Chromosome Aberrations in Field Strains of *Blattella germanica*

by

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Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Masters of Science

in

Entomology

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July, 1993

Blacksburg, Virginia

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(ABSTRACT)

Resistant and susceptible field strains of the German cockroach were compared for possible chromosome aberrations. Resistant strain females produced significantly higher numbers of aberrant oothecae (> 5 unhatched eggs) than the susceptible strains. Chromosome aberrations found in the susceptible strains were attachments (autosome-autosome, autosome-x) and fragments that did not reappear in outcrosses. Attachments (autosome-autosome, autosome-x), fragments, three translocation configurations that did not reappear in outcrosses and two reciprocal translocation heterozygotes occurred in the resistant srtrains. These two translocations have been tentatively identified as T(12:8)/12:8 from the Bowl strain and T(11:6)/11:6 from the K851 strain. T(12:8)/12:8 exhibits random disjunction at metaphase I. There were no differences related to susceptible vs. resistant strains in the frequency of chromosome aberrations from the aberrant oothecae.

There was no evidence, except in the K851 strain, to support a relationship between egg arrest and chromosome aberrations, or the hypothesis that chromosome aberrations result from the selective pressure of insecticides. It is suggested by this study that translocations are the most common type of "floating" polymorphism in the German cockroach. The first occurrence of three known phenotypic mutants, bent bristle, yellow body, and pallid eye, and one new phenotypic mutant, colorless eye, in field strains are reported by this study.

Acknowledgements

I wish to extend thanks to my committee members, Drs. Mary H. Ross, Donald G. Cochran, and Asim Esen, all of whom were very generous with their time and ideas. I especially would like to thank my advisor, Dr. Ross for her patience and support. Special thanks to Mrs. Nancy Boles and Mrs. Elizabeth Watson of the cockroach lab for all their help with my roaches, squashes, and photos and their support. The advice and comments of Dr. Heather Wren were very much appreciated. Thanks also to Cindy Schlossnagle for her caring and support. I would also like to thank my fellow graduate students, especially Colleen Cannon, Deb Davidson, David Judge, and Karen Walker for their help, advice, and support. I also thank the Patricia Roberts Harris Fellowship committee for honoring me with their award.

I would like to extend my most heartfelt thanks to Ronald Jarrell for his friendship and his help in getting this through the mainframe. Thanks also to Lloyd Eldred, Andrew Shoemaker, Fred Homan, Scott Gosik, and Helen Madden for their time, friendship, and computer expertise. I would also like to thank Denise Rich, Tessa Dalton, and Ellen Edge for their encouragement, love, and support when I needed it most. Finally, I would like to thank my father, John Crouch, for his support that first year, my stepmother, Terri Crouch, for her encouragement, my brother, Brad, for his love, and most importantly my mother, Trudy Crouch, for her belief, love, and "never say die" attitude, which kept me going till the end.

Table of Contents

List of Illustrations

List of Tables

Introduction

Insects have played an important role in cytogenetic studies. Polytene chromosomes, sex inheritance, linkage maps, and the first description of an inversion are a few of the contributions made using the fruit fly, *Drosophila melanogaster* (King 1975). Much cytogenetic work has also been done on natural populations of Orthoptera. Because of the large size and low numbers of their chromosomes and their variety of form and geographic distribution, the Orthoptera have been very useful for studies of evolution and population cytogenetics (Hewitt 1979). Significant research, both genetic and cytogenetic, has also been done with close relatives of the Orthoptera, the Blattodea or cockroaches. This group is not as popular for study perhaps because of the relatively high number of chromosomes found in many of the species (White 1976).

There are approximately 4000 species of cockroaches that have been described, and it is estimated that there are about the same number of species which have not been described (Cornwall 1968). One of the best known species of cockroaches is *Blattella germanica* (L.), the German cockroach. *B. germanica* is a common pest species which probably originated in East Asia and has subsequently spread worldwide through commercial travel (Roth 1985). Because it prefers a warm, moist, environment, the German cockroach is generally found in cohabitation with man.

Through the use of insecticides an effort has been made to control populations of *B. germanica*. Insecticides have become a selective force on these natural populations. This has resulted in a variation of resistance in natural populations, from low to very high and to one or more insecticides (Cochran 1989). Limited cytogenetic studies of two field-collected strains of *B. germanica* have suggested that changes at the karyotypic level may accompany the development of resistance as populations respond to selection pressure. In these studies, irregularities in meiotic cells were more frequently found in a field-collected, resistant strain than in a susceptible strain. More cytogenetic studies could look to the possibility of a relationship between resistance and chromosomal aberrations in natural populations (Ross 1986). They could also address the question of whether one type of chromosomal rearrangement predominates, e.g., "karyotypic orthoselection" (White 1973).

The objectives of this study were: (1) to search for and, if found, investigate the nature and frequency of chromosome aberrations in field-collected strains of the German cockroach, (2) to compare the frequency of chromosome aberrations in susceptible field strains to highly resistant field strains, (3) if a major chromosomal mutation is found: to identify the type and the chromosomes involved, and (4) to determine whether one particular type of chromosome rearrangement is more common than others.

Literature Review

Introduction

Cytogenetic study on natural populations of insects has provided many examples of chromosomal rearrangements. For example, natural populations of *Drosophila* have contained deficiencies, duplications, inversions, isochromosomes, and ring chromosomes (Swanson, et. al. 1981). Studies of natural populations of Orthoptera have shown chromosome varients such as inversions, translocations, centric fusions and fissions, sex chromosome rearrangements, supernumerary B-chromosomes, and supernumerary segments (Hewitt 1979). Chromosomal variation in natural populations of some Orthoptera has been shown to be affected by the environment. In the grasshopper, *Caledia captiva*, the position of the centromere on every chromosome varied along a latitudinal cline from medial locations in the northern populations to more distal or terminal ones in the southern populations (Shaw, et. al. 1987). Yadav and Yadav (1989) suggest that in acridioid grasshoppers, not all chromosomal aberrations in natural populations are spontaneous or from ionizing radiation, but can be forced in by environmental factors (pollutants). Translocations have been found in natural populations of the Blattodea, close relatives of the Orthoptera. Unlike the

Orthoptera though, there have been no examples of inversions, fusions, or dissociations (White 1976).

Blattodea Cytogenetics

The chromosome numbers of the Blattodea range from n = 8 to n = 40 with n = 19 (haploid in females) the most common (Cohen and Roth 1970). The tendency is for metacentric or submetacentric chromosomes with a few species having acrocentric or subacrocentric chromosomes (White 1976). The chromosomes of species with high diploid numbers are generally smaller than those with lower diploid numbers (Cochran and Ross 1967). Translocation heterozygosity has been shown in *Periplaneta americana* and *Blaberus discoidalis*. Rings and chains of four and sometimes six and eight were found at first metaphase in male meiosis of these two species (John and Lewis 1957, 1959). This evidence for translocation heterozygosity was attributed to inbreeding in the populations studied (John and Lewis 1958). Inversions, fusions, extra heterochromatic segments, dissociations, and supernumerary chromosomes have not been found in cockroaches (White 1976).

The closest known relative of *B. germanica* is the Asian cockroach, *B. asahinai* Mizukubo. It is the only species known to hybridize with *B. germanica*. The chromosomal morphology is very similar overall. The only differences found between the two are the result of a non-reciprocal translocation of the nucleolar organizing region from the X chromosome in *B. germanica* to the short arm of chromosome 12 in *B. asahinai*, and the smaller size of the X in *B. asahinai* (Ross 1988). This is evidence of a low level of interspecific divergence and of relatively recent divergence (Ross 1988).

German Cockroach Cytogenetics

The normal karyotype of *B. germanica* is 2n = 24 for females and 2n = 23 for males (Cochran and Ross 1967; Cohen and Roth 1970). Sex determination is of the XX:XO type with heterogametic males (Cochran and Ross 1967). The X chromosome is unpaired and heterochromatic in male meiosis. The chromosomes for most of the Blattellidae, which includes *B. germanica*, are principally metacentric and submetacentric with a few acrocentrics (Cohen and Roth 1970).

Identification of the wild-type chromosomes of the German cockroach is primarily by length. Chromosomes are numbered 1 thru 12 (haploid number) with 1 (the X chromosome) the shortest and 12 the longest (Cochran and Ross 1969). Identification is also facilitated by staining patterns in standard orcein preparations and by location of the centromere, which is shown by a C-banding procedure. Chromosomes 3, 4, 5, 7, 8, and 11 are metacentric; chromosomes 6, 9, 10, and 12 are submetacentric; chromosome 2 is acrocentric; and chromosome 1 is probably metacentric (Keil and Ross 1984).

Chromosome Rearrangements

In the German cockroach, known chromosome rearrangements are almost entirely limited to reciprocal translocations. There have been more than 20 translocations isolated. Most were found following exposure to ionizing radiation (Ross and Cochran 1975a); two were isolated from field-collected strains. The first was T(9:10)/9:10 *Pw* from the Elo strain, a pyrethrins-resistant strain (Ross and Cochran 1966). The second was in the Carver strain which is also highly resistant to several insecticides (Ross 1986). Translocated chromosomes were identified by measurement of length and by banding patterns.

Translocations in the German cockroach have provided much information. They have been used to correlate linkage groups with particular chromosomes (Ross and Cochran 1975a). A sex difference in fecundity were shown to be associated with T(7:12)/7:12 (Ross and Cochran 1975b). Significant sex differences in percentage of hatch was shown when T(9:11)/9:11, which has since been changed to T(8:9)/8:9 (Ross and Cochran 1981), was crossed reciprocally with wild-type (Cochran and Ross 1974). Single reciprocal translocations in *B. germanica* have also provided information on sterility and male competitiveness (Ross and Cochran 1975a) and have been used to compare the pertinant features of translocations having one chromosome in common (Cochran and Ross 1977b). Phenotypic changes are associated with two translocations; T(9:10)/9:10 *Pw* with pronotal winglets (Ross 1964) and T(4:6)/4:6 *Cu* with curly wings (Ross and Cochran 1989).

The single reciprocal translocations found in *B. germanica* made it possible to synthesize and study double translocation heterozygotes. A preliminary study on double translocations showed that they might be capable of suppressing populations (Ross and Cochran 1976). The translocations T(8:9)/8:9 and T(3:12)/3:12 were used to form a new double translocation heterozygote. In matings to wild-type, double translocation-carrying males were shown to cause sterility in a high percentage of first ootheca from embryonic trapping. The percentage of embryonic trapping was higher in the case of the double translocation-carrying male than in the double translocation-carrying female (Ross and Cochran 1977).

A translocation involving three independent chromosomes, a stable ring-of-six, appeared in the German cockroach following irradiation (Cochran and Ross 1977a). This translocation was first designated as T(4:8:10)/4:8:10 then changed to T(4:5:10)/4:5:10 (Ross and Cochran 1981). T(4:5:10)/4:5:10 males, when crossed with wild type, came close to causing complete sterility from embryonic trapping (Cochran and Ross 1977a). This ring along with T(8:9)/8:9 was used in the synthesis of a double translocation heterozygote. This double was a promising genetic control mechanism because of its effect on sterility (Ross and Cochran 1981). A three chromosome double translocation heterozygote T(3:7:12)/3:7:12 was developed by crosses between T(3:12)/3:12 and T(7:12)/7:12 (Ross and Cochran 1979). This double translocation heterozygote was released into

a laboratory population of German cockroaches. This reduced population numbers following releases into one generation (Ross 1980).

