BASIC STUDIES OF CHIASMA FREQUENCY IN MALE *Blattella germanica* (L.)

by

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INTRODUCTION

Variation in sexually reproducing organisms is produced by three mechanisms: mutation; the independent assortment of chromosomes; and crossing-over within bivalent pairs during meiosis. Of these three mechanisms, the significance of the third is least well established by biologists. The adaptive significance of cross-over rates and variation in those rates both within and between individuals, populations, and species is very poorly understood.

The problem is confounded by the dual nature of chiasmata, the physical manifestations of genetic crossing-over. Chiasmata are indicative of recombination and undoubtedly have selective value in that sense. They also function in mediating desynapsis after diplotene in the absence of the synaptonemal complex. Two classical concepts of the nature and behavior of chiasmata; terminalization (a process by which chiasmata move to the ends of bivalents) and interference (regulation of chiasma spacing by distance and sequence of formation) have had the effect of obscuring the selective aspects of the problem by placing chiasmata in a solely physical context. Recently these concepts have come into disrepute (Jones, 1974b; 1978). This has opened the way for studies of the selective advantage of an organism's positioning and frequency of chiasmata.

Studies of chiasmata in Blattella germanica have relevance on two counts. Genetic and cytogenetic studies of this cockroach have progressed to a point where analysis of chiasma frequency can begin to

answer questions concerning linkage, chromosome structure, and disjunction patterns of translocation heterozygotes that are otherwise unresolvable (Ross & Cochran, 1975a). This information is also valuable in a comparative sense. The animals generally examined in chiasma and recombination studies are relatively advanced phylogenetically; e.g. Drosophila, various grasshoppers, mice, etc. Data from a relatively primitive insect like B. germanica could provide a meaningful contrast.

The objectives of this study are three fold. The first is to determine the feasibility of further studies of chiasma frequency in this insect. Is the variability of a degree that reasonable sample sizes can be used to gather meaningful data? Secondly, to establish the baseline chiasma frequency and variability for the wild-type laboratory stock, the VPI strain. Thirdly, to examine a translocation stock to gain insight into its possible intrachromosomal and interchromosomal effects on chiasma frequency. Bivalent specific studies are precluded at this time by an inability to unambiguously identify specific bivalents in late meiotic prophase.

LITERATURE REVIEW

Theories and models of crossing-over:

Sutton in 1902 and 1903 (Dunn, 1969) asserted that Mendel's newly rediscovered law of independent assortment could only apply to genes on different chromosomes. The number of freely assorting genes would therefore be limited to the number of chromosomes in an individual. The discovery of more than the original seven independently assorting genes described by Mendel made Sutton's hypothesis untenable. DeVries made the first attempt to reconcile the existence of multiple independently assorting genes with the chromosome theory of inheritance (Kushev, 1974). He hypothesized that homologous chromosomes exchange segments. In 1892, Rückert observed that homologous chromosomes were attached at specific locations during late prophase in meiosis. He termed these points chiasmata after their "X" shaped configuration (Kushev, 1974).

Janssens, working with a salamander, Batrachoseps attenuatus, developed the chiasmatype theory of recombination by integrating the observations of Rückert and the hypothesis of DeVries. This hypothesis held that homologous chromosomes twist around each other as they conjugate and shorten throughout meiotic prophase. As they split apart the twists cause breaks in the chromatids. Occasionally the breaks would not rejoin in the original fashion but would join with the opposing homologue to form a chiasma which would become visible as the repulsion of the chromosomes continued. Janssens apparently

understood that complete linkage of all genes on a chromosome was unlikely to be adaptive over time (Kushev, 1974). The chiasmatype theory provided a means to effect recombination of these genes.

The classical theory of chiasma formation (Wenrich, 1917) was the principal opposition to the chiasmatype theory. This hypothesis envisioned no exchange of genetic material and chiasmata as only manifestations of the chromatids twisting around one another.

Morgan's early work with Drosophila began the conversion of Janssens' hypothesis to a theory (Dunn, 1969). Morgan made two important contributions to the study of recombination. He postulated that the degree of linkage observed in genetic studies was a function of the physical distance between loci on a chromosome (Morgan, 1911) and introduced the term, "crossing-over", to describe the exchange of identical segments between homologues leading to recombination of linked genes (Morgan and Cattell, 1912).

Belling (1928) postulated that exchanges between homologues occurred at pachytene as a consequence of breaks during leptotene. He believed these breaks occurred at random between a certain minimum and maximum distance from each other. The formation of chiasmata thus became a random event dependent on the probability of two breaks occurring in direct opposition minus the probability that these breaks would rejoin with the adjacent sister-chromatid or with the original broken end.

