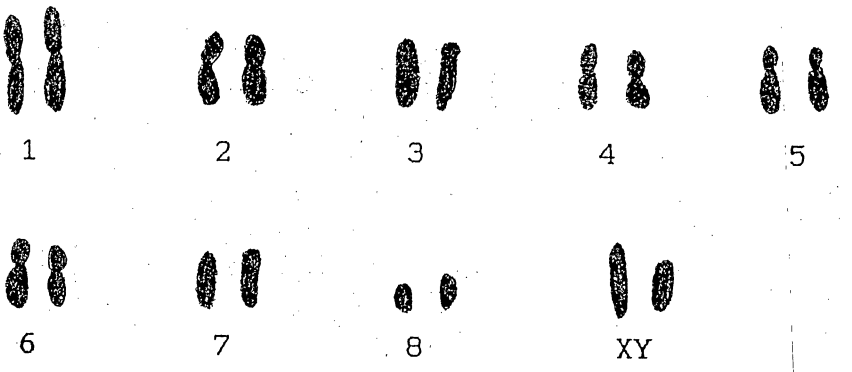
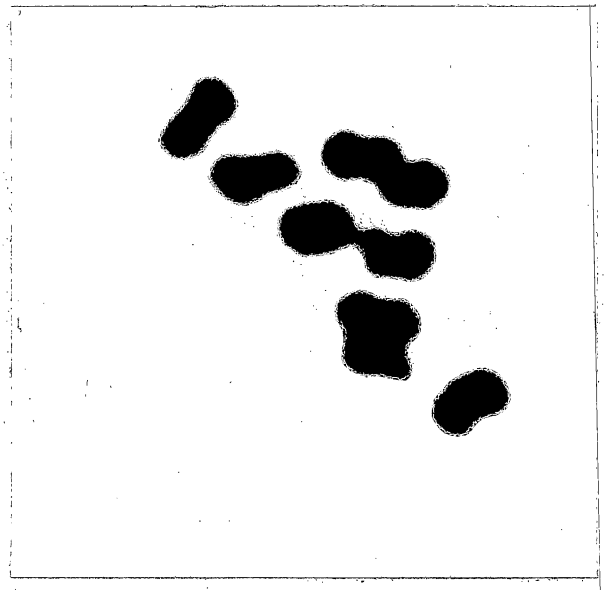

1
2
3
4
5
6
7
8
XY

Figure 20

(a) - (b). Metaphase I of Tribolium brevicornis with 9 bivalents, no heteromorphic sex chromosome identified. 3,200X.



(a)

(b)

Table 2. The Five Species-Groups of *Tribolium* And Their Geographical Origin. (From Sokoloff 1972)

Species-group and species	Country or Region of Origin
1. *brevicornis* species-group	
T. brevicornis	California
T. linsleyi Hinton	Mexico
T. parallelus	Western N. America
T. gebieni Uyttenb	?
T. carinatum dubium Hinton	Argentina
T. uezumii Nakane	Honshu Japan
2. *confusum* species-group	
T. anaphe Hinton	Africa
T. confusum Duval	Africa in origin, now widespread
T. destructor Uyttenb	Africa in origin, now in Europe and N. America
T. semicostata (Gebien) (= *T. giganteum* Hinton)	Africa
T. downesi Hinton	Africa
T. beccarii Gridelli (= *T. downesi*?)	Africa
T. semele Hinton	Africa
T. sulmo Hinton	Africa
T. indicum	Africa and India
T. indicum f. seres Hinton	Africa
T. indicum f. ares Hinton	India
T. thusa Hinton	Africa
3. *alcine* species-group	
T. alcine Hinton	Madagascar
T. quadricollis (Fairmaire) (= *T. dolon* Hinton)	Madagascar
T. ceto Hinton	Madagascar
4. *castaneum* species-group	
T. castaneum (Herbst)	Cosmopolitan
T. madens (Charp.)	'Nearly Cosmopolitan'
T. audax Halsted	N. America
T. freemani Hinton	Kashmir
T. cylindricum Hinton	Malay Peninsula
T. politum Hinton	Doerian Islands
T. waterhousei Hinton	Australia
T. parki Hinton	Larat Island
T. apiculum Neboiss	Australia
5. *myrmecophilum* species-group	
T. myrmecophilum Lea	Australia
T. antennatum Hinton	Australia

Table 3. Chromosomally sampled species of *Tribolium*, including chromosome number and meioformula.

Species	Cells counted: Mitoses	Meioses	Chromosome number	Meioformula
T. castaneum	22	17	20	9 + Xy_p
T. freemani	12	18	20	9 + Xy_p
T. madens	14	16	30	9 + Xy_p + (BII 3 + BI 2)
T. audax	12	19	24	9 + Xy_p + (BII 1 + BI 1)
T. confusum	2	25	18	8 + neo-XY
T. anaphe	11	16	18	9, with no heteromorphic sex chromosome identified.
T. destructor	3	18	18	9, with no heteromorphic sex chromosome identified.
T. brevicornis	2	26	18	9, with no heteromorphic sex chromosome identified.