In translocation heterozygotes as well as in wild type, chiasmata appear to be located distally, one chiasma per arm (Keil and Ross 1983). In translocation quadrivalents, the chiasmata maintain the rings-of-four through diakenesis and metaphase I, aiding identification of adjacent and alternate disjunction (Ross and Cochran 1989). From these two basic types, four types of orientation, adjacent-1, alternate-1, adjacent-2, and alternate-2 can be observed. Endrizzi (1974) showed evidence for all four from reciprocal translocations from cotton. Though it was argued that there were only three orientations, adjacent-1 and adjacent-2 and alternate-1 (Boussy 1982), all four types of disjunction were shown in *B. germanica* (Cochran 1976, Cochran 1983). In translocations showing random disjunction (50% adjacent, 50% alternate) the frequencies of the four types were in a 2:1:1:2 ratio of adjacent-1, alternate-1, adjacent-2, alternate-2 respectively (Cochran 1976). Where directed disjunction favored alternate disjunction in the translocations studied, there was an increase in alternate-2 cells compared to the other orientations (Cochran 1977). In the analysis of the double reciprocal translocation T(8:9)/8:9 and T(3:12)/3:12 and the double translocation heterozygote T(3:7:12)/3:7:12, changes in the ratios appear to be genetically controlled (Ross and Cochran 1977, 1979). When the interchange heterozygote T(8:9)/8:9 was selected for matings with 50% embryonic lethality, near homozygosity for random disjunction was the result, thus eliminating the directed (alternate) disjunction (Diez et al. 1984). This was thought to stem mainly from the presence of non-distal chiasmata. It was suggested that normal chiasma were associated with discrete ratios (Diez et al. 1984).

The frequency of adjacent disjunction in translocations was very similar to the number of unhatched eggs that did not complete development (Ross and Cochran 1983). When reduced to a certain level, the viable embryos in an ootheca cannot open the keel at hatch. This type of sterility, called "embryonic trapping", occurs as a side effect of high lethality (Ross and Cochran 1986). As stated by Keil and Ross (1977) "sterility from embryonic trapping is governed primarily by the frequency of embryonic lethals within the individual ootheca." Since it is possible to determine the

frequency of egg arrest, presumably due to effects of translocations, from the external view of the ootheca, separation of wild-type and translocation egg cases is possible (Ross and Cochran 1987). In T(3:12)/3:12 there is a close correlation between the frequency of adjacent-1 vs. adjacent-2 and eggs which ceased development at stage I and stage VII respectively (Ross and Cochran 1981). It has been suggested that the nature of the stage I defects is due to certain loci and the possible involvement of regulatory genes and that other translocation heterozygotes with prevalent stage I arrest will show varied genetic disturbances (Ross and Nguyen-Tan 1982). With early arrest associated with aneuploid eggs in *B. germanica* translocations that involved different chromosomes, it has been inferred that the genes responsible for normal embryonic development are carried on most chromosomes (Ross and Cochran 1989).

The only chromosome mutation found in the German cockroach other than reciprocal translocations was a deficiency involving chromosome 9. It was designated DF(9) *Pw* because the phenotype was like that of the prowing translocation, T(9:10)/9:10 *Pw* (Ross and Cochran 1975a).

Cytogenetic Studies on Field-Collected Strains

Ross (1986) compared the karyotypes from two field-collected strains. One, the Carver strain, was highly resistant to several insecticides. The other strain, Fairbanks, was insecticide susceptible and karyotypically similar to the VPI strain, a susceptible laboratory strain, except for a terminal knob on chromosome 4 in Fairbanks strain males, and a small heterochromatic fragment which appeared attached to no one specific bivalent in 15% of the cells observed. The karyotype of the Carver strain, however, showed many examples of chromosome aberrations. Pseudo-multiples, fragments, univalents, deletions, attachment or insertion of fragments into unique positions, and a reciprocal translocation involving chromosome 12 and probably chromosome 6 were found. Most of these aberrations can be attributed to an increased tendency for the fusion of heterochromatin (Ross 1986).

Materials and Methods

Field-Collected Strains and Crosses

The field-collected strains of *B. germanica* used were divided into two groups dependent on pesticide resistance characteristics. The susceptible strains were either completely susceptible or their resistance to one or more insecticides no more than what is generally regarded as "tolerance." The susceptible group consisted of the following field-collected strains:

1. Fairbanks (1985)

2. N3 (1987)

3. Young (1986)

4. Hennis (1986)

The resistant strains and major types of resistance consisted of the following (pers. comm., Dr. D. G. Cochran)

1. Reddick; > 100× to pyrethrins, 60× to bendiocarb, > 100× to allethrin (1986)

2. K851; > 100× to pyrethrins, allethrin, phenothrin, and permethrin; 23× to malathion (1987)

3. Garland; > 100× to pyrethrins; 40× to malathion, and bendiocarb (1986)

4. N5; > 100× to pyrethrins, and allethrin; > 60× to fenvalerate (1987)

5. Montgomery Gardens; > 140× to pyrethrins and bendiocarb (1986)

The VPI strain, a standard susceptible laboratory strain, was used as the wild-type strain for karyotypic comparison. VPI strain males were out-crossed to females used in this study. This ensured heterozygosity for major chromosome mutations or other heritable aberrations that had partial lethal effects seen in egg cases of field-strain females. In addition, studies were made to determine the basis of a heritable trait(s) characterized by an approximate 50% lethality that originated from two ootheca-bearing females from the Bowl strain. The strain was collected in 1981 and was highly resistant to bendiocarb (> 60x) and slightly resistant to propoxur. Lines had been maintained by selection of females with approximately 50% egg arrest. Nymphs from one of these lines, designated Bowl 3, were provided for study.

General Rearing

The field-collected strains were maintained in glass battery jars (3.8 l) fitted with screen wire cages. Individual females and their progeny were reared in quart glass jars fitted with a screen wire cage. Water and dog food pellets were supplied as necessary. Clean jars, both battery and quart, were supplied once a week. The VPI strain was maintained in at least seven battery jars with periodic mixing. The stocks were maintained at 25-28°C with a photo-period of approximately 14L:10D.

Selection of Oothecae For Study

For each of the field strains, except the Bowl strain, nymph jars were set up. Nymphs were taken from each field strain and housed in battery jars. Females with their first ootheca were removed from these jars for observation. Around 200 females were used per strain.

When approximately three weeks old, each ootheca was observed with a dissecting microscope after briefly anesthetizing the female with CO_2. Eggs within each oothecal compartment were compared; determining whether they were developing normally, or if in some, development had been arrested. Females whose ootheca had less than five, or no, eggs showing early arrest were considered normal and discarded. Females whose ootheca had five or more eggs showing early arrest were selected for cytological observations on their male progeny. The number and stage of egg arrest in these oothecae were noted. In three week old oothecae most eggs were beyond stage VII, where the dorsal vessel is observable and longitudinal rows of white dots are conspicuous (Tanaka 1976). If all the eggs were before stage V, the ootheca was considered immature and observed again one week later.

Ootheca-bearing females that were selected for study were reared singly in quart jars. Their progeny were separated into males and females at the third instar, and their number noted. Third instar females were identified by a medial notch in the ninth sternum and the eighth overlapped by the seventh, which causes it to resemble lateral lobes (Ross and Cochran 1960). The male nymphs were used for chromosome observations. The sibling female nymphs were outcrossed to VPI males if chromosome aberrations were found in the males.

The sibling females had their oothecae observed as previously described. If one or more females had an ootheca in which five or more eggs did not complete development, the resulting male progeny were used for chromosome observation. If a chromosome aberration reappeared, the sibling females were again crossed to VPI males in order to maintain the trait. If all the females had

normal oothecae, or the chromosome aberration did not reoccur in the outcrosses, the cockroaches were discarded.

Chromosome Preparation and Observation

Observations were on meiotic cells of third-fourth instar male nymphs. At least eight males from hatch of each ootheca were observed. Pachytene and diplotene cells were examined for chromosome aberrations especially for evidence of major chromosomal rearrangements. These rearrangements are generally deletions or deficiencies, duplications, inversions, and translocations. A deficiency is the loss of a block of chromatin from a chromosome. It can be terminal or interstitial and either type, if large enough, can be seen at pachytene. A duplication, here used in a gross context, is an extra piece of the normal complement of a chromosome. Duplications can be in tandem, reverse tandem, or as a displaced piece. An inversion is a portion of a chromosome that has become rearranged in reverse order as a consequence of chromosome breakage. There are two types, paracentric, which is confined to one arm of the chromosome, and pericentric, which involves the centromere. Most translocations are reciprocal. A reciprocal translocation is an exchange of blocks of chromatin between two non-homologous chromosomes. Translocation heterozygotes can be identified by "cross-like" configurations at pachytene/diplotene.

Cells for observation were stained by a standard orcein perparation (Cochran and Ross 1969). The preparations were observed under phase contrast with a Zeiss Photomicroscope III and photographed with T-Max 100 or Plus-X film. Cells were from the testes of third-fourth instar males. The testes were dissected into a drop of 15% acetic acid on a clean slide. Excess fat and other debris were removed carefully. The acetic acid was removed and replaced with orcein stain for five minutes. The stain was then removed and replaced with 15% acetic acid. Testes were squashed under a coverslip using thumb pressure. The coverslip was ringed with clear fingernail polish.

Observations on Embryonic Development

In stage I of egg development, it is not possible to tell if an egg has been fertilized. Therefore oothecae with five or more eggs showing early arrest (including some stage I) were used to determine if the eggs were fertilized. Oothecae from as many field-strains as possible (K851, Bowl, Young, Montgomery Gardens, Garland, Hennis) were observed.

The selected oothecae were put in a water bath of 80°C for 45 minutes. The oothecal coverings were split along the keel and pulled back till most of the embryo was exposed. The oothecae were placed in Kahle's fixative for 18-24 hours, then washed in distilled water, two changes of one hour each. They were placed in HCl for 10 minutes. The oothecae were dipped into distilled water then placed in Feulgen's stain for 10 minutes. They were put into 20% ethyl alcohol (ETOH) for 24 hours which was replaced with fresh 20% ETOH if necessary. The 20% solution was replaced with 60% ETOH for 24 hours and then replaced with 80% ETOH for 24 hours. The 80% solution was then replaced with 100% ETOH for 5 minutes. As many stage I eggs as possible were dissected out of the ootheca and mounted in Canada balsam along with a few of the more advanced, normal eggs for comparison.

Results

Oothecae of Field Strains

The first ootheca produced by females of each of the susceptible and resistant strains was examined. The number of oothecae observed ranged from 161 in N3 (susceptible) to 240 in K851 (resistant) (Table 1). In the susceptible strains,the percentage of oothecae that were aberrant (with 5 or more eggs arrested in development) ranged from a low of 1.5% in the Fairbanks strain to 4.5% in the Hennis strain. The percentage of aberrant oothecae in the resistant strains ranged from 7.1% in the N5 strain to 11.3% in the K851 strain (Table 1). This percentage was higher in resistant than susceptible strains. The pooled data of susceptible and resistant strains showed that the frequency of aberrant oothecae was significantly higher in resistant strains than in susceptible strains (Table 2).

The average number of eggs per ootheca ranged from a low of 30 in the Young strain to a high of 40 in the Fairbanks strain (Table 1). The Fairbanks, Garland, and Young strains differed significantly from each other. Otherwise, there were no significant differences between strains and no consistent trend towards a difference related to susceptible vs. resistant strains.

The average number of nymphs hatched per ootheca ranged from 20 in the Young strain to 29 in the Hennis strain (Table 1). The Hennis strain differed significantly from the Young strain and the Reddick strain. Otherwise, there were no significant differences between strains and no consistent trend towards a difference related to susceptible vs. resistant strains. The sex ratio was 1:1 in nymphs of all strains.

The total of percentage hatch and the percentage of eggs arrested for each strain were in reasonable agreement with 100 percent (Table 1). For example, in the N5 strain on Table 1, 71 percent hatch plus 26 percent eggs arrested gives 97 percent. Percent hatch was exclusive of oothecae that did not hatch, while the percentage of eggs arrested was inclusive of these oothecae. Not all of aberrant ootheca found in the susceptible and resistant strains hatched. Some of the females carrying the egg case died, other females dropped the egg case prematurely. In the susceptible strains, three of 26 (11.5%) aberrant ootheca did not hatch. In the resistant strains, 19 of 65 (29.2%) aberrant ootheca did not hatch. In all cases of unhatched oothecae, the percentage of incompletely developed eggs was between 18-39%. Though the appearence of the unhatched egg cases suggested embryonic trapping, the percentage of incompletely developed eggs was too low for this to be the case (Keil and Ross 1977).