The direct correlation of chiasmata and genetic cross-overs was not achieved until Mather (1933) demonstrated the phenomenon with triploid Drosophila. This correlation has been demonstrated

subsequently with techniques of increasing sophistication (Brown and Zohary, 1955; Taylor, 1965; Jones, 1974; and Tease, 1978).

Darlington (1934) determined the total number of chiasmata from a series of Zea mays cells and converted this count to genetic map units by calculating that one chiasma equals fifty map units. The total map length was then distributed among the ten chromosomes of the Zea karyotype on the basis of length at pachytene. In this manner he obtained an approximate genetic length for each chromosome. No linkage group had a map length greater than that calculated from chiasma frequencies. The observed map lengths were a minimum as none of the chromosomes were completely marked with mutants. At the time this was considered powerful evidence for the chiasmatype theory.

Two major models as elaborations of Janssens' partial chiasmatype theory have attempted to place chiasma formation in the context of modern biochemistry. Both models have three factors in common: (1) chiasma formation is preceded by dissociation of one base chain from DNA in each of two homologous chromosomes, (2) formation of hybrid DNA made of one base chain from one chromatid and one from a homologous chromatid, and (3) intragenic recombination or gene conversion resulting from breaks within the base sequence of a single gene. The process requires opposition of identical regions and the nicking of the DNA of homologous chromosomes at exactly the same point.

In the Whitehouse model, the two base chains dissociate at identical points but with opposite polarity (Whitehouse, 1963; Whitehouse and Hastings, 1965). Newly synthesized chains fill the gap and ultimately reassociate with the chain originally displaced. The ends

rejoin and the unpaired original chain degrades. Holliday (1964) hypothesized that the dissociating chains have the same polarity and thus are able to join immediately with the base chain that remains attached to the homologous chromatid. This half-chromatid chiasma is resolved when two of the four base chains break and rejoin at the chiasma.

Biochemical investigations have confirmed the presence of mechanisms capable of producing these hypothesized events. Endonuclease nicking enzymes have been discovered in Lilium (Howell and Stern, 1971) that produce single chain breaks positioned to create 5'-hydroxyl and 3'-phosphoryl termini. The peak of highest activity of this enzyme was found to be in pachytene. The annealing of the broken ends is preceded by a phosphorylation of the 5' termini by a polynucleotide kinase with a narrow activity peak at mid-pachytene. The annealing phase of the process is accomplished by a polynucleotide ligase that joins 3' ends to 5' ends. This enzyme appears active from interphase throughout pachytene (Howell and Stern, 1971; Stern and Hotta, 1977). While the simple presence of an enzyme system is not enough to define a biochemical process, the association of these enzymes is powerful evidence for the timing of crossing-over in pachytene and appears to lend support to the Holliday model.

Genetic and environmental control of crossing-over:

Once the correlation between chiasmata and genetic crossing-over was established, the control of recombination could be investigated cytologically and genetically. The early work toward this goal has

been reviewed by Bodmer and Parsons (1962).

Chinnici (1971a), working with Drosophila melanogaster, reported a bidirectional selection scheme that resulted in the production of a high recombinant stock and a low recombinant stock. The sex linked genes, sc and cv were used as the primary markers. No evidence was found to indicate that the changes in recombination between sc and cv were offset by compensatory changes in adjacent regions. From the gradual nature of the response to selection, Chinnici (1971b) concluded that the control of crossing-over in Drosophila is polygenic.

Shaw (1972,1974) was able to demonstrate a similar situation in Schistocerca gregaria by a bidirectional selection applied to cytological determinants of crossing-over. However, Shaw concluded that the genes governing chiasma frequency were almost selectively neutral. Shaw drew this conclusion from analysis of variance data which indicated a high degree of variance for the trait.

Factors that have been shown to cause deviations from baseline chiasma frequencies belong to two general categories. Artificial selection indicates the genetic control of chiasma formation. Other factors are environmental in nature. Shaw (1971) found in Schistocerca that 40% of the individuals examined showed significant differences in chiasma frequency for primary spermatocytes taken from different follicles of the same testis. Shaw postulated differences in local oxygen concentration and variations in the composition of the haemolymph bathing the gonads. He also found differences in chiasma frequency of primary spermatocytes removed from an individual over time, although no trend either up or down could be discerned.