Table 4. Measurements* of chromosomes, Tribolium destructor

		Cell Number 1	2	3	4	5	6	Mean ±	Standard Deviation
Chromosome	1	3.5	2.5	3.5	4.0	4.0	3.5	3.50 ±	0.548
	2	3.5	2.5	2.5	3.0	3.0	3.5	3.00 ±	0.447
	3	3.0	2.5	2.5	3.0	3.0	3.5	2.33 ±	0.516
	4	2.5	1.5	2.0	1.5	2.0	2.5	2.00 ±	0.447
	5	2.5	1.5	2.0	1.5	1.5	1.5	1.75 ±	0.418
	6	2.0	1.5	1.5	1.5	1.5	1.5	1.5 ±	0.214
	7	1.5	1.5	1.5	1.5	1.5	1.5	1.0 ±	0.204
	8	1.5	1.5	1.5	1.5	1.5	1.5	1.0 ±	0.214
	9	1.5	1.0	1.0	1.0	1.0	1.0	1.0 ±	0.204

* Measurements in microns.

Table 5. Measurements* of chromosomes, Tribolium freemani. Chromosome number 10 is the Xy_p.

		Cell Number 1	2	3	4	5	6	Mean ±	Standard Deviation
Chromosome	1	2.5	3.0	2.5	3.5	3.0	3.5	3.17 ±	0.577
	2	2.5	2.5	2.5	3.0	2.5	3.5	3.00 ±	0.500
	3	2.5	2.0	2.0	2.5	2.0	2.5	2.33 ±	0.289
	4	2.0	2.0	2.0	2.0	2.0	2.0	2.00 ±	0.000
	5	1.5	2.0	1.5	2.0	2.0	2.0	1.83 ±	0.289
	6	1.0	2.0	1.5	2.0	2.0	2.0	1.83 ±	0.289
	7	1.0	1.0	1.0	2.0	2.0	2.0	1.67 ±	0.577
	8	1.0	1.0	1.0	2.0	2.0	1.5	1.50 ±	0.500
	9	1.0	1.0	1.0	1.0	.75	1.5	1.67 ±	0.289
	10	.75	1.0	.75	1.0	.75	1.0	0.917 ±	0.144

* Measurements in microns.

Table 6. Measurements* of chromosomes, Tribolium anaphe.

		Cell Number 1	2	3	4	5	6	Mean ±	Standard Deviation
Chromosome	1	2.0	3.0	3.0	3.0	3.5	2.0	2.75 ±	0.612
	2	1.5	3.0	2.5	2.5	3.0	2.0	2.42 ±	0.584
	3	1.5	2.5	3.0	2.5	3.0	2.0	2.42 ±	0.584
	4	1.5	2.5	2.5	2.5	2.5	2.0	2.25 ±	0.419
	5	1.5	2.0	2.0	2.0	2.5	1.5	1.92 ±	0.376
	6	1.0	1.5	2.0	1.5	2.0	1.5	1.58 ±	0.376
	7	1.0	1.5	2.0	1.5	2.0	1.0	1.50 ±	0.447
	8	.75	1.5	1.5	1.5	1.5	1.0	1.29 ±	0.332
	9	.75	.75	1.5	1.0	1.5	.75	1.04 ±	0.368

* Measurements in microns.

Table 7. Measurements* of chromosomes, Tribolium brevicornis

		Cell Number 1	2	3	4	5	6	Mean ±	Standard Deviation
Chromosome	1	3.5	3.5	3.0	3.0	4.5	4.0	3.58 ±	0.584
	2	3.5	3.0	3.5	4.0	3.0	3.5	3.42 ±	0.376
	3	3.0	3.5	3.0	3.0	3.0	3.0	3.08 ±	0.204
	4	3.0	3.0	3.0	3.0	3.0	3.0	3.00 ±	0.000
	5	3.0	3.0	3.0	3.0	3.0	3.0	3.00 ±	0.000
	6	2.5	3.0	3.0	3.0	2.5	2.5	2.75 ±	0.274
	7	2.5	2.5	2.5	2.5	2.5	2.5	2.50 ±	0.000
	8	2.5	2.5	2.0	2.5	2.5	2.5	2.42 ±	0.204
	9	2.0	2.5	2.0	2.5	2.0	2.0	2.17 ±	0.258

* Measurements in microns.

Table 8. Measurements* of chromosomes, Tribolium castaneum. Chromosome number 10 is the Xy_p.

		Cell Number							
		1	2	3	4	5	6	Mean ±	Standard Deviation
Chromosome	1	4.0	4.0	3.5	4.0	4.0	3.5	3.83 ±	0.258
	2	3.5	3.5	3.0	4.0	4.0	3.0	3.50 ±	0.447
	3	3.5	3.0	3.0	3.5	3.0	3.0	3.17 ±	0.258
	4	3.0	3.0	3.0	3.0	2.5	3.0	2.92 ±	0.204
	5	3.0	3.0	3.0	2.5	2.5	3.0	2.83 ±	0.258
	6	2.5	3.0	2.5	2.5	2.5	2.5	2.58 ±	0.204
	7	2.5	2.5	2.5	2.0	2.0	2.5	2.33 ±	0.258
	8	2.0	2.0	2.5	2.0	2.0	2.5	2.17 ±	0.258
	9	2.0	2.0	2.0	2.0	2.0	2.0	1.92 ±	0.204
	10	1.5	1.0	1.0	2.0	1.5	1.5	1.42 ±	0.376

* Measurements in microns.

Table 9. Measurements* of chromosomes, Tribolium confusum. Chromosome number 1 is the neo-XY.