Table 3 shows the stages in which eggs were arrested before development was completed. In the aberrant oothecae, eggs were arrested at one of three stages, stage I (white), stage III (cloudy), or stage VII (green line) (Tanaka 1976). In the susceptible Fairbanks and N3 strains, the majority of eggs were arrested at stage I. In the susceptible Young strain, 100% of eggs were arrested at stage I. Of the resistant strains, two of the five, K851 and Reddick, also had the majority of aberrant eggs arrested at stage I. In one susceptible strain, Hennis, and three resistant strains, Garland, Montgomery Gardens, and N5, the majority of aberrant eggs were arrested in stage III. The percent of stage VII arrest ranged from 6.4% in the Reddick strain to 20.9% in the Montgomery Gardens strain. In two strains, the Fairbanks and Young, egg arrest was limited to early stages (I-III). Overall, most eggs ceased development relatively early, i.e. by about the seventh day out of a normal development period of 26-28 days.

Chromosome Aberrations in Field Strains

Only a small percentage of the total number of oothecae per strain yielded an egg case from which chromosome aberrations occurred in the male nymphs (Table 4). The number of aberrant egg cases from which chromosome aberrations occurred in male progeny ranged from zero in N3 and N5 to four in the Reddick strain. When the total number of aberrant oothecae and the total number of aberrant oothecae which yielded chromosome aberrations for both susceptible and resistant strains were pooled and compared, the difference between susceptible and resistant strains was not significant ($0.75 < P < 0.90$) on a 2×2 contingency table.

A list of the chromosome aberrations found in the different field strains is shown in Table 5. The aberrations found consisted of fragments, attachments (autosome - autosome and autosome - x), early separation of chromosomes, early desynapsis, and translocations. Examples of attachments, the most prevalent aberration found in the field strains studied, are shown in Figures 1 & 2. Attachments were apparently due to fusion of heterochromatin on non-homologous chromosomes. Examples of other chromosome aberrations are shown in Figures 3 & 4. Examples of the translocations which were apparently spontaneous non-recovered after the first generation are shown in Figure 5. The frequency in which these chromosome aberrations were found in the male progeny from aberrant ootheca and in the cells of these individual males is shown in Table 6. These frequencies show no consistent trend toward a difference related to susceptible vs. resistant strains. However, of all the chromosome aberrations found in the susceptible and resistant strains, only a translocation heterozygote from the Bowl 3 resistant strain (Figure 6) and a translocation heterozygote from the K851 resistant strain (Figure 10) reappeared in outcrosses with the VPI strain. The K851 male that produced the translocation heterozygote that reappeared in outcrosses was from a different ootheca than the male that produced the translocation in K851.

The Bowl 3 translocation (Figure 6) was tentatively identified as T(12:8)/12:8 through measurements of chromosome length and banding patterns from orcein stained preparations. Comparisons to the karyotype of VPI chromosomes were used to compile a karyotype of the Bowl 3 translocation (Ross 1986). The break in chromosome 12 appeared to be midway down its length, in the longer arm of the chromosome. A comparison of chromosome 12 from the translocation to a normal chromosome 12 is shown in Figures 7 & 8. Chromosome 12 is highly distinctive and there was no doubt concerning its identification. The break in chromosome 8 appeared to be at the midpoint of the chromosome, presumably near the centromere region. The identification of chromosome 8 was less certain although it was supported by identification of other chromosomes in the Bowl 3 karyotype.

Metaphase I/anaphase I cells were observed in males heterozygous for the Bowl 3 translocation, T(12:8)/12:8. The totals of adjacent and alternate disjunction in the cells of five males are listed in Table 7. There was a 1:1 ratio of adjacent vs. alternate disjunction indicating random disjunction of the Bowl 3 translocation. Examples of alternate and adjacent cells at metaphase I from T(12:8)/12:8 are shown in Figure 9. The most prevalent type of adjacent cell was the "music-note" adjacent-1 cell shown in Figure 9A. Most of the alternate cell types which were not distinguishable as alternate-1 or alternate-2 appeared as in Figure 9D.

The K851 translocation was tentatively identified as T(11:6)/11:6 by observations on chromosome lengths and staining patterns (Figure 10). Because chromosomes in the K851 translocation heterozygote karyotype were, in part, found in a state of early desynapsis or were falling apart, it was hard to identify chromosomes by comparisons with the VPI karyotype. It was necessary to put together a karyotype of normal K851 diplotene chromosomes (Figure 11) that was used for comparison in the compilation of the K851 translocation karyotype. In the K851 translocation karyotype, early seperation of chromosomes, especially chromosomes 9, 7, and 4 (Figure 10), occurred frequently. In the normal karyotype, a trend toward early separation was also observed, for example, in chromosomes 3 and 4 (Figure 11).

Analysis of Apparent Stage I Eggs

Stage I of egg development in German cockroaches lasts for five days and covers early cleavage divisions to the formation of a segmented germ band (Tanaka 1976). From the outer view of the egg case, only the whitish-appearing yolk is visible in stage I. It could not be determined with certainty whether eggs that appeared to be in stage I were fertilized. The possibility that eggs categorized as stage I were unfertilized was considered.

Feulgen stained whole mounts of apparent stage I eggs from aberrant oothecae with at least five eggs arrested were observed. In the case of the translocations (Bowl 3 and K851 strains), one ootheca each was used for the whole mounts. Whole mounts from the Garland, Young, and Montgomery Gardens strains were observed using three oothecae from each strain. Likewise, two oothecae each were used from the Hennis, N5, and N3 strains. Examples of these whole mounts are shown in Figure 12. Eggs shown in Figure 12 B, C, D are from the K851, Hennis, and Garland strains respectively. Most of the eggs had cleavage energids scattered through the yolk (Figure 12B). In some, germ bands had formed, but none were developing normally. A few of the whole mounts had only fat droplets in the yolk and no germ band. Most of the apparent stage I eggs were considered fertilized because of the presense of cleavage energids and germ bands in the eggs.

Morphological Polymorphisms

Table 8 lists morphological polymorphisms found in the field-collected strains during this study. Orange body, a very common polymorph, appeared in one susceptible strain and three resistant strains. Three polymorphs which appeared in the field-collected strains seemed to be identical to the yellow-body, bent bristle, and pallid-eye mutants described by Ross and Cochran (1975). Both

yellow-body and bent bristle appeared in the Young strain, a susceptible strain. Pallid-eye also appeared in a susceptible strain, N3. A polymorphism called at present "colorless eye" also appeared in the N3 strain. Colorless eye mutants were crossed to both pearl-eye and pallid-eye to see if it was a new mutant. The F_1 progeny had the normal black eye color. The colorless eye appeared in the F_2 progeny and was distinguishable from both pearl- and pallid-eye. Thus, it appears that "colorless eye" is recessive and that it is not an allelle of either pallid-eye or pearl-eye.

Table 1. Survey for possible lethal effects in oothecae.

Strain	No. of oothecae studied	Aberrant oothecae no. (%)[1]	Avg. no. eggs per aberrant oothecae[2]	Avg. no. nymphs per ootheca[2] (%hatch)[4]	Avg. % eggs arrested during development[5]
Fairbanks (S)	193	3 (1.5)	40 ± 3.4[a]	27 ± 1.2 (67)[ab]	25
Hennis (S)	200	9 (4.5)	38 ± 5.4[ab]	29 ± 5.7 (76)[a]	26
N3 (S)	161	7 (4.3)	33 ± 1.6[cd]	24 ± 2.2 (73)[abc]	22
Young (S)	166	7 (4.2)	30 ± 3.9[d]	20 ± 3.8 (69)[c]	28
Garland (R)	200	16 (8.0)	35 ± 3.1[c]	25 ± 5.5 (72)[abc]	28
K851 (R)	240	27 (11.3)	36 ± 4.3[abc]	25 ± 3.0 (68)[abc]	22[3]
Montgomery Gardens (R)	207	17 (8.2)	37 ± 4.5[abc]	28 ± 5.4 (73)[ab]	27
N5 (R)	196	14 (7.1)	38 ± 4.0[ab]	27 ± 5.9 (71)[ab]	26
Reddick (R)	210	20 (9.5)	33 ± 5.3[cd]	23 ± 5.0 (70)[bc]	25

(S) = Susceptible field-collected strain.
(R) = Resistant field-collected strain.
[1] with ≥ 5 eggs arrested in development.
[2] Means followed by the same letter are not significantly different at the .05 level (experimentwise) using Tukey's HSD test (Zar, 1984, pp. 186-190).
[3] Inclusive of translocation with 33% egg arrest.
[4] Exclusive of ootheca that did not hatch.
[5] Inclusive of ootheca that did not hatch.

Table 2. Comparison of aberrant oothecae in insecticide-susceptible and insecticide-resistant field collected strains.

	Normal	Aberrant[a]	Total
Susceptible	694	26	720
Resistant	959	94	1053
Total	1653	120	1773

[a] significantly different ($P < 0.001$); 2×2 contingency table.

Table 3. Frequency of the stages of egg arrest in eggs that did not complete development.

Strain	% eggs per ootheca arrested stage I	% eggs per ootheca arrested stage III	% eggs per ootheca arrested stage VII
Fairbanks (S)	63.0	37.0	-
Hennis (S)	8.9	78.3	12.8
N3 (S)	71.7	18.3	15.0
Young (S)	100.0	-	-
K851[a] (R)	79.5	4.3	18.2
Garland (R)	10.2	78.8	11.0
Montgomery Gardens (R)	13.3	65.8	20.9
N5 (R)	17.1	75.5	7.4
Reddick (R)	71.8	21.8	6.4

(S) = Susceptible
(R) = Resistant
[a] inclusive of translocation egg case

Table 4. Comparison of aberrant oothecae with chromosome aberrations in susceptible and resistant field-collected strains.

Strain	No. of females with aberrant ootheca		
	No. ootheca hatched	No. with chromosome aberration(s) in male progeny	% with chromosome aberration(s) from total oothecae of strain
Fairbanks (S)	2	1	0.5
Hennis (S)	9	1	0.5
N3 (S)	5	0	0.0
Young (S)	7	1	0.6
K851 (R)	22	3	1.3
Garland (R)	13	3	1.5
Montgomery Gardens (R)	14	2	1.0
N5 (R)	10	0	0.0
Reddick (R)	16	4	1.9

(S) = Susceptible
(R) = Resistant

Table 5. Chromosome aberrations.

Strain	Aberration
Fairbanks (S)[a]	attachments (a-a)[b]
Hennis (S)[a]	attachments (a-a)[b], fragments, early desynapsis
Young (S)	attachments, early desynapsis
K851 (R)[a]	translocation heterozygote, attachments (a-a)[b], fragments
Garland (R)[a]	translocation configuration (one cell), attachments (a-a)[b]
Montgomery Gardens (R)[a]	fragment, attachments (a-a)[b]
Reddick (R)[a]	attachments (a-a)[b], translocation heterozygote
Bowl 3 (R)[a]	translocation heterozygote, attachments (a-a)[b]
Bowl 2/Bowl 5 (R)[a]	translocation configuration (one cell)

(S) = Susceptible
(R) = Resistant
[a] shows autosome - X attachments common to *B. germanica*
[b] autosome - autosome attachments

Table 6. **Frequency of chromosome aberrations.**

Strain males[b]	No. of males studied[a]	Males with chromosome aberration No. (%)	Frequency (%) of chromosome aberrations in cells of individual.
Fairbanks (S)	8	2 (25)	36.6
Hennis (S)	8	3 (37.5)	40.0
Young (S)	8	1 (12.5)	20.0
K851 (R)	26	7 (26.6)	42.7[c]
Garland (R)	24	5 (25.7)	21.6
Montgomery Gardens (R)	16	3 (18.7)	20.0
Reddick (R)	32	6 (18.7)	17.7
Bowl 3 (R)	12	4 (33.3)	58.8[c]

(S) = Susceptible
(R) = Resistant
[a] Total from all aberrant oothecae with chromosome aberrations (see Table 4, col. 2).
[b] Minimum of 15 cells counted per male.
[c] Exclusive of translocations.

Table 7. Frequency of alternate and adjacent disjunction at metaphase I in the translocation heterozygote tentatively identified as T(12:8)/12:8.