Barker (1960) showed clinal variation in chiasma frequency for two species of British grasshoppers with regard to altitude and latitude. Myrmeleotettix maculatus populations from northern high altitudes of Great Britain showed a lower chiasma frequency (14.36 per cell) than low altitude southern populations (15.18 per cell). Three medium and low altitude populations exhibited appropriately intermediate chiasma frequencies. The other grasshopper examined by Barker, Chorthippus parallelus, exists at relatively uniform altitudes but still showed a cline from the northern population (14.54 per cell) to the southern population (15.51 per cell). There were no differences in chiasma frequency associated with the time of year or between the years the populations were sampled for both species.

Recently, King and Hayman (1978) found seasonal variation in the chiasma frequency of an Australian gekko, Phyllodactylus marmoratus. The frequency of total and interstitial chiasmata varied cyclicly throughout the year. Total chiasma frequency peaked in July and August while interstitial chiasmata were at a maximum in October. King and Hayman believe that the cycle is driven mainly by seasonal variation in ambient temperatures.

White (1934), Henderson (1962), and Shaw (1971) documented reductions of chiasma frequencies at elevated temperatures. All three studies used grasshoppers as the experimental animals. Henderson and Shaw demonstrated high proportions of univalents after exposure to 40°C. They attributed this to a failure of chiasmata to form or hold the bivalents in close approximation at diplotene. Shaw's analysis showed a particular lack of uniformity in response and recovery from

temperature fluctuations and heat shocks.

Inbreeding has a major influence on chiasma frequency. Both inbred plant and animal lines have been shown to develop reduced chiasma frequency with respect to outbred lines of the same species (Shaw, 1971; Rees and Thompson, 1958). Homozygosity from inbreeding probably generates greater variability between individuals and reduces mean chiasma frequency (Shaw, 1971). In highly inbred populations genetic advantages accruing from recombination will become fewer as homozygosity increases. Chiasma frequencies would be expected to drop to a minimum necessary for orderly desynapsis. Chiasmata could also be expected to become localized about the telomeric regions where their effects would be minimized (Zarchi et al, 1972; Hillel et al, 1973).

Variation in chiasma frequency can be produced through mutations. This adds to the evidence provided by selection experiments that chiasma formation is under direct genetic control. The best evidence in this instance comes from plant studies. Maguire (1978), Tease and Jones (1976), and Jones (1974), utilizing: Zea mays, Crepis capillaris, and various rye genotypes, respectively, have demonstrated a general phenomenon of chiasma frequency reduction by various mutations. The observed effects range from total suppression of chiasmata in single chromosomes with associated compensation in other bivalents to complete suppression of chiasma formation with the formation of univalents.

A similar class of meiotic mutants has been described in detail for Drosophila by Lindsley and Sandler (1977). Mutants influencing

meiosis in the broad sense and chiasma formation and maintenance in particular were described. An asynaptic mutant's effect on chiasma distribution was reported by Rees (1957) in the locust, Schistocerca gregaria.

Cytogenetically, chiasma frequency may be affected by numerical and structural mutations. The literature on numerical mutations in general and supernumerary segments and chromosomes specifically is voluminous. The studies of Hewitt and John (1964,1967) and John and Hewitt (1966) serve as specific examples. White (1973) has written a very complete chapter on this topic. The supernumerary or B-chromosomes of the British grasshopper, Myrmeleotettix maculatus, were examined by Barker (1960) and by Hewitt and John (1964,1967) and found to raise cellular chiasma frequencies significantly. The effect produced was far greater than the simple increase of a single chiasma in the predominantly heterochromatic supernumerary chromosome. John and Hewitt (1966) reported a polymorphism for a supernumerary segment in the grasshopper, Chorthippus parallelus, that produced an interchromosomal effect on chiasma frequency. Both in a heterozygous and homozygous state the extra heterochromatin, in this case located on the small S8 chromosome(s), raised the cellular number of chiasmata significantly while maintaining the same variance as individuals with normal karyotypes. The chiasma frequency of the S8 bivalent itself never rose above one.

Lucchesi and Suzuki (1968) reviewed the effect of inversions on chiasma formation. Cross-overs are suppressed in bivalents heterozygous for inversions. However, in many organisms this is

compensated for by an interchromosomal increase in chiasmata, the Schultz-Redfield effect. The mechanisms producing this phenomenon are not well understood. The effect is highly variable: no expression with some inversions, partial expression in others (Suzuki, 1962), expression only in inversion homozygotes (Valentin, 1978), or uniform expression as increased chiasma frequency in most cases. Usually the interchromosomal effect is strongest when the inversions occupy proximal or distal regions of the involved chromosome. Suzuki (1963) concluded that the rearrangement of heterochromatic blocks is the crucial factor influencing expression.