		Cell Number 1	2	3	4	5	6	Mean ± Standard Deviation
Chromosome	1	4.0	4.5	4.0	4.0	3.5	4.5	4.08 ± 0.376
	2	3.5	4.0	3.5	4.0	3.5	4.0	3.75 ± 0.274
	3	3.0	4.0	3.5	3.5	3.5	4.0	3.58 ± 0.376
	4	3.0	4.0	3.0	3.0	3.0	3.0	3.17 ± 0.408
	5	3.0	3.0	3.0	3.0	3.0	3.0	3.00 ± 0.000
	6	3.0	3.0	3.0	3.0	2.5	3.0	2.92 ± 0.204
	7	2.5	3.0	3.0	2.5	2.5	2.5	2.67 ± 0.258
	8	3.0	3.0	2.5	2.0	2.5	2.5	2.58 ± 0.376
	9	2.5	2.5	2.5	2.0	2.0	2.0	2.25 ± 0.273

* Measurements in microns.

Table 10. Measurements* of chromosomes, Tribolium audax. Chromosome number 10 is the Xy_py.

		Cell Number							
		1	2	3	4	5	6	Mean ±	Standard Deviation
Chromosome	1	3.5	3.0	4.0	3.5	3.5	4.0	3.58 ±	0.376
	2	3.0	3.0	3.0	3.5	3.0	3.5	3.17 ±	0.258
	3	2.5	3.0	2.5	3.0	3.0	3.5	2.92 ±	0.376
	4	2.5	2.5	2.5	2.5	2.5	3.0	2.58 ±	0.204
	5	2.5	2.5	2.0	2.5	2.5	2.5	2.42 ±	0.214
	6	2.0	2.5	2.0	2.0	2.5	2.5	2.25 ±	0.274
	7	2.0	2.5	2.0	2.0	2.5	2.5	2.25 ±	0.274
	8	2.0	2.0	2.0	2.0	2.0	2.0	2.00 ±	0.000
	9	2.0	1.5	2.0	2.0	2.0	2.0	1.92 ±	0.204
	10	1.5	1.5	1.5	1.5	1.5	1.5	1.50 ±	0.000
	11	1.0	1.0	1.5	1.0	1.0	1.0	1.08 ±	0.204
	12	1.0	1.0	1.0	1.0	1.0	1.0	1.00 ±	0.000

* Measurements in microns.

Table 11. Measurements* of chromosomes, Tribolium madens.

		Cell Number 1	2	3	4	5	6	Mean ± Standard Deviation
Chromosome	1	4.0	4.0	4.5	4.5	4.5	4.0	4.25 ± 0.274
	2	4.5	4.0	4.5	4.0	4.0	4.0	4.17 ± 0.258
	3	3.0	2.5	2.5	3.0	4.0	3.0	3.75 ± 0.683
	4	2.5	3.0	2.5	2.5	2.5	3.0	2.67 ± 0.274
	5	3.5	2.0	2.5	2.5	2.5	2.5	2.58 ± 0.516
	6	2.0	2.0	2.5	2.5	2.5	2.5	2.33 ± 0.274
	7	2.5	2.0	2.0	2.0	2.0	2.0	2.08 ± 0.204
	8	2.0	2.0	2.0	2.5	2.0	2.0	2.08 ± 0.258
	9	1.5	2.0	2.5	1.5	1.5	1.5	1.75 ± 0.258
	10	1.5	1.5	2.0	1.5	1.0	1.5	1.50 ± 0.000
	11	1.5	1.0	1.5	1.0	.75	1.0	1.13 ± 0.258
	12	.75	1.0	1.5	1.0	1.0	.75	1.00 ± 0.137
	13	.75	1.0	1.0	1.0	.75	1.0	0.92 ± 0.102
	14	.75	.75	1.0	1.0	.75	1.0	0.88 ± 0.129
	15	.75	.75	1.0	.75	.75	.75	0.79 ± 0.102

* Measurements in microns.

TABLE 12. ANOVA, chromosomes 1-9, eight species of Tribolium.

Chromosome	f value	Significant difference
1	7.33	Yes
2	11.90	Yes
3	7.15	Yes
4	1.72	No
5	16.70	Yes
6	2.77	Yes
7	14.75	Yes
8	-16.33	Yes
9	37.95	Yes

$f_{\alpha} = 2.25$

Table 13. Interspecific comparison of chromosome #1. Tukey's analysis*.

Species compared	q value	Significant difference
T. madens vs. T. anaphe	8.02	*
T. madens vs. T. freemani	6.68	*
T. madens vs. T. destructor	4.01	No
T. madens vs. T. brevicornis	3.58	No
T. madens vs. T. audax	3.58	No
T. madens vs. T. castaneum	2.25	No
T. madens vs. T. confusum	0.91	No
T. confusum vs. T. anaphe	7.11	*
T. confusum vs. T. freemani	5.78	*
T. confusum vs. T. destructor	3.10	No
T. confusum vs. T. brevicornis	2.67	No
T. confusum vs. T. audax	2.67	No
T. confusum vs. T. castaneum	1.34	No
T. castaneum vs. T. anaphe	5.78	*
T. castaneum vs. T. freemani	4.44	No
T. castaneum vs. T. destructor	1.76	No
T. castaneum vs. T. brevicornis	1.34	No
T. castaneum vs. T. audax	1.34	No

Table 13. cont. Chromosome #1. Tukey's analysis*.