Male	No. of cells with adjacent disjunction	No. of cells with alternate disjunction	% of cells adjacent/alternate	Total
1	33	25	57/43	58
2	53	52	51/49	105
3	29	45	39/61	74
4	18	15	55/54	33
5	26	23	53/47	49
Total	159	160		319

Table 8. Morphological mutations in field-collected strains.

Strain	Mutation(s)[a]
Fairbanks (S)	orange-body
N3 (S)	pallid-eye, "colorless eye"[b]
Young (S)	bent bristle[b], yellow body[b]
Garland (R)	orange-body
Montgomery Gardens (R)	orange-body
N5 (R)	orange-body

(S) = Susceptible
(R) = Resistant
[a] identified by phenotype except "colorless eye."
[b] new to field-collected strains.

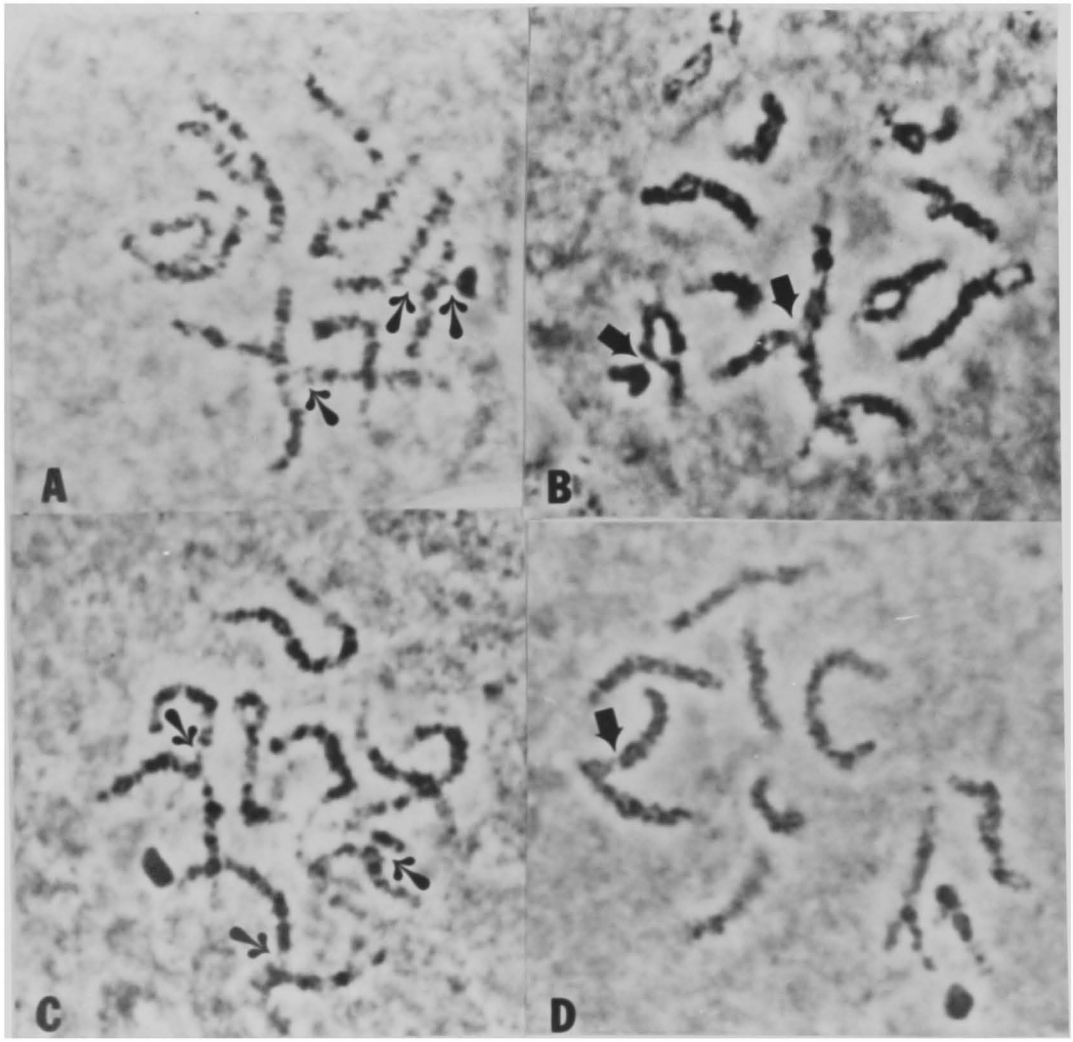


Figure 1. **Chromosome aberrations in male meiocytes; attachments in field-collected strains:** Arrows indicate attachment sites. A and B) from the Hennis strain; C) the Fairbanks strain; D) the K851 strain.

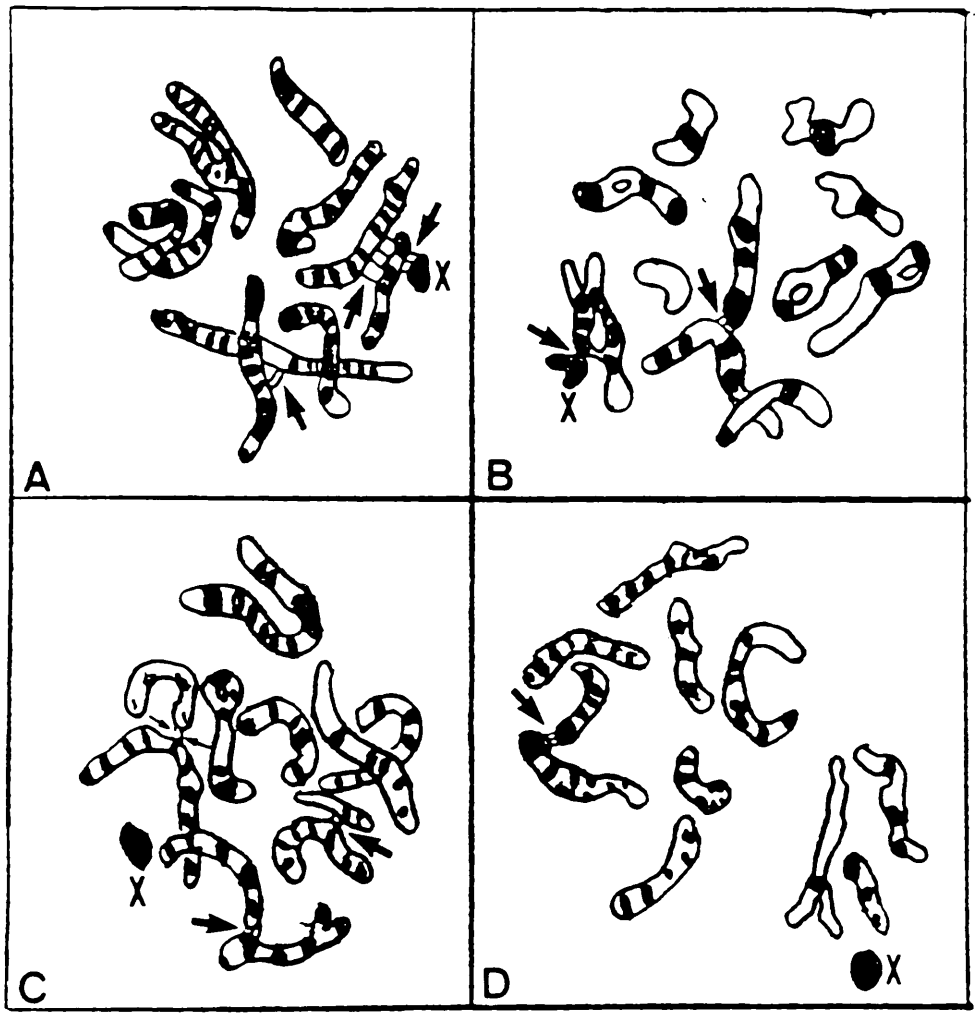


Figure 2. Interpretive drawing of Fig. 1: A and B) are from the Hennis strain; C) the Fairbanks strain; D) the K851 strain. Arrows indicate attachment sites. 'X' indicates the X chromosome.

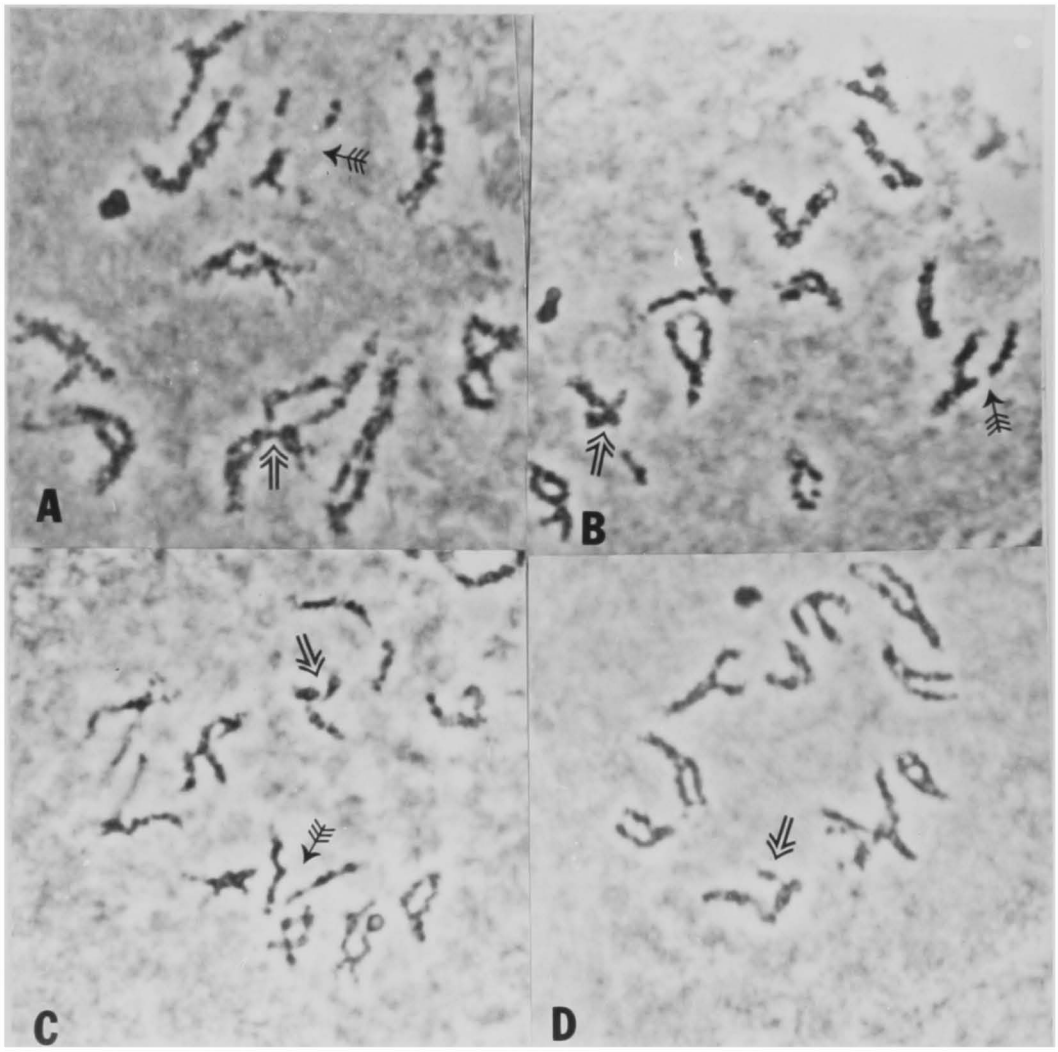


Figure 3. Chromosome aberrations in male meiocytes; attachment and other aberrations in field-collected strains: A) Incipient breakage of chromosome 3 (feathered arrow) and attachment in K851 strain; B) break in what may be chromosome 11 (feathered arrow) and fragment in the K851 strain; C) desynapsis (feathered arrow) and fragment in the Hennis strain; D) fragment in the Garland strain.