Translocations also have various types of interchromosomal effects on chiasma frequency. Translocations have been reported to raise or lower, or to have no effect on chiasma frequency. Ramel (1962) reported no significant effect on chiasma frequency in either direction for simple translocation heterozygotes. Hewitt and John (1964) reported a similar effect in Chorthippus in which a translocation involving two of the longest chromosomes failed to alter the number of chiasmata in a cell. This translocation formed a ring-of-four consistently. They also reported another translocation involving a long and a medium sized chromosome that appeared to cause an overall decrease in the number of chiasmata per cell. This association formed both rings and chains of four at diplotene. Translocations in Drosophila generally have depressed levels of recombination in the involved arms, thus lowering the overall cellular frequency (Lucchesi and Suzuki, 1968).

The most frequent effect of translocations is a tendency to raise chiasma frequency both within the association and in the non-involved bivalents. White (1963) reported a complex translocation in Moraba scurra involving four non-homologous chromosomes, i.e., an association of eight in meiosis, that significantly increased crossing-over. Short arms that normally form a single chiasma formed multiples with high regularity to produce the elevated frequencies. The translocation was very regular in its meiotic behavior and tended to form large chains and ring multiples. Lewis and John (1963) found that chiasmata formed in the very short acrocentric arms of Chorthippus only when they were involved in a translocation. Hewitt (1967) found an interchange involving a long and a very short heteropycnotic chromosome that increased chiasmata in all of the bivalents except the two smallest. The latter consistently formed only one chiasma. Normal chiasma frequencies in Chorthippus range from 14-16.62 chiasmata per cell in contrast to a mean of 19.80 chiasmata for those cells bearing the translocation.

Hinton's (1965) investigations of 26 Drosophila melanogaster translocations with genetic markers uncovered highly variable effects on recombination. Of six intensively investigated lines, two increased recombination while four remained ambiguous. The twenty translocations casually examined showed ambiguity with decreases and increases in chiasma frequency. Double and triple cross-overs were abundant in the high recombination lines. Non-cross-over areas were less frequent than in normal flies. Increases in single cross-over events with concomitant decreases of double, triple, and non-cross-over

events characterized the low recombination lines. Hinton believed a disruption of the position of chiasma frequency modifying or controlling genes through the rearrangement of chromosome segments may be responsible for these effects. Alternatively, Hewitt (1967) implicated heterochromatin rearrangements caused by the translocations as the causal agent. Suzuki (1963) drew similar conclusions with regard to inversions and their interchromosomal effects. The effects of supernumerary chromosomes and segments that are largely heterochromatic seem to support the latter hypothesis although Hinton's and Hewitt's views are not mutually exclusive. The mechanism producing the interchromosomal effect is unclear in the heterochromatin rearrangement hypothesis as well.

The control of chiasma frequency appears to have a component positively correlated with the physical length of individual chromosomes and chromosome segments. This has been termed interference. Haldane's (1931) analysis of Vicia fabae confirmed both the existence and genetic importance of chiasma interference. The existence of interference was inferred statistically from the non-random distribution of chiasmata along bivalents. If interference does not exist, chiasmata would follow a Poisson distribution where the variance is equal to the mean. Shaw (1971) found the distribution of chiasmata in some populations of Stethophyma grossum to be random, indicating a lack of interference.

As regions of high crossing-over intensity are often interspersed with regions of low cross-over intensity (Mather, 1936), it appears the intensity of interference lessens with distance.

Belling (1928) suggested that in Lilium chiasmata are formed preferentially in certain regularly spaced regions of the chromosome. This phenomenon would produce the same statistical effect as interference, yet be dependent upon an entirely different mechanism. Exaggeration of chromosome distances through highly localized areas more subject to mutation than others will also produce results spuriously similar to those expected from interference. Mather (1938) postulated two parameters: "d" the distance from a fixed point on the chromosome, either a telomere or the centromere and the first formed chiasma, and "i" the interference distance within which the formation of subsequent chiasmata would be precluded. Mather believed these to be constants of wide significance.

Henderson (1963) and Fox (1973) using cytological material from Schistocerca gregaria have found both "d" and "i" to vary in two important ways. First, both these distances vary from bivalent to bivalent. Secondly, they vary within bivalents with respect to the number of chiasmata present. The observation that chiasma frequency increases with increasing chromosome length is a prediction of the interference hypothesis, yet in Schistocerca the length of a bivalent increases with increasing numbers of chiasmata.