Species compared	q value	Significant difference
T. brevicornis + T. audax	4.44	No
T. brevicornis vs. T. anaphe	4.44	No
T. brevicornis + T. audax	3.10	No
T. brevicornis vs. t. freemani	3.10	No
T. brevicornis + T. audax	0.43	No
T. brevicornis vs. T. destructor	0.43	No
T. destructor vs. T. anaphe	4.01	No
T. destructor vs. T. freemani	2.67	No
T. freemani vs. T. anaphe	1.34	No

q_c= 4.52

* Indicates a significant difference.

Table 14. Interspecific comparison of chromosome #2. Tukey's analysis*.

Species compared	q value	Significant difference
T. madens vs. T. anaphe	69.23	*
T. madens vs. T. freemani	55.77	*
T. madens vs. T. destructor	46.15	*
T. madens vs. T. audax	38.46	*
T. madens vs. T. brevicornis	30.77	*
T. madens vs. T. castaneum	26.92	*
T. madens vs. T. confusum	17.31	*
T. confusum vs. T anaphe	51.92	*
T. confusum vs. T. freemani	38.46	*
T. confusum vs. T. destructor	28.85	*
T. confusum vs. T. audax	21.15	*
T. confusum vs. T. brevicornis	13.46	*
T. confusum vs. T. castaneum	9.62	*
T. castaneum vs. T. anaphe	42.30	*
T. castaneum vs. T. freemani	28.85	*
T. castaneum vs. T. destructor	19.23	*
T. castaneum vs. T. audax	11.54	*
T. castaneum vs. T. brevicornis	3.84	No
T. brevicornis vs. T. anaphe	38.46	*
T. brevicornis vs. T. freemani	25.00	*
T. brevicornis vs. T. destructor	15.38	*
T. brevicornis vs. T. audax	7.69	*

Table 14. cont. Chromosome #2. Tukey's analysis*.

Species compared	q value	Significant difference
T. audax vs. T. anaphe	30.77	*
T. audax vs. T. freemani	17.31	*
T. audax vs. T. destructor	7.69	*
T. destructor vs. T. anaphe	23.08	*
T. destructor vs. T. freemani	9.61	*
T. freemani vs. T. destructor	-9.61	*

* Indicates a significant difference

$q_{\alpha} = 4.52$

Table 15. Interspecific comparison of chromosome #3. Tukey's analysis*.

Species compared	q value	Significant difference
T. confusum vs. T. freemani	8.21	*
T. confusum vs. T. destructor	7.72	*
T. confusum vs. T. anaphe	8.27	*
T. confusum vs. T. audax	4.07	No
T. confusum vs. T. brevicornis	3.09	No
T. confusum vs. T. castaneum	2.53	No
T. confusum vs. T. madens	2.53	No
T. madens vs. T. freemani	8.20	*
T. madens vs. T. destructor	5.19	*
T. madens vs. T. anaphe	4.63	*
T. madens vs. T. audax	1.54	No
T. madens vs. T. brevicornis	0.80	No
T. madens vs. T. castaneum	0.00	No
T. castaneum vs. T. freemani	5.68	*
T. castaneum vs. T. destructor	5.19	*
T. castaneum vs. T. anaphe	4.63	*
T. castaneum vs. T. audax	1.54	No
T. castaneum vs. T. brevicornis	0.56	No
T. brevicornis vs. T. freemani	5.12	*
T. brevicornis vs. T. destructor	4.63	*

Table 15. cont. Chromosome #3. Tukey's analysis*.

Species compared	q value	Significant difference
T. brevicornis vs. T. anaphe	4.07	No
T. brevicornis vs. T. audax	0.99	No
T. audax vs. T. freemani	4.14	No
T. audax vs. T. destructor	3.64	No
T. audax vs. T. anaphe	3.09	No
T. anaphe vs. T. freemani	1.05	No
T. anaphe vs. T. destructor	0.56	No
T. destructor vs. T. freemani	0.49	No

* Indicates a significant difference.

$q_{\alpha} = 4.52$

Table 16. Interspecific comparison of chromosome #5. Tukey's analysis*.

Species compared	q value	Significant difference
T. brevicornis vs. T. destructor	9.69	*
T. brevicornis vs. T. freemani	9.07	*
T. brevicornis vs. T. anaphe	8.37	*
T. brevicornis vs. T. audax	4.50	No
T. brevicornis vs. T. madens	2.56	No
T. brevicornis vs. T. castaneum	1.32	No
T. castaneum vs. T. destructor	8.37	*
T. castaneum vs. T. freemani	7.75	*
T. castaneum vs. T. anaphe	7.05	*
T. castaneum vs. T. audax	3.18	No
T. castaneum vs. T. madens	1.24	No
T. madens vs. T. destructor	7.13	*
T. madens vs. T. freemani	6.50	*
T. madens vs. T. anaphe	5.81	*
T. madens vs. T. audax	1.94	No
T. audax vs. T. destructor	5.19	*
T. audax vs. T. freemani	4.57	*
T. audax vs. T. anaphe	3.88	No
T. anaphe vs. T. destructor	1.32	No
T. anaphe vs. T. freemani	0.53	No

Table 16. cont. Chromosome #5. Tukey's analysis*.

Species compared	q value	Significant difference
T. freemani vs. T. destructor	0.62	No
T. confusum vs. T. destructor	9.69	*
T. confusum vs. T. freemani	9.07	*
T. confusum vs. T. anaphe	8.37	*
T. confusum vs. T. audax	4.50	No
T. confusum vs. T. madens	2.56	No
T. confusum vs. T. castaneum	1.32	No

* Indicates a significant difference.