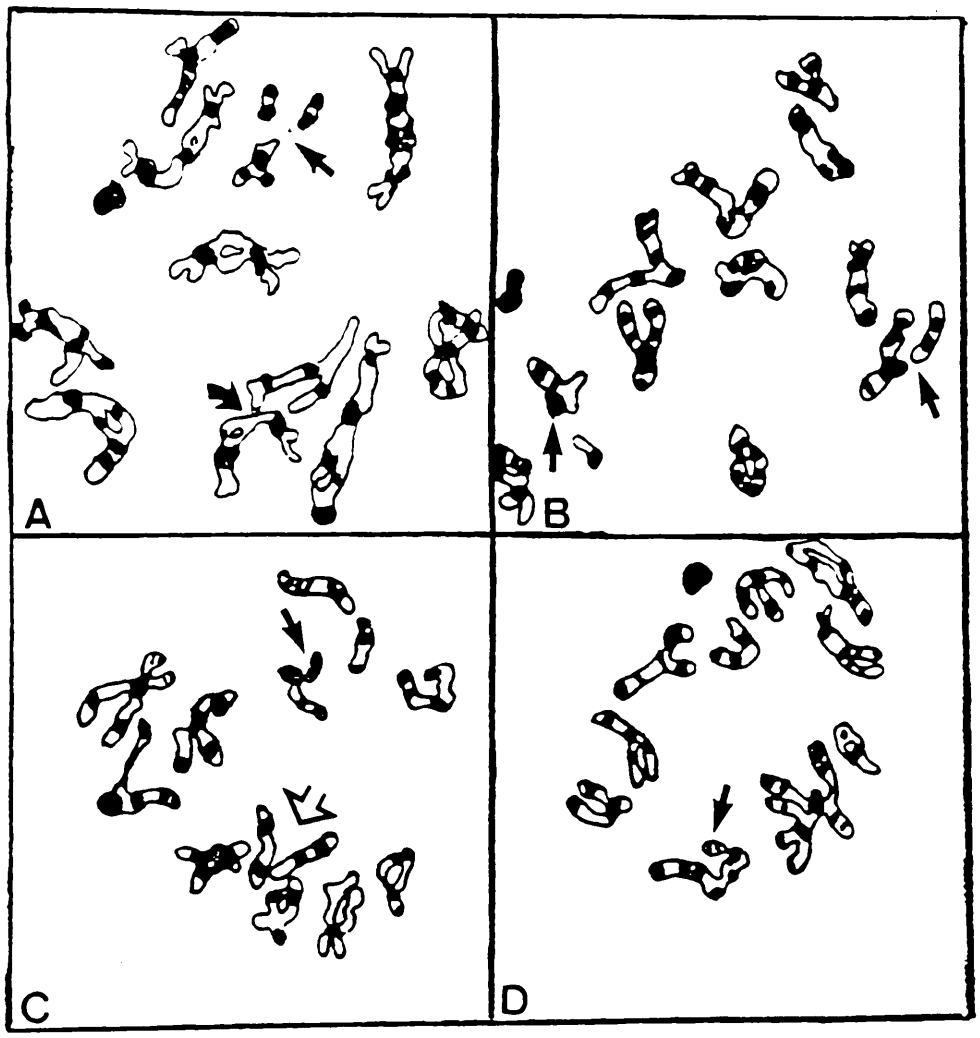


Figure 4. **Interpretive drawing of Fig. 3:** A and B) the K851 strain; C) the Hennis strain; D) the Garland strain. Solid arrow indicates fragments and breaks. Open arrow indicates desynapsis. Curved arrow indicates attachments. Tentatively identified chromosomes are indicated by number. 'X' indicates the X chromosome.

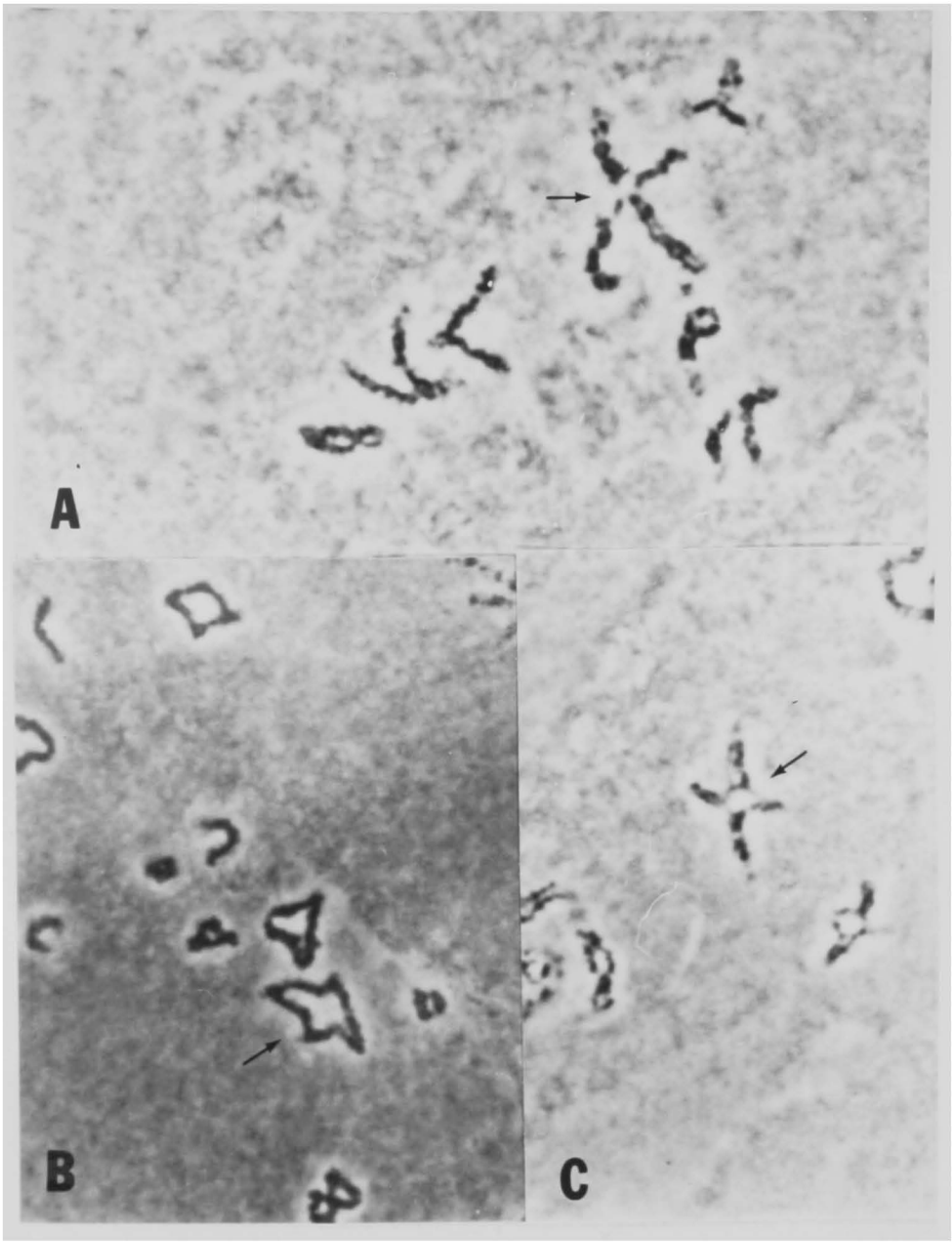
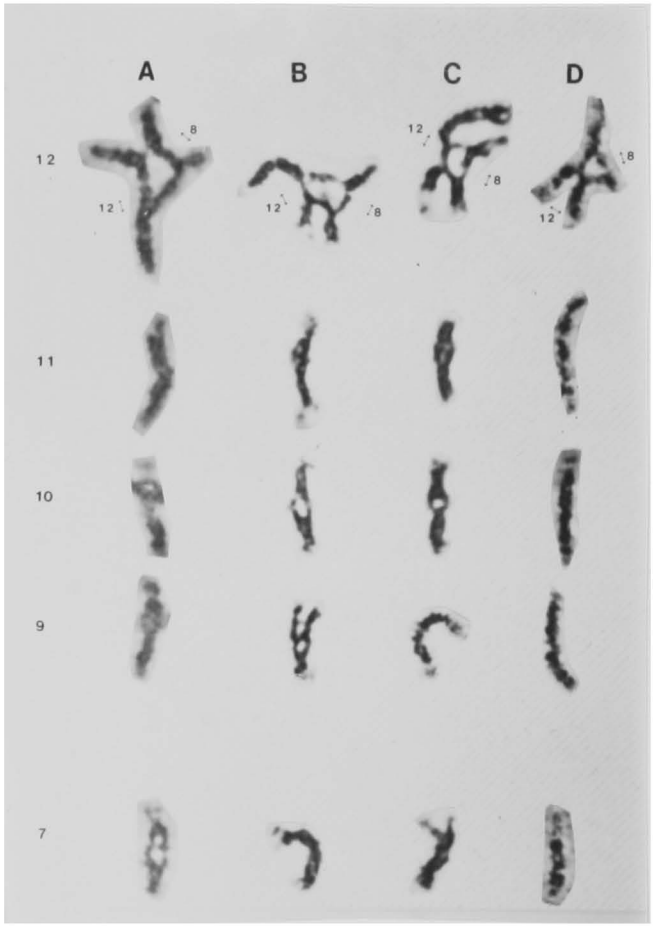
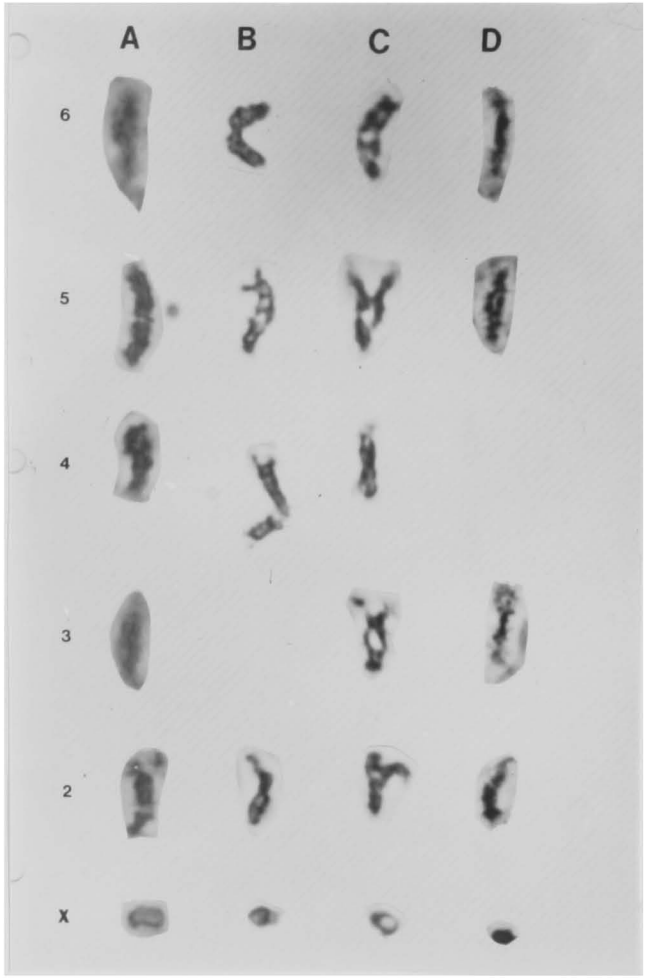


Figure 5. **Translocation heterozygotes in male meiocytes:** A) Garland translocation configuration, one cell only; B) Reddick translocation heterozygote; C) K851 #4 translocation heterozygote.

Figure 6. Orcein-stained meiotic karyotype of the Bowl 3 translocation tentatively identified as T(12:8)/12:8: Chromosomes from four cells (A-D) are numbered from 12, the longest, to X (Number 1), the shortest.