Sequential formation of chiasmata is basic to the action of interference. In Schistocerca the first chiasma appears to form near the telomere as distal chiasmata are the most prevalent (Henderson, 1963; Fox, 1973). Shaw and Knowles (1976) assert that in Caledia, a genus of Australian and Pacific grasshoppers, the sequence of formation is reversed, i.e. from centromere to telomere. They invoke

similar evidence. The mid-sized bivalents that form both single and double chiasmata tend to have a concentration of chiasmata near the centromere when there are two chiasmata. Single chiasmata tend to be more distally located but still within the proximal third of the bivalent.

Jones (1974) has questioned the propriety of this type of data in determining sequence of formation. It may be, he reasons, that telomeric or centromeric regions have a higher probability of forming chiasmata unrelated to the sequence of formation. The possibility that chiasmata are not sequentially formed and variations in the magnitude of interference indicate that a physical regulatory mechanism cannot solely explain chiasma distribution. Recombination is more probably regulated by a combination of interference and overriding genetic control. This combination can be modified by natural selection to produce chiasmata at an optimal location and frequency in terms of individual fitness.

Cytogenetics of Blattella germanica:

B. germanica males have 22 autosomes and a single X chromosome that exists as a univalent in meiosis. Females are determined by two sex chromosomes which synapse to form a small bivalent (Cohen and Roth, 1970; White, 1976). The meiotic karyotype has been determined by the use of length measurements at late pachytene (Cochran and Ross, 1969). The bivalents of the VPI and other wild type strains were measured with an ocular micrometer. Standardization was achieved by measuring bivalents only in those cells where the longest measured 12-14

microns. Twelve distinct classes resulted from these measurements corresponding with the number of bivalents and the sex chromosome. There is a steady progression from the smallest to the largest autosomes. Certain bivalents can be distinguished visually in addition to the heterochromatic X. Chromosome 12 is the longest in the karyotype and can generally be distinguished on that basis. Bivalents two and seven have one indistinct telomere: the other telomere in bivalent seven is very distinct. Bivalent three has an interstitial area that stains only faintly (Ross and Cochran, 1975a). Centromere position is difficult to determine accurately in meiosis. Mitotic figures published by Cohen and Roth (1970) clearly show all chromosomes to be metacentric to submetacentric.

Meiosis has been well described in B. germanica (Cochran and Ross, 1969; Ross and Cochran, 1975a) and is typical. This contrasts with the observations of Lewis and John (1957a) and John and Lewis (1959) on Periplaneta americana and Blaberus discoidalis. These two cockroaches lack a typical diplotene-diakinesis and have a premetaphase stretch. Functionally this telescopes the process of spermatogenesis by moving pachytene cells directly into prematephase. This particular feature of meiotic prophase has led many observers to believe that meiosis was achiasmate in these species (White, 1976). John and Lewis (1957b) conclusively refuted this claim.

Typical recombination has been repeatedly shown in B. germanica through genetic studies (Ross and Cochran, 1975a). There is an apparent tendency toward close linkage of the known mutants. Ross and Cochran (1975) also reported a rough estimate of chiasma frequency,

1.3 per bivalent. Chiasma position has not been investigated adequately. White (1976) suggests that published figures show a tendency toward distal localization.

Translocations in B. germanica are characterized by breakpoints in the vicinity of the centromere and high disjunction frequency. Most translocations associate as rings-of-four through metaphase I indicating at least one chiasma in each arm. Some translocations do break up into chains or bivalents with moderate frequency. The determining factor is apparently the length of the pairing arm: i.e. interchanges with short arms have a higher propensity to form chains and bivalents (Ross and Cochran, 1975a). This may reflect a lack of chiasma formation in such arms. The translocation used in this study, T(8;9), was described by Cochran and Ross (1974). It is a typical translocation in B. germanica, probably involving the interchange of nearly whole chromosome arms. It does exhibit an apparent sex difference in the frequency of alternate vs adjacent disjunction. Alternate disjunction predominate in the males.

MATERIALS AND METHODS

Cockroach stocks and rearing procedures:

The studies of chiasma frequency changes with successive oothecae utilized the VPI strain, a wild type strain of Blattella germanica which has been in culture at this institution since 1947.

A stock heterozygous for a translocation involving chromosomes eight and nine, both mid-length chromosomes (Cochran and Ross, 1974) was also examined. Males used in this investigation were drawn from two different backcross systems. In one, T(8;9), heterozygotes were identified by the mutant ruby-eye, ru, an allele on chromosome nine closely linked with the translocation breakpoint. The second backcross system utilized black-body, Bl, an allele on chromosome eight, to mark the cockroaches bearing the translocation. The cockroaches in the latter system were homozygous for rose-eye. In both backcross systems those cockroaches bearing a translocation appeared normal for the mutant marker used and could easily be selected.