$q_{\alpha} = 4.52$

Table 17. Interspecific comparison of chromosome #6. Tukey's analysis*.

Species compared	q value	Significant difference
T. confusum vs. T. destructor	11.35	*
T. confusum vs. T. anaphe	11.35	*
T. confusum vs. T. freemani	9.92	*
T. confusum vs. T. audax	5.68	*
T. confusum vs. T. madens	5.68	*
T. confusum vs. T. castaneum	2.88	No
T. confusum vs. T. brevicornis	1.44	No
T. brevicornis vs. T. destructor	9.92	*
T. brevicornis vs. T. anaphe	9.92	*
T. brevicornis vs. T. freemani	8.47	*
T. brevicornis vs. T. audax	4.24	No
T. brevicornis vs. T. madens	4.24	No
T. brevicornis vs. T. castaneum	1.44	No
T. castaneum vs. T. destructor	8.47	*
T. castaneum vs. T. anaphe	8.47	*
T. castaneum vs. T. freemani	7.03	*
T. castaneum vs. T. audax	2.79	No

Table 17. cont. Chromosome #6. Tukey's analysis*.

Species compared	q value	Significant difference
T. castaneum vs. T. madens	2.79	*
T. audax vs. T. destructor	5.68	*
T. audax vs. T. anaphe	5.68	*
T. madens vs. T. destructor	5.68	*
T. madens vs. T. anaphe	5.68	*
T. audax vs. T. freemani	4.24	No
T. madens vs. T. freemani	4.24	No
T. freemani vs. T. destructor	1.44	No
T. freemani vs. T. anaphe	1.44	No

* Indicates a significant difference.

$q_{\alpha} = 4.52$

Table 18. Interspecific comparison of chromosome #7. Tukey's analysis*.

Species compared	q value	Significant difference
T. confusum vs. T. destructor	9.76	*
T. confusum vs. T. freemani	9.14	*
T. confusum vs. T. anaphe	9.14	*
T. confusum vs. T. madens	4.60	*
T. confusum vs. T. audax	3.28	No
T. confusum vs. T. castaneum	2.66	No
T. castaneum vs. T. destructor	7.11	*
T. castaneum vs. T. freemani	6.48	*
T. castaneum vs. T. anaphe	6.48	*
T. castaneum vs. T. madens	1.95	No
T. castaneum vs. T. audax	0.63	No
T. audax vs. T. destructor	6.48	*
T. audax vs. T. freemani	5.86	*
T. audax vs. T. anaphe	5.86	*
T. audax vs. T. madens	6.02	*
T. madens vs. T. destructor	5.16	*
T. madens vs. T. freemani	4.53	*
T. madens vs. T. anaphe	4.53	*
T. anaphe vs. T. destructor	0.63	No
T. anaphe vs. T. freemani	0.00	No

Table 18. cont. Chromosome #7. Tukey's analysis*.

Species compared	q value	Significant difference
T. brevicornis vs. T. destructor	8.44	*
T. brevicornis vs. T. freemani	7.81	*
T. brevicornis vs. T. anaphe	7.81	*
T. brevicornis vs. T. madens	3.28	No
T. brevicornis vs. T. audax	1.95	No
T. brevicornis vs. T. castaneum	1.33	No
T. brevicornis vs. T. confusum	1.33	No

* Indicates a significant difference.

$q_{\alpha} = 4.52$

Table 19. Interspecific comparison of chromosome #8. Tukey's analysis*.

Species compared	q value	Significant difference
T. confusum vs. T. anaphe	10.57	*
T. confusum vs. T. destructor	9.51	*
T. confusum vs. T. freemani	9.51	*
T. confusum vs. T. audax	4.75	*
T. confusum vs. T. madens	3.36	No
T. confusum vs. T. castaneum	3.36	No
T. confusum vs. T. brevicornis	1.31	No
T. brevicornis vs. T. anaphe	9.26	*
T. brevicornis vs. T. destructor	8.20	*
T. brevicornis vs. T. freemani	8.20	*
T. brevicornis vs. T. audax	3.44	No
T. brevicornis vs. T. castaneum	2.05	No
T. brevicornis vs. T. madens	2.05	No
T. madens vs. T. anaphe	7.21	*
T. madens vs. T. destructor	6.15	*
T. madens vs. T. freemani	6.15	*
T. madens vs. T. audax	1.39	No
T. castaneum vs. T. anaphe	7.21	*
T. castaneum vs. T. destructor	6.15	*
T. castaneum vs. T. freemani	6.15	*

Table 19. cont. Chromosome #8. Tukey's analysis*.

Species compared	q value	Significant difference
T. castaneum vs. T. audax	1.39	No
T. audax vs. T. anaphe	5.82	*
T. audax vs. T. destructor	4.75	*
T. audax vs. T. freemani	4.75	*
T. destructor vs. T. anaphe	1.07	No
T. freemani vs. T. anaphe	1.07	No

* Indicates a significant difference.

$q_{\alpha} = 4.52$

Table 20. Interspecific comparison* of chromosome #9. Tukey's analysis*.