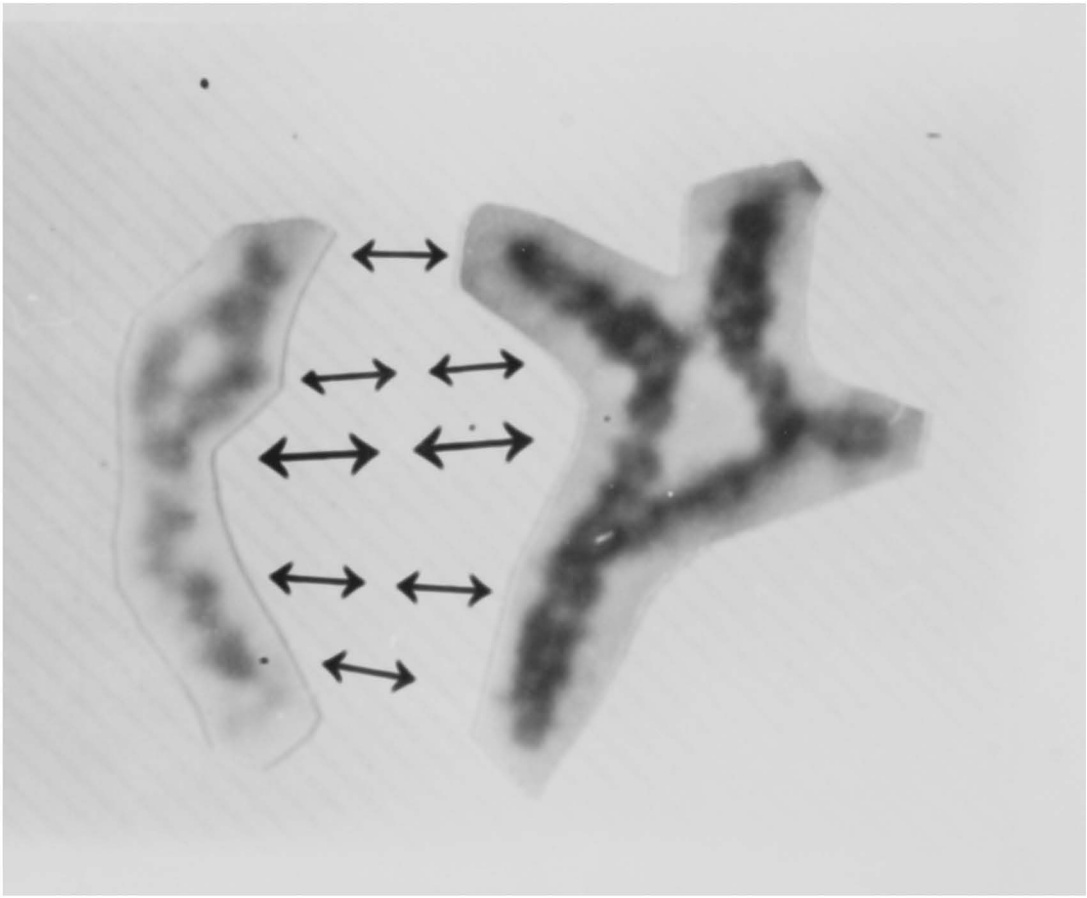


Figure 7. Wild-type chromosome 12 (left) compared to chromosome 12 (right) in the translocation tentatively identified as T(12:8)/12:8: Arrows indicate similar regions on the chromosomes.

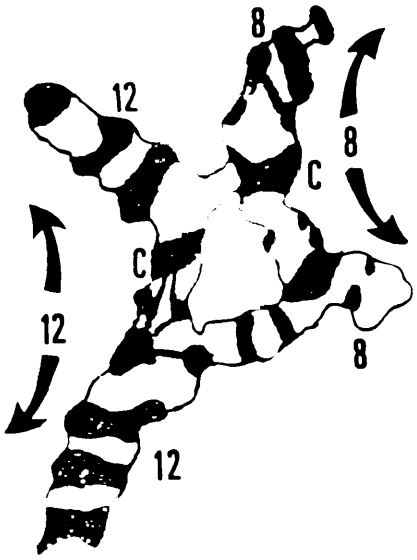


Figure 8. Interpretive drawing of T(12:8)/12:8 from Fig. 7: Numbers indicate regions of each chromosome. 'C' indicates the centromere of each chromosome.

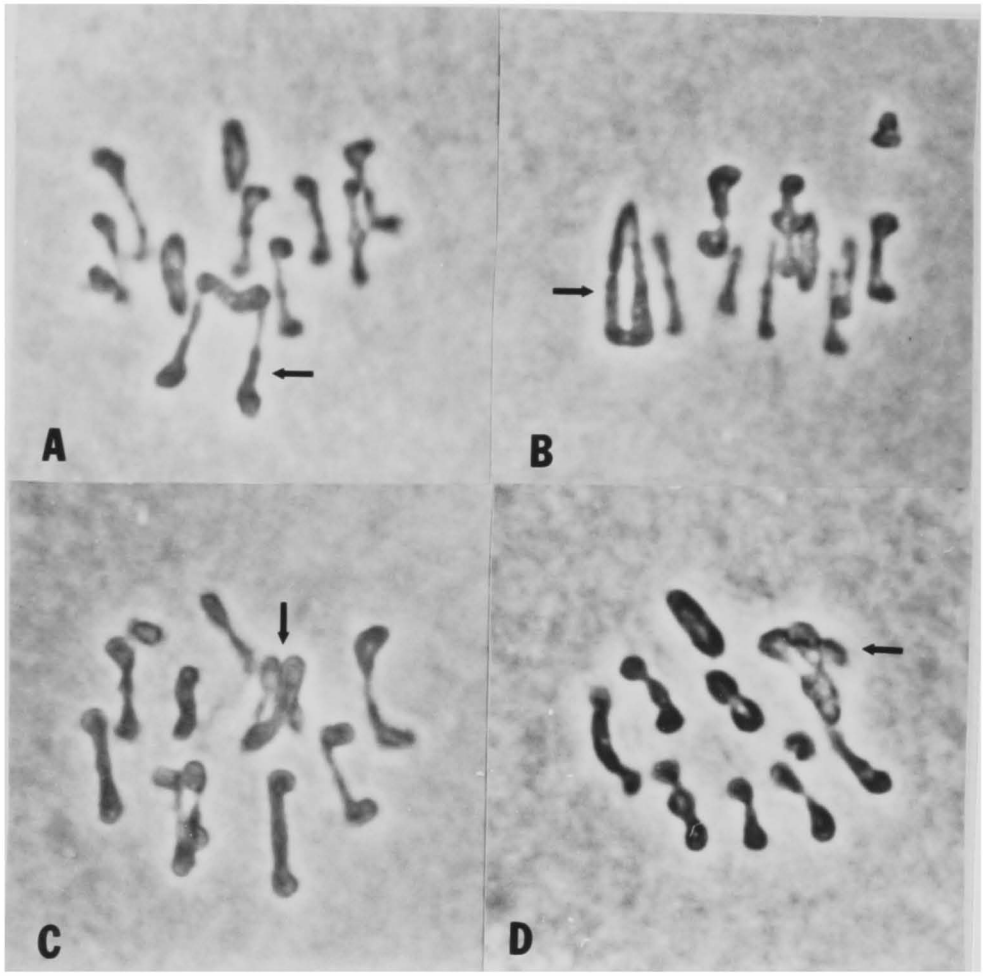
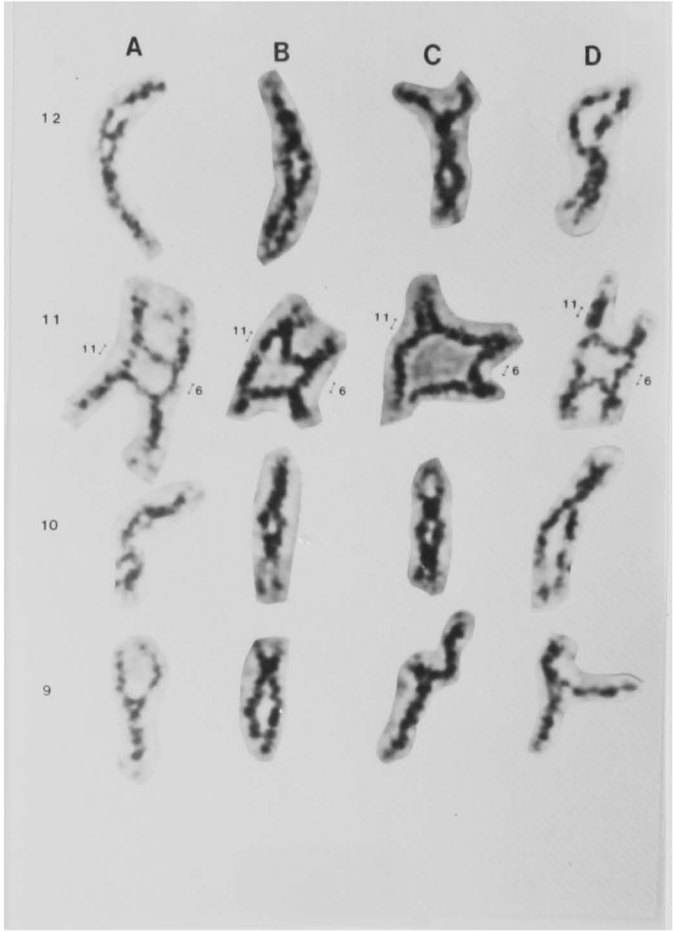


Figure 9. Metaphase I cell types from Bowl 3 T(12:8)/12:8 translocation: A) adjacent-1; B) adjacent-2; C) alternate cell type; D) alternate cell type.

Figure 10. Orcein-stained meiotic karyotype of the K851 translocation tentatively identified as T(11:6)/11:6: Chromosomes from four cells (A-D) are numbered from 12, the longest, to X (Number 1), the shortest.



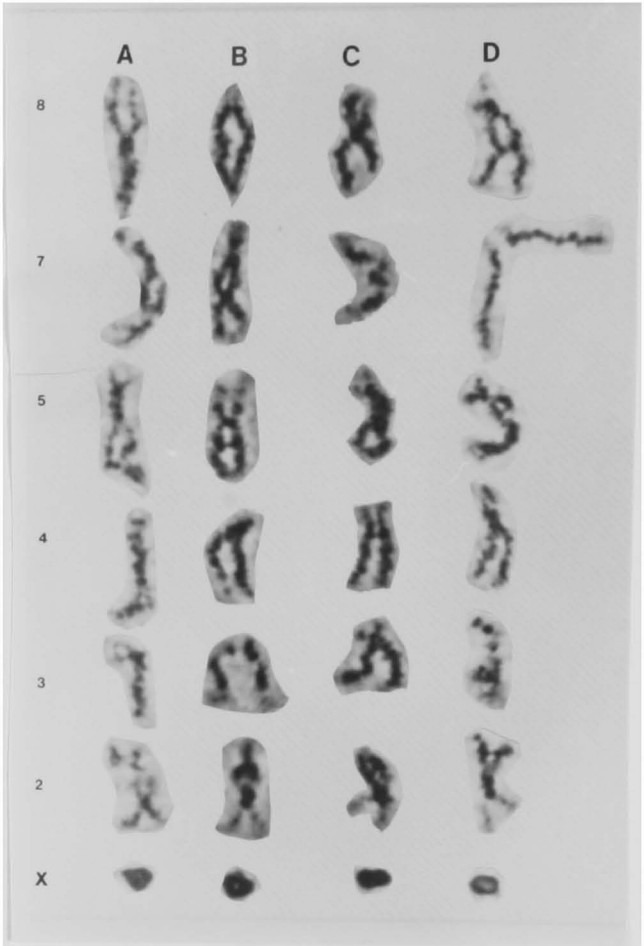
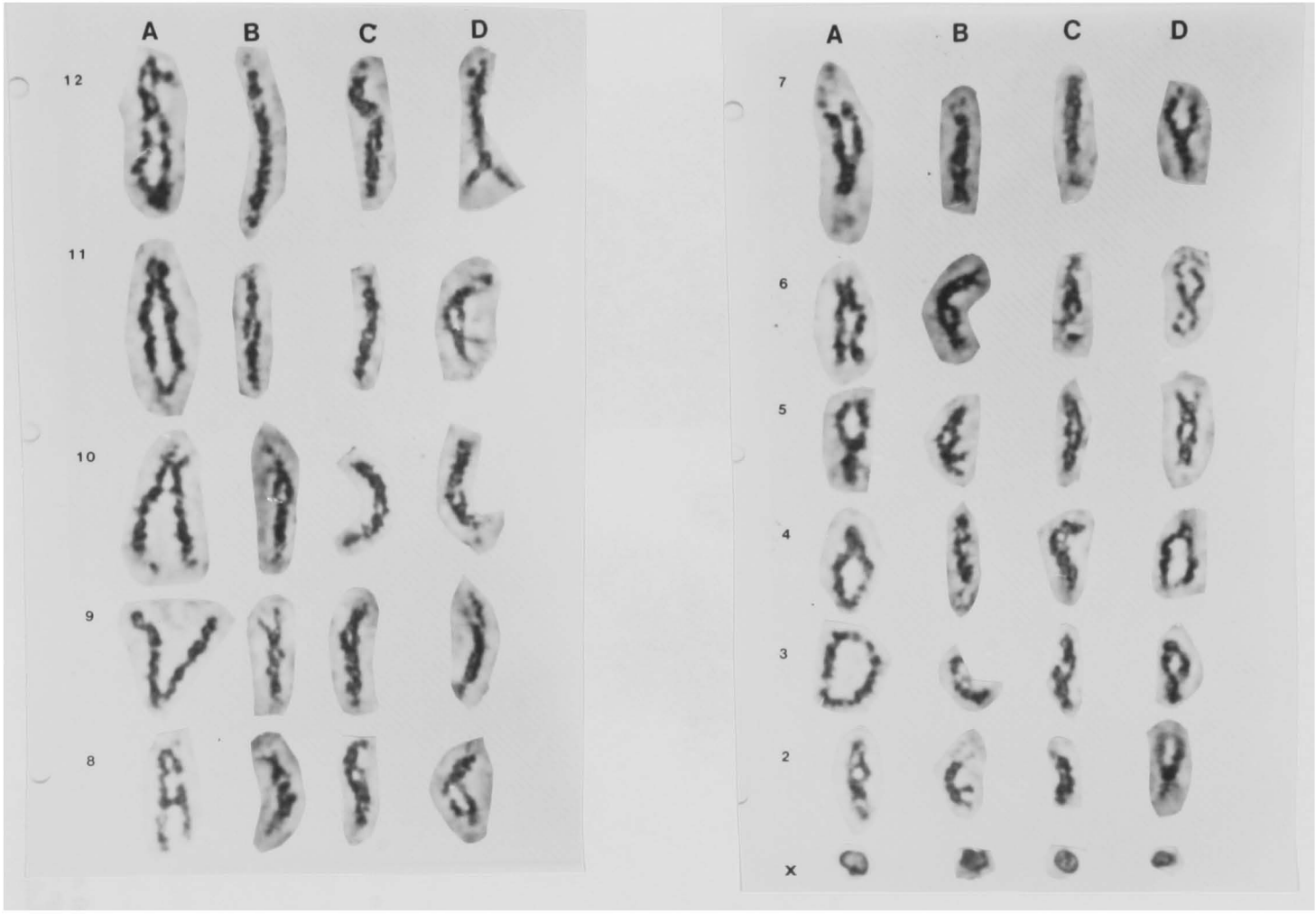