Fifty male and fifty female large nymphs of the VPI strain were removed from the general laboratory cultures and allowed to mature. The sexes were kept separate until the majority had become adults. They were then allowed to interbreed in quart-sized Mason jars, 16-17 pairs per jar. The jars were provided with a folded wire screen, a water vial, dog food, and a cheese cloth top. The cockroaches were placed in a clean jar with fresh dog food and a new water vial weekly. The vial was refilled once during the week if necessary.

The maturation of the females' oothecae was carefully monitored. At weekly intervals VPI strain females whose oothecae had hatched were transferred to another jar to bear their second ootheca. When nearly all the females had hatched their first oothecae the males in the jars were discharged, leaving only the nymphs in the jars. After hatch of the second oothecae the females were again separated from the nymphs and allowed to bear a third ootheca. Up to 25% of the females died or became infertile prior to the hatch of the third ootheca. Due to high female mortality and reduced production of nymphs, the females were discarded after their third ootheca.

Upon reaching the third to fourth instar the nymphs were sexed and placed in separate jars. A random sample of the males was used for slide preparation. The remaining VPI strain cockroaches were maintained in culture and formed the basis for subsequent nymph production for cytogenetic analysis. To guard against any possible effects due to inbreeding, adult male cockroaches from the VPI laboratory cultures were included when the sexes were reunited. Approximately equal numbers of sibling males and males from the breeder colonies were placed with the females.

T(8;9) heterozygotes were backcrossed reciprocally to the appropriate mutant marker using about 20 pairs per jar, supplied and maintained as above. The females were allowed to bear one ootheca and were discarded with the adult males. The nymphs were sexed and selected for phenotype at the third instar and placed in one of four jars; males with and without the mutant marker and similarly for females. A random sample of male nymphs heterozygous for the

translocation was selected for cytogenetic analysis. The remaining cockroaches were placed in a reciprocal backcross with additional males from the appropriate laboratory cultures to maintain nymph production.

All rearing procedures for both the VPI strain and T(8;9) were carried out in a constant temperature and humidity cabinet. The cabinet was maintained at 26°C with 65-85% relative humidity. A 12:12 dark - light cycle was used.

Cytogenetic methods:

Previous studies (Cochran and Ross, 1969) have shown that the testes of third to fourth instar cockroaches frequently yield large numbers of cells in late meiotic prophase I. Consequently only numphs corresponding to that age/size class were examined. Males were removed from the rearing jars in batches of one dozen or more and killed using ethyl ether. The abdominal tergites were cut down the midline with fine pointed scissors. The wound was opened using an insect pin and the testes removed using forceps with one curved tip.

The testes of the German cockroach are composed of four lobes each. They are located in the distal quarter of the abdomen on each side of the midline. The left testis rests in a hollow surrounded by fat body tissue while the right rests over a lateral loop of the ventriculus, also surrounded by fat body tissue.

The testes were removed and placed on a clean glass slide in a drop of 15% acetic acid. Each slide contained both testes of one insect, the right designated as "a" and the left "b". As much adhering body fat and other debris as possible was removed. The acetic acid

drop was removed with a paper towel. A drop of acetic orcein stain was placed on each testis and allowed to remain for five minutes or longer. The stain was wiped off and the testes resuspended in 15% acetic acid. A cover slip was applied and the preparation squashed under layers of paper toweling using thumb pressure. The coverslips were sealed with clear nail polish.

The slides were either examined immediately or stored under refrigeration. The preparations remained in good condition for up to one week. Observations were carried out with phase contrast optics using a green substage filter to enhance contrast and minimize halo. The slides were first scanned at low power (240X) for areas of large concentrations of primary spermatocytes in prophase I. The oil immersion objective (2400-1500X) was used to examine individual cells.

The number of diplotene-diakinesis cells counted for each individual was based on the results of the pilot study (see below). No implicit upper limit was imposed although examination of cells was terminated as soon as I became aware that sufficient numbers had been obtained. To produce a balanced design, the numbers of individuals examined in each group (successive oothecae and translocation) were kept constant. Each cell's chiasmata were recorded as the number of ring bivalents, indicative of one chiasma in each arm of the chromosome. As each autosomal bivalent has a minimum of one chiasma, the average frequency per bivalent was calculated from the tally of the ring bivalents with the use of a set of constants. Bivalents with three chiasmata, which form a "figure-of-eight" arrangement, were tallied as two rings with additional notation of their occurrence.

It is possible to express these data as average total chiasmata per cell, although in this study chiasma frequency per bivalent seemed to offer conceptual clarity. No clear precedent was apparent from the literature.