Species compared	q value	Significant difference
T. confusum vs. T. freemani	14.94	*
T. confusum vs. T. anaphe	14.94	*
T. confusum vs. T. destructor	14.44	*
T. confusum vs. T. madens	7.16	*
T. confusum vs. T. audax	4.07	No
T. confusum vs. T. castaneum	4.07	No
T. confusum vs. T. brevicornis	0.99	No
T. brevicornis vs. T. freemani	13.95	*
T. brevicornis vs. T. anaphe	13.95	*
T. brevicornis vs. T. destructor	13.45	*
T. brevicornis vs. T. madens	6.17	*
T. brevicornis vs. T. audax	3.09	No
T. brevicornis vs. T. castaneum	3.09	No
T. audax vs. T. freemani	10.86	*
T. audax vs. T. destructor	10.37	*
T. audax vs. T. madens	3.09	No
T. castaneum vs. T. freemani	10.86	*
T. castaneum vs. T. anaphe	10.86	*
T. castaneum vs. T. destructor	10.37	*
T. castaneum vs. T. madens	3.09	No

Table 20. cont. Chromosome #9. Tukey's analysis*.

Species compared	q value	Significant difference
T. madens vs. T. freemani	7.78	*
T. madens vs. T. anaphe	7.78	*
T. madens vs. T. destructor	7.28	*
T. destructor vs. T. freemani	0.49	No
T. destructor vs. T. anaphe	0.49	No

* Indicates a significant difference.

$q_{\infty} = 4.52$

Table 21. Intraspecific comparison of chromosomes, Tribolium destructor. Tukey's analysis*.

Chromosomes compared	q value	Significant difference
1 vs. 9	16.92	*
1 vs. 8	14.55	*
1 vs. 7	14.55	*
1 vs. 6	13.43	*
1 vs. 5	12.24	*
1 vs. 4	10.49	*
1 vs. 3	8.18	*
1 vs. 2	3.49	No
2 vs. 9	13.43	*
2 vs. 8	11.04	*
2 vs. 7	11.04	*
2 vs. 6	9.93	*
2 vs. 5	8.74	*
2 vs. 4	6.99	*
2 vs. 3	4.68	*
3 vs. 9	8.74	*
3 vs. 8	6.36	*
3 vs. 7	6.36	*
3 vs. 6	5.24	*
3 vs. 5	4.08	No
3 vs. 4	2.31	No
4 vs. 9	6.43	*
4 vs. 8	4.06	No
4 vs. 7	4.06	No
4 vs. 6	2.92	No
4 vs. 5	1.75	No
5 vs. 9	4.68	*
5 vs. 8	2.31	No
5 vs. 7	2.31	No
5 vs. 6	1.19	No
6 vs. 9	3.49	No
6 vs. 8	1.12	No
6 vs 7	1.12	No

* Indicates a significant difference.

$q_{\alpha} = 4.64$

Table 22. Intraspecific comparison of chromosomes, Tribolium freemani. Tukey's analysis*.

Chromosomes compared	q value	Significant difference
1 vs. 9	14.85	*
1 vs. 8	11.97	*
1 vs. 7	11.36	*
1 vs. 6	9.47	*
1 vs. 5	8.86	*
1 vs. 4	8.18	*
1 vs. 3	5.68	*
1 vs. 2	1.89	No
2 vs. 9	12.95	*
2 vs. 8	10.07	*
2 vs. 7	9.47	*
2 vs. 6	7.58	*
2 vs. 5	6.97	*
2 vs. 4	6.29	*
2 vs. 3	3.79	No
3 vs. 9	9.17	*
3 vs. 8	6.29	*
3 vs. 7	5.68	*
3 vs. 6	3.79	No
3 vs. 5	3.18	No
3 vs. 4	2.50	No
4 vs. 9	6.67	*
4 vs. 8	3.79	No
4 vs. 7	3.18	No
4 vs. 6	1.29	No
4 vs. 5	0.68	No
5 vs. 9	5.99	*
5 vs. 8	3.11	No
5 vs. 7	2.50	No
5 vs. 6	0.60	No
6 vs. 9	5.38	*
6 vs. 8	2.50	No
6 vs. 7	1.89	No
7 vs. 9	3.48	No
7 vs. 8	0.61	No
8 vs. 9	2.88	No
9 vs. 10	0.91	No
8 vs. 10	3.79	No
7 vs. 10	4.39	No

* Indicates a significant difference.
$q_{\alpha} = 4.64$

Table 23. Intraspecific comparison of chromosomes, Tribolium anaphe. Tukey's analysis*.

Chromosomes compared	q value	Significant difference
1 vs. 9	9.05	*
1 vs. 8	7.72	*
1 vs. 7	6.61	*
1 vs. 6	6.19	*
1 vs. 5	4.39	No
1 vs. 4	2.64	No
1 vs. 3	1.75	No
1 vs. 2	1.75	No
2,3 vs. 9	7.30	*
2,3 vs. 8	5.98	*
2,3 vs. 7	4.87	*
2,3 vs. 6	4.44	No
2,3 vs. 5	2.65	No
2,3 vs. 4	0.89	No
4 vs. 9	6.40	*
4 vs. 8	5.08	*
4 vs. 7	3.97	No
4 vs. 6	3.54	No
4 vs. 5	1.75	No
5 vs. 9	4.66	*
5 vs. 8	3.33	No
5 vs. 7	2.22	No
5 vs. 6	1.79	No
6 vs. 9	2.86	No
6 vs. 8	1.53	No
6 vs. 7	0.42	No
7 vs. 9	2.43	No
7 vs. 8	1.11	No
8 vs. 9	1.32	No

* Indicates a significant difference.