Figure 11. Orcein-stained meiotic karyotype of the field-collected strain, K851 Chromosomes from four cells (A-D) are numbered from 12, the longest, to X (Number 1), the shortest.



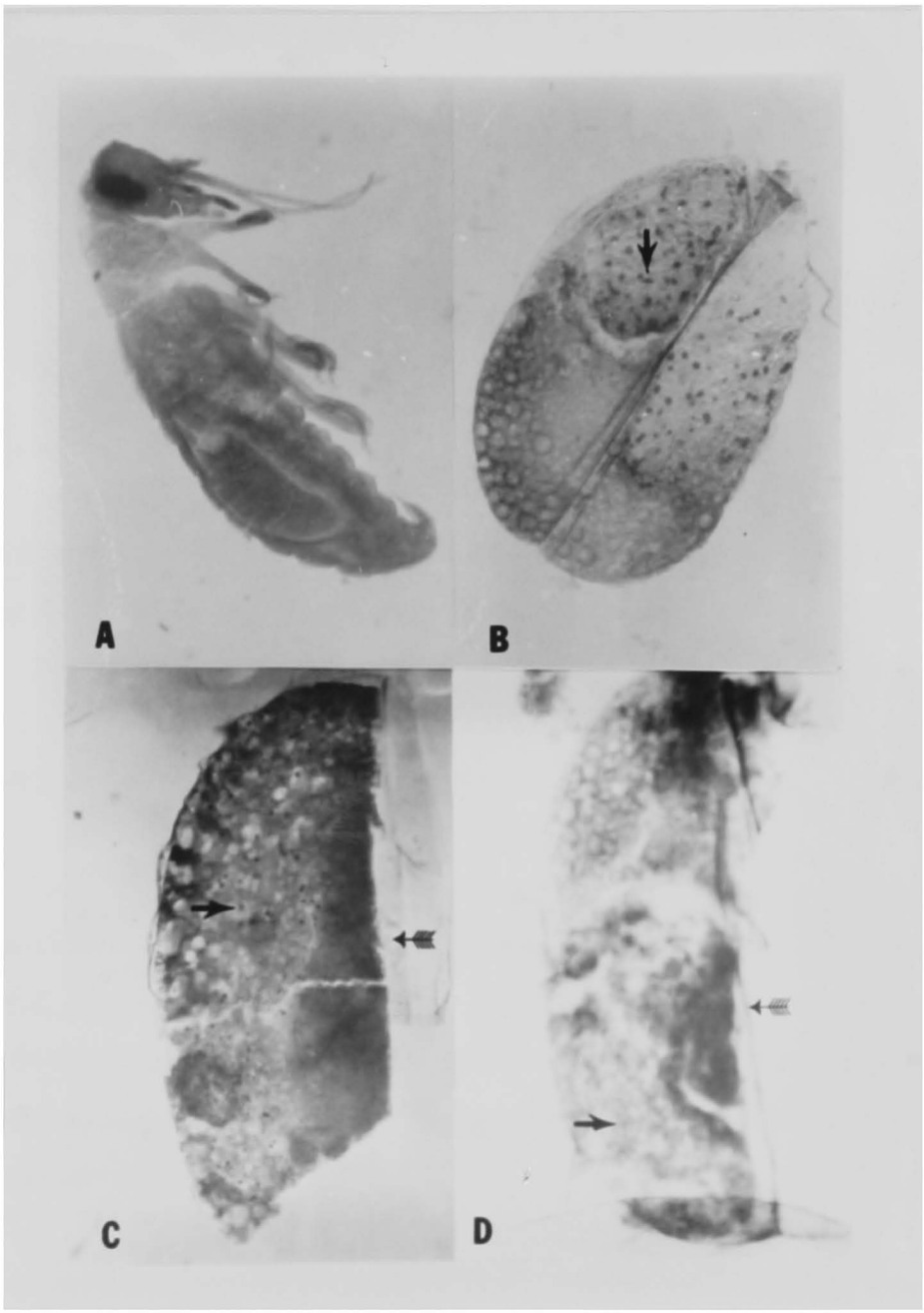


Figure 12. Normal embryo (A) for comparison to aberrant embryos: B) Egg arrested after a few cleavage divisions from T(12:8)/12:8, arrow indicates cleavage energids. C and D) eggs arrested with abnormally developed germ bands (feathered arrows). Arrows indicate cleavage energids in yolk.

Discussion

White has stated that " evolution is, ultimately a complex sequence of changes in the chromosomal DNA " and that " to understand the processes involved in the differentiation of populations, species and higher categories, we need to know what is happening in the karyotype itself " (1973). Adaption is also an aspect of evolution and there is no doubt that *B. germanica* has managed to adapt to an array of insecticides. This has resulted in a variation of resistance in natural populations.

Comparisons between susceptible and resistant field strains were a major part of this study. With the ease of viewing egg development through the shell of an egg case, it was possible to survey the oothecae of resistant and susceptible field strains for the occurence of partially developed eggs. Egg cases with five or more embryos arrested before hatch were assumed to be aberrant and to possibly have chromosome aberration(s).

There was no notable difference in the stages of embryo arrest between the susceptible and resistant strains as a whole. In all strains, both susceptible and resistant, most eggs were arrested in either stage I or stage III. Stage I arrest, however, is indistingushible from the appearence of unfertilized eggs, or white eggs. Observations on Feulgen-stained whole mounts of apparent stage I eggs from both resistant and susceptible strains showed mainly fertilized eggs, though unfertilized eggs were

also found. While most stage I eggs were considered fertilized, unfertilized eggs can not be ruled out as having been counted as stage I at a very low frequency.

Very few phenotypic mutations have been reported for field strains (orange-body, pallid-eye). The present data (Table 8) support earlier studies that suggest orange-body is the most frequently occurring polymorphism in natural populations of the German cockroach. This study also reports the first occurrence of three traits that are phenotypically identical to known mutants, bent bristle, yellow body, and pallid eye, and the discovery of one new mutant, "colorless eye", in field strains.

There was a significant difference between the number of aberrant oothecae produced by the resistant strains (higher) and the susceptible strains (lower). It is suggested that this difference is related to the development of resistance to insecticides but the underlying mechanism is not known. Perhaps with increased resistance, the frequency of mutation in a gene or genes, or chromosomal mutations that have deleterious effects on embryonic development, also increases.

Though there was a significant difference between susceptible and resistant strains in the number of aberrant oothecae, there was no consistant trend toward a difference related to susceptible vs. resistant strains in other characteristics. The latter include the number of nymphs hatched, the average number of eggs per ootheca, and the ratio of male to female nymphs. More importantly, there was no consistant trend toward a difference related to susceptible vs. resistant strains in the frequency of chromosomal abnormalities in males hatched from aberrant oothecae.

In Ross's study comparing two field strains (1986), both the susceptible and the resistant strain showed chromosomal aberrations with aberrations in nearly all males of the resistant strain. In the present study, the only difference seemed to lie in the type of aberration present. The susceptible strains had attachments (Fairbanks and Young), and attachments and fragments (Hennis). The resistant strains had translocations with attachments and fragments (K851 and Bowl3), spontaneous or non-recovered translocations with attachments and/or fragments (K851, Garland, Reddick), or just attachments and fragments (Montgomery Gardens). Perhaps, like the Carver

strain, these aberrations can be attributed to an increased tendency for fusion of heterochromatin (Ross 1986).

There were five occurrences of chromosome configurations typical of reciprocal translocations observed in this study. One was a translocation configuration that occurred in one cell only in the Garland (resistant) strain. Ross has also observed translocation configurations in two males of the Bowl strain (pers. comm.). In this study, two of the five occurrences were not recovered, but the males were apparently translocation heterozygotes, one in the Reddick and one in the K851 (both resistant) strains. The Reddick translocation was found in one male and the two translocation configurations found in K851 were floating free of a cell. None of the females from the same hatch as the K851 male that produced the spontaneous translocation produced an aberrant ootheca while the Reddick females did, yet there was no translocation found in the meiotic cell preparations. It is difficult to explain these translocations. Certainly, human error must be considered. However, spontaneous translocations occurring in single individuals have been reported in several grasshopper species (John and Hewitt 1963, Coleman 1947). Two males were reciprocal translocation heterozygotes, one from K851 (a different male from the one that produced the spontaneous translocation) and one from Bowl 3 (resistant strains). Both were recovered in outcrosses, as expected.

With the addition of the tentatively identified T(12:8)/12:8 and T(11:6)/11:6 translocation heterozygotes, there is now a total of four translocations isolated from natural populations of the German cockroach. The translocation T(12:8)/12:8 is the fifth appearence of the longest chromosome, number 12, in a translocation heterozygote. It can be noted that the breakpoint for chromosome 12 in T(12:8)/12:8 is almost identical to the one in the T(7:12)/7:12 translocation heterozygote (Ross and Cochran 1975b). Chromosome 11, though the second longest chromosome next to chromosome 12, only appears in one other translocation, T(11:12)/11:12 (Cochran and Ross 1977). It is likewise the second occurrence of chromosome 8 in a translocation heterozygote, the first being T(8:9)/8:9; and also the second occurrence of chromosome 6, T(4:6)/4:6 Cu (Ross and Cochran 1989). From the translocations found in this study and in others,

it can be inferred that translocations are the most common type of "floating" chromosomal polymorphism in the German cockroach (Ross 1988).