Statistical methods:

As the experimental cockroaches were maturing and acclimatizing to the regulated conditions of the rearing chamber, I conducted a pilot study using third and fourth instar nymphs from the VPI strain laboratory colonies. The purpose of this study was to determine an optimal sampling scheme with regard to the number of cells per cockroach and the number of cockroaches per group. A total of twenty cockroaches with a range of 9 to 272 cells per cockroach were examined. The resulting mean chiasma frequency and the sample variance were assumed to be equivalent to the true or population mean and variance for the purpose of analysis. The variance component was considered to be an over-estimate as the cockroaches had been reared in a variable environment and were products of oothecae from females of unknown age and history.

These preliminary data were analyzed using an iterative technique for optimal sample size determination (Sokal and Rohlf, 1969, p. 247) where one standard deviation was used as the desired level of sensitivity. This method is relatively insensitive to changes in desired significance level (α) and probabilities that differences found will be significant (P). It is sensitive, however, to changes in the ratio, σ/δ, where σ is the population standard deviation and

δ is the smallest difference one wishes to detect. In my analysis this ratio was set equal to one. The formula used is as follows:

$$n \geq 2(\sigma/\delta)^2 \{t_{\alpha[v]} + t_{2(1-P)[v]}\}^2$$

Where n is the optimal sample size, v is the number of degrees of freedom of the sample standard deviation (a(n-1); a = 3 groups, n = 20 cockroaches) and t is the Student's t statistic for the given α and v.

After examining the cockroaches reared under controlled conditions, the resulting mean chiasma frequency per bivalent and associated standard deviation per cockroach were analyzed for fidelity to the assumptions implicit in analysis of variance (ANOVA). Briefly these are as follows: random sampling of individuals, independence of the error terms of the variables, normal distribution of the variables, and homogeneity of the variances.

Random sampling of the individual cockroaches was described above. The independence of the error terms was tested using the runs test (Sokal and Rohlf, 1969, p. 626). Normality was tested in two ways. First the g_1 and g_2, kurtosis and skewness, statistics were calculated using library programs (Barr et al, 1976). Secondly the data were broken into classes of .04 chiasma per bivalent for the VPI strain cockroaches and .02 chiasma per bivalent for the T(8;9) heterozygotes. The resulting frequency distributions were compared to normal distributions generated using sample means and standard deviations. Two quantitative methods were used in this comparison in addition to visual examination of the plots. The F_{max} and Kolomogorov-Smirnov statistics tested the deviations of the observed frequency

data from the expected distributions. The homogeneity of variances was tested using the F test as performed by SAS library programs (Barr et al, 1976) and Bartlett's test for heteroscedasticity (Sokal and Rohlf, 1969, p. 110).

The assumptions having been satisfied, an analysis of variance was run on the three sets of data from the successive ootheca study. The analysis was carried out using only the mean chiasma frequency per cockroach and not the data from individual cells because of small variances at the cell level. Additional manipulation was necessary before the analysis of variance technique could be applied to the comparison between the translocation data and the data from the first oothecae of the VPI strain. The translocation data were modified so as to exclude those chiasmata directly associated with the ring-of-four of the translocation. The raw number of chiasmata per cell was reduced by two to account for the ring of four or by a single chiasma in the case of a chain configuration. Comparisons between the VPI strain first oothecal data and the translocation data were then possible using analysis of variance.

RESULTS

General observations:

The procedure used to prepare the testes resulted in generally well spread chromosomes. The actively dividing cells were found commonly on the periphery of the squashed testis. Thumb pressure squashes did not rupture many cell membranes and thus bivalents from single cells were not difficult to isolate. If sufficient pressure was not exerted, folded and twisted bivalents appeared which could be mistaken for ring bivalents indicative of a chiasma in each arm. Such slides were resquashed to better flatten and extend the bivalents.

During the course of the investigation a total of 190 insects were examined of which 80 yielded adequate numbers of cells in diakinesis to warrant detailed examination, i.e. 42.1%. The percentage of cockroaches with suitable numbers of cells in diakinesis varied with the group examined: first oothecae, 40.8%; second oothecae, 35.1%; third oothecae, 48.8%; and the translocation oothecae, 46.5%. These differences are probably due to my increasing ability to discern the appropriate time to sacrifice the animals.

The process of meiosis/spermatogenesis in B. germanica is synchronous with groups of 25-100 cells in a testis at the same stage of active cell division. Discounting interphase, cells arrested in various stages of meiotic prophase I were in the majority. Metaphase and anaphase I were the stages next most commonly observed. The only figures indicative of the second division of meiosis were in

metaphase II.