$q_{\alpha} = 4.64$

Table 24. Intraspecific comparison of chromosomes, Tribolium brevicornis. Tukey's analysis*.

Chromosomes compared	q value	Significant difference
1 vs. 9	10.93	*
1 vs. 8	8.99	*
1 vs. 7	8.37	*
1 vs. 6	6.43	*
1 vs. 5,4	4.49	No
1 vs. 3	3.88	No
1 vs. 2	1.39	No
2 vs. 9	9.53	*
2 vs. 8	7.59	*
2 vs. 7	6.98	*
2 vs. 6	5.04	*
2 vs. 5,4	3.10	No
2 vs. 3	2.48	No
3 vs. 9	7.05	*
3 vs. 8	5.12	*
3 vs. 7	4.49	No
3 vs. 6	2.56	No
3 vs. 5,4	0.62	No
4,5 vs. 9	6.43	*
4,5 vs. 8	4.49	No
4,5 vs. 7	3.88	No
4,5 vs. 6	1.94	No
6 vs. 9	4.49	No
6 vs. 8	2.56	No
6 vs. 7	1.94	No
7 vs. 9	2.56	No
7 vs. 8	0.62	No
8 vs. 9	1.94	No

* Indicates a significant difference.

$q_{\alpha} = 4.64$

Table 25. Intraspecific comparison of chromosomes, Tribolium castaneum. Tukey's analysis*.

Chromosomes compared	q value	Significant difference
1 vs. 9	36.73	*
1 vs. 8	31.92	*
1 vs. 7	28.85	*
1 vs. 6	24.04	*
1 vs. 5	19.23	*
1 vs. 4	17.50	*
1 vs. 3	12.69	*
1 vs. 2	6.35	*
2 vs. 9	30.38	*
2 vs. 8	25.58	*
2 vs. 7	22.50	*
2 vs. 6	17.69	*
2 vs. 5	12.88	*
2 vs. 4	11.16	*
2 vs. 3	6.35	*
3 vs. 9	24.04	*
3 vs. 8	19.23	*
3 vs. 7	16.15	*
3 vs. 6	11.34	*
3 vs. 5	6.53	*
3 vs. 4	4.81	*
4 vs. 9	19.23	*
4 vs. 8	14.42	*
4 vs. 7	11.35	*
4 vs. 6	6.54	*
4 vs. 5	1.73	No
5 vs. 9	17.50	*
5 vs. 8	12.69	*
5 vs. 7	9.62	*
5 vs. 6	4.81	*
6 vs. 9	12.69	*
6 vs. 8	7.88	*
6 vs. 7	4.81	*
7 vs. 9	7.88	*
7 vs. 8	3.08	No
8 vs. 9	4.81	*
9 vs. 10	9.62	*

* Indicates a significant difference.

$q_c = 4.64$

Table 26. Intraspecific comparison of chromosomes, Tribolium confusum. Tukey's analysis*.

Chromosomes compared	q value	Significant difference
1 vs. 9	14.64	*
1 vs. 8	12.00	*
1 vs. 7	11.28	*
1 vs. 6	9.28	*
1 vs. 5	8.64	*
1 vs. 4	7.28	*
1 vs. 3	4.00	No
1 vs. 2	2.64	No
2 vs. 9	12.00	*
2 vs. 8	9.36	*
2 vs. 7	8.64	*
2 vs. 6	6.64	*
2 vs. 5	6.00	*
2 vs. 4	4.64	No
2 vs. 3	1.36	No
3 vs. 9	10.64	*
3 vs. 8	8.00	*
3 vs. 7	7.28	*
3 vs. 6	5.28	*
3 vs. 5	4.64	No
3 vs. 4	3.28	No
4 vs. 9	7.36	*
4 vs. 8	4.72	*
4 vs. 7	4.00	No
4 vs. 6	2.00	No
4 vs. 5	1.36	No
5 vs. 9	3.36	No
5 vs. 8	3.36	No
5 vs. 7	2.64	No
5 vs. 6	0.64	No
6 vs. 9	5.36	*
6 vs. 8	2.72	No
6 vs. 7	2.00	No
7 vs. 9	3.36	No
7 vs. 8	0.72	No
8 vs. 9	2.64	No

* Indicates a significant difference.

$q_{\alpha} = 4.64$

Table 27. Intraspecific comparison of chromosomes, Tribolium audax. Tukey's analysis*.

Chromosomes compared	q value	Significant difference
1 vs. 9	17.00	*
1 vs. 8	14.36	*
1 vs. 7,6	12.09	*
1 vs. 5	10.55	*
1 vs. 4	9.09	*
1 vs. 3	6.00	*
1 vs. 2	3.45	No
2 vs. 9	11.73	*
2 vs. 8	10.90	*
2 vs. 7,6	8.64	*
2 vs. 5	7.09	*
2 vs. 4	5.64	*
2 vs. 3	2.55	No
3 vs. 9	9.18	*
3 vs. 8	8.36	*
3 vs. 7,6	6.18	*
3 vs. 5	4.55	No
3 vs. 4	3.09	No
4 vs. 9	6.09	*
4 vs. 8	5.27	*
4 vs. 7,6	3.00	No
4 vs. 5	1.45	No
5 vs. 9	4.64	No
5 vs. 8	3.82	No
5 vs. 7,6	1.55	No
6,7 vs. 9	3.09	No
6,7 vs. 8	2.27	No
8 vs. 9	0.82	No
5 vs. 10	8.36	*
6,7 vs. 10	6.82	*
8 vs. 10	4.55	No
9 vs. 10	3.73	No
9 vs. 11	7.55	*
10 vs. 11	3.82	No
11 vs. 12	0.73	No
9 vs. 12	8.27	*
10 vs. 12	4.55	No

*Indicates a significant difference.