In the Bowl 3 translocation, there was a 50/50 frequency of alternate to adjacent disjunction at metaphase I, indicating random disjunction. Random disjunction can also be indicated by approximately 50% egg arrest in the oothecae (Ross and Cochran 1983). The Bowl 3 translocation carrying males hatched from an ootheca in which 50% of the eggs were arrested before completing development. Based on Cochran's (1976) study, the translocation is probably characterized by a ratio of 2:1:1:2, adjacent-1, alternate-1, adjacent-2, alternate-2, disjunction respectivly. Directed disjunction can be indicated by a low percentage of egg arrest in the oothecae (Ross and Cochran 1983). It can be speculated then that the K851 translocation, because it occurred in males that hatched from an ootheca with only 16% egg arrest, was characterized by a preponderance of alternate disjunction.

Translocations are rare in animal populations (White 1976). Inbreeding and selection experiments with translocation heterozygotes show an absence of gross abnormalities suggesting that the German cockroach is resistant to changes resulting from a reduction in population size only (Ross 1988). This would seem to eliminate the idea that translocations were found in resistant strains because the strains had been in the laboratory for a few years and been inbred. In addition, though the susceptible VPI strain has been in the laboratory for more than 40 years, no major chromosomal mutations have been found. In this study, the susceptible and resistant strains had been in the laboratory for appproximately three to five years.

The genic balance is maintained in nature by eliminating deleterious mutations and selecting useful ones. In the evolution of the *B. germanica* karyotype translocations have been the only major chromosomal mutation found in field strains at present. In a review, John (1976) suggests that for a species to have adopted a system that includes polymorphisms for interchanges, it must have been under considerable pressure to do so. In field strains of *B. germanica*, the strongest recent selection pressure has been from insecticides. Thus, it may not be from chance alone that all translocations

found so far are in insecticide resistant strains. Similarily, Yadav and Yadav (1989) suggest that chromosome aberrations in natural populations of some grasshoppers are not spontaneous but are caused by enviromental factors, including insecticides.

White has stated that in the order Blattodea there is no possibility of recognizing a "primitive karyotype". If, looking only at field strains and assuming the VPI susceptible lab strain as a "primitive karyotype", or base karyotype, in situations where stress (irradiation, insecticides) is placed on populations, it may be that translocation heterozygotes predominate as a mechanism of "karyotypic orthoselection" (White 1973). Aberrations in the K851 strain seem to support these suggestions. These aberrations were close to those in the resistant strain studied by Ross (1986). K851 was the most highly resistant strain of the study and, within this strain, three chromosomes tended to fall apart, two chromosomes had breaks, a translocation configuration occurred in one male, and one ootheca was aberrant because the mating involved a translocation heterozygote. K851 had the highest individual frequency (42.7%) of chromosome aberrations. Only data from this strain seems to support a hypothesis that chromosome aberrations can result from the selective pressure of insecticides.

There was no evidence found in this study to support a relationship between egg arrest and chromosome aberrations except, possibly, in the K851 strain. Chromosome abnormalities may have been equally as frequent in cells of males from normal-appearing oothecae. However, the difference between the frequency of aberrant oothecae in susceptible and resistant strains indicate that aberrant oothecae must be a heritable trait. Why, therefore, was there no reappearence in outcrosses of progeny from aberrant oothecae, except for the crosses of some of the female siblings, of the translocation-carrying males? It could be explained, perhaps, by suggesting that aberrancy was caused by a recessive trait or by a trait associated only with males. In either case, the trait would not be expressed in outcrosses to the VPI strain. Not only a larger sample, but also a closer look at more freshly collected strains and the individual strain resistances to specific insecticides should be considered when looking at aberrancy in field strains. If development of insecticide resistance and occurrence of chromosomal aberrations are related as suggested by Ross (1986), this

study's lack of strong support for this hypothesis, though it does not rule out the possibility, may have been due to elimination of deleterious traits during laboratory rearing.

Bibliography

Boussy, I. A. 1982. "Alternate-2" disjunction does not exist. Genetics 100 : 505-509.

Cochran, D. G. 1976. Disjunction types and their frequencies in two heterozygous reciprocal translocations of *Blattella germanica* (L.). Chromosoma (Berl.). 59 : 129-135.

Cochran, D. G. 1977. Patterns of disjunction frequencies in heterozygous reciprocal translocations from the German cockroach. Chromosoma (Berl.). 62 : 191-198.

Cochran, D. G. 1983. Alternate-2 disjunction in the German cockroach. Genetics. 104 : 215-217.

Cochran, D. G. 1989. Monitoring for insecticide resistance in field-collected strains of the German cockroach, *Blattella germanica* (Dictyoptera:Blattellidae). J. Econ. Entomol. 82 : 336-341.

Cochran, D. G. and M. H. Ross. 1967. Preliminary studies of the chromosomes of twelve cockroach species (Blattaria: Blattidae, Blattellidae, Blaberidae). Ann. Ent. Soc. Am. 60 : 1265-1272.

Cochran, D. G. and M. H. Ross. 1969. Chromosome identification in the German cockroach. Wild type and mutant stocks. J. Heredity. 60 : 87-92.

Cochran, D. G. and M. H. Ross. 1974. Cytology and genetics of T(9:11) in the German cockroach, and its relationship to other chromosome 9 traits. Can. J. Cytol. 16 : 639-649.

Cochran, D. G. and M. H. Ross. 1977a. Cytology and genetics of a stable ring-of-six translocation in the German cockroach. J. Heredity. 68 : 172-178.

Cochran, D. G. and M. H. Ross. 1977b. The cytogenetics of T(11;12), a reciprocal translocation in the German cockroach. J. Heredity. 68 : 379-382.

Cohen, S. and L. M. Roth. 1970. Chromosome numbers in the Blattaria. Ann. Ent. Soc. Am. 63 : 1520-1547.

Coleman, L. C. 1947. Chromosome abnormalities in an individual of *Chorthippus longicornis* (*Acrididae*). Genetics 32 :435-447.

Cornwall, P. B. 1968 The Cockroach. Vol. 1. Hutchison and Company. New York.

Diez, M. and M. H. Ross and D. G. Cochran. 1984. Genetically controlled differences in orientation in an interchange herterozygote of the German cockroach. J. Heredity. 75 : 107-112.

Endrizzi, J. E. 1974. Alternate-1 and alternate-2 disjunctions in heterozygous reciprocal translocations. Genetics. 77 : 55-60.

Hewitt, G. M. 1979. Animal Cytogenetics. Vol 3. Insecta 1. Orthoptera. Gebruder Borntraeger, Berlin and Stuttgart.

John, B. 1976. Myths and mechanisms of meiosis. Chromosoma (Berl.). 54 : 295-325.

John, B. and G. M. Hewitt. 1963. A spontaneous interchange in *Chorthippus* with extensive chiasma formation in an interstial segment. Chromosoma (Berl.). 14 : 638-643.

John, B. and K. R. Lewis. 1957. Studies on *Periplaneta americana* L. I. Experimental analysis of male meiosis. Heredity. 11 : 1-9.

John, B. and K. R. Lewis. 1958. Studies on *Periplaneta americana* L. III. Selection for heterozygosity. Heredity. 12 : 185-197.

John, B. and K. R. Lewis. 1959. Selection for interchange heterozygosity in an inbred culture of *Blaberus discordalis* (Serville). Genetics. 44 : 251-267.

Keil, C. B. and M. H. Ross. 1977. An analysis of embryonic trapping in the German cockroach. Ent. Exp. & Appl. 22 : 220-226.

Keil, C. B. and M. H. Ross. 1983. Chiasma frequency in male *Blattella germanica* (Dictyoptera; Blattellidae). Can. J. Genet. Cytol. 25 : 65-71.

Keil, C. B. and M. H. Ross. 1984. C-banded meiotic karyotype of *Blattella germanica* from prophase I cells. J. Heredity. 75 : 185-190.

King, R. C. 1975. Handbook of Genetics. Vol. 3. Plenum Press. New York.

Ross, M. H. 1964. Pronotal wings in *Blattella germanica* (L.) and their possible evolutionary significance. Am. Mid. Nat. 71 : 161-180.

Ross, M. H. 1980. A laboratory experiment involving releases of a double translocation heterozygote into a field derived population of the German cockroach. Ann. Entomol. Soc. Am. 73 : 18-23.

Ross, M. H. 1986. Cytogenetics of *Blattella germanica*: comparison of an insecticide-resistant and an insecticide- susceptible field strain. Can. J. Cytol. 28 : 835-843.

Ross, M. H. 1988. Cytological studies of *Blattella germanica* and *Blattella asahinai*. I. A possible genetic basis of interspecific divergence. Genome. 30 : 812-819.

Ross, M. H. and D. G. Cochran. 1960. A simple method for sexing nymphal German cockroaches. Ann. Ent. Soc. Am. 53(4) : 550-551.

Ross, M. H. and D. G. Cochran. 1966. Genetic variability in the German cockroach. I. Additional genetic data and the establishment of tentative linkage groups. J. Heredity. 57 : 221-226.

Ross, M. H. and D. G. Cochran. 1975a. The German cockroach, *Blattella germanica. In* Handbook of genetics, Vol 3. *Edited by* R. C. King. Plenum press, New York.

Ross, M. H. and D. G. Cochran. 1975b. Two new reciprocal translations in the German cockroach. Cytology and genetics of T(3;12) and T(7;12). J. Heredity. 66 : 79-89.

Ross, M. H. and D. G. Cochran. 1976. Sterility and lethality in crosses involving two translocation heterozygotes of the German cockroach, *Blattella germanica.* Experientia. 32 : 445-447.

Ross, M. H. and D. G. Cochran. 1977. Analysis of a double reciprocal chromosome translation in the German cockroach. J. Heredity. 68 : 231-237.

Ross, M. H. and D. G. Cochran. 1979. Properties of a 3-chromosome double translocation heterozygote in the German cockroach. J. Heredity. 70 : 259-266.

Ross, M. H. and D. G. Cochran. 1981. Synthesis and properties of a double translocation heterozygote involving a stable ring-of-six interchange in the German cockroach. J. Heredity. 72 : 39-44.

Ross, M. H. and D. G Cochran. 1983. Genetics and Cytogenetics of the German cockroach. *In* Cytogenetics and Genetics of Vectors. *Edited by* R. Pal, J.B. Kitzmiller, and T. Kanda. Kodansha, Tokyo.

Ross, M. H. and D. G. Cochran. 1989. Mini review: Genetics of the German cockroach. Comp. Biochem. Physiol. 94A(4) : 551-554.

Ross, M. H. and G. C. Nguyen-Tan. 1982. Aberrant egg development from matings of interchange heterozygotes of the German cockroach. J. Heredity. 72 : 154-158.

Roth, L. M. 1985. A taxonomic revision of the genus *Blattella* Caudell (Dictyoptera, Blattaria: Blattellidae). Entomolgia Scandinavica, Supplement 22.

Shaw, D. D. and D. J. Coates and M. L. Arnold. 1988. Complex patterns of chromosomal variation along a latitudinal cline in the grasshopper *Caledia captiva* Genome. 30 : 108-117.

Swanson, C. P. and T. Merz and W. J. Young. 1981. Cytogenetics. The chromosome in division, inheritance, and evolution. Prentice Hall, Inc. New York.

Tanaka, A. 1976. Stages in the embryonic development of the German cockroach, *Blattella germanica* Linne (Blattaria, Blattellidae). Kontyu, Tokyo. 44(4) : 512-525.

White, M. J. D. 1973. Animal cytology and evolution. Third edition. Cambridge University Press. Cambridge.

White, M. J. D. 1976. Blattodea. *In* Animal Cytogenetics. Vol. 3 Insecta 2. Blattodea, Mantodea, Isoptera, Grylloblattodea, Phasmatodea, Dermoptera and Embioptera. *Edited by* B. John, H. Bauer, S, Brown, H. Kayano, A. Levan, and M. White. Gebruder Borntraeger, Berlin and Stuttgart.

Yadav, J. S. and A. S. Yadav. 1981. On chromosomal aberrations in the natural populations of some grasshoppers. Genet. Iber. 41 : 37-46.

Vita

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