$q_{\alpha} = 4.64$

Table 28. Intraspecific comparison of chromosomes, Tribolium madens. Tukey's analysis*.

Chromosomes compared	q value	Significant difference
1 vs. 9	7.35	*
1 vs. 8,7	6.18	*
1 vs. 6	5.70	*
1 vs. 5	4.50	No
1 vs. 4	4.27	No
1 vs. 3	3.08	No
1 vs. 2	0.23	No
2 vs. 9	7.12	*
2 vs. 8,7	5.95	*
2 vs. 6	5.47	*
2 vs. 5	4.27	No
2 vs. 4	4.05	No
2 vs. 3	2.85	No
3 vs. 9	4.27	No
3 vs. 8,7	3.11	No
3 vs. 6	2.62	No
3 vs. 5	1.42	No
3 vs. 4	1.19	No
4 vs. 9	3.08	No
4 vs. 8,7	1.91	No
4 vs. 6	1.42	No
4 vs. 5	0.23	No
5 vs. 9	2.85	No
5 vs. 8,7	1.28	No
5 vs. 6	1.89	No
6 vs. 9	1.65	No
6 vs. 8,7	0.48	No
7,8 vs. 9	1.17	No
9 vs. 10	0.48	No
9 vs. 11	4.21	No
10 vs. 11	3.36	No
9 vs. 12	1.91	No
10 vs. 12	1.42	No
11 vs. 12	0.37	No
12 vs. 13	0.23	No
11 vs. 13	0.59	No
10 vs. 13	1.65	No
12 vs. 14	0.34	No
11 vs. 14	0.71	No
12 vs. 15	0.59	No
13 vs. 15	0.37	No
14 vs. 15	0.26	No

* Indicates a significant difference.

$q_{\alpha} = 4.64$

LITERATURE CITED

Baragaño, R. (1978) Nuevo metodo para el estudio de cromosomas en Coleoptera a partir de hemocitos de estado larvarios. Bol. Serv. Plagas 4: 23-33.

Blackwood, M. (1956). The inheritance of B-chromosomes in Zea mays. Heredity 10: 353.

Brown, W. (1972). Textbook of cytogenetics. C.V. Mosby, St. Louis.

Camacho, J. Cabrero, J. (1982). Supernumerary segments in five species of grasshoppers (Orthoptera: Acridoidea). Genetica 59: 113-117.

Catcheside, D.G. (1956). The genetics of B-chromosomes in maize. Heredity 10: 345.

Cochran, D.G., and Ross, M.H. (1969). Chromosome identification in the German cockroach. J. Hered. 60: 87-92.

Crowson, R.A. (1981). The biology of Coleoptera.. Academic Press, London.

Crowson, R.A. (1967). The natural classification of the family Coleoptera. Classey, Middlesex.

Garber, E.D. (1972). Cytogenetics: An introduction. McGraw-Hill, New York.

Gresson, R. (1948). Essentials of general cytology. English Universities Press, London.

Johannisson, R. (1977). A method for making permanent slides of squashed spermatocytes in Drosophila. Drosophila Inf.Service 52: 169.

Jones, Jack Colvard. (1962). Current concepts concerning insect hemolymphs. Amer. Zool. 2: 209-246.

Juan, C. and Petitpierre, E. (1988). A chromosome survey of north African and eastern Mediterranean Tenebrionids (Coleoptera). Cytobios 54: 85-94.

Lewis, K.R. and John, B. (1963). Chromosome marker. Little, Brown and Co., Boston.

Moore, Richard D. and Sokoloff, Alexander. (1982). The determination of chromosome number in Tribolium brevicornis and Tribolium anaphe. Tribolium Inf. Bull. 22: 116-117.

Ostergren, G. (1947). Heterochromatic B-chromosomes in Anthoxanthum. Hereditas 33: 261.

Roseland, Craig R., Beeman, Richard W., Kramer, Karl J., and Hopkins, Theodore L. (1983). Microinjection of amino acids in Tribolium. Tribolium Inf. Bull. 23: 132.

Ross, M.H. (1986). X chromosome origin of a supernumerary-like segment in Blatella germanica. Theor. Appl. Genet. 73: 316-319.

Smith, S.G. (1953). Chromosome numbers of Coleoptera. Heredity, Lond., 7: 31-48.

Smith, S.G. (1952b). The cytology of some tenebrionoid beetles (Coleoptera). J. Morph. 91: 325-364.

Sokoloff, Alexander. (1972). The biology of Tribolium with special emphasis on genetic aspects vol. 1 Clarendon Press, Oxford.

Swanson, Carl P., Merz, Timothy, and Young, William J. (1967). Cytogenetics. Prentice-Hall, Englewood Cliffs.

Virkki, N. (1974).Evolution of extremely complicated sex chromosome systems in Blaps. J. Agric. Univ. Peurto Rico, 18: 140-149.

White, M. (1954). Animal cytology and evolution 2nd ed. University Press, Cambridge.

White, M. (1976). John, B. (ed). Animal cytogenetics vol 3. insecta 2. Borntrager, Berlin.