Diakinesis was found to be the most reliable stage for scoring chiasma frequency. As I gained experience with the material, I found I could distinguish true cross-overs from simple overlaps and incomplete repulsions of the chromosomes. Chiasmata in B. germanica are either terminal in position or terminalization of interstitial chiasmata is complete and very rapid (Fig. 1). Ambiguities concerning interstitial chiasmata and the possible occurrence of two chiasmata in one arm were resolved by incidental examination of pachytene figures. Rapid terminalization of one or both of an arm's chiasmata could mask three chiasma bivalents if diplotene-diakinesis cells alone were examined. No widespread evidence of interstitial or three chiasma events were discovered in cells from the VPI strain. Only two bivalents with three chiasmata were found. Both were at diplotene and clearly showed two chiasmata in one arm of what was probably chromosome twelve. These bivalents were in a characteristic figure-of-eight configuration.

Pilot study:

The cytogenetic analysis of twenty randomly selected male numphs yielded a mean chiasma frequency per bivalent of 1.3512 and a standard deviation 0.1320. Using an iterative approach (Sokal and Rohlf, 1969, p. 246) an optimal sample size of 56 cells per insect was determined. Two iterations were sufficient to stabilize the calculated value of n (sample size). The optimum number of cockroaches per group to be examined was calculated from a graph (Fig. 2) of σ,

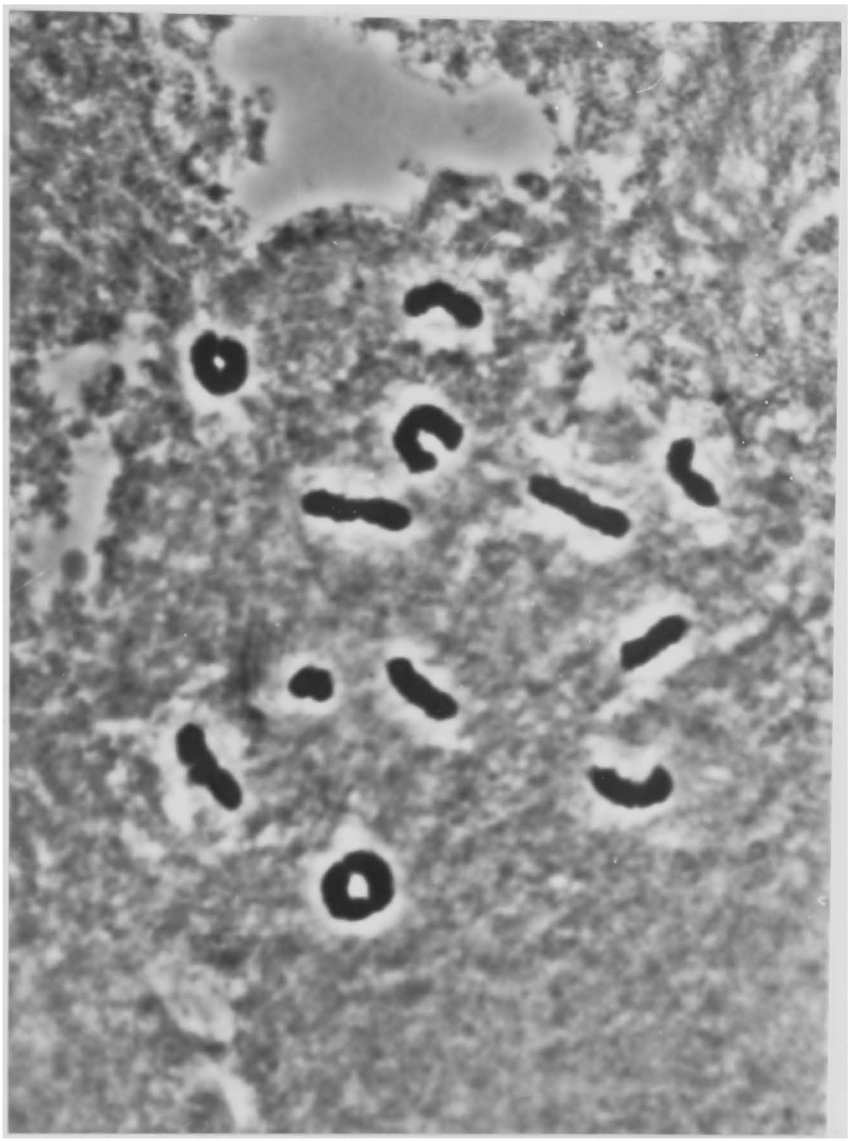

Figure 1. Blattella germanica first oothecal spermatocyte at diakinesis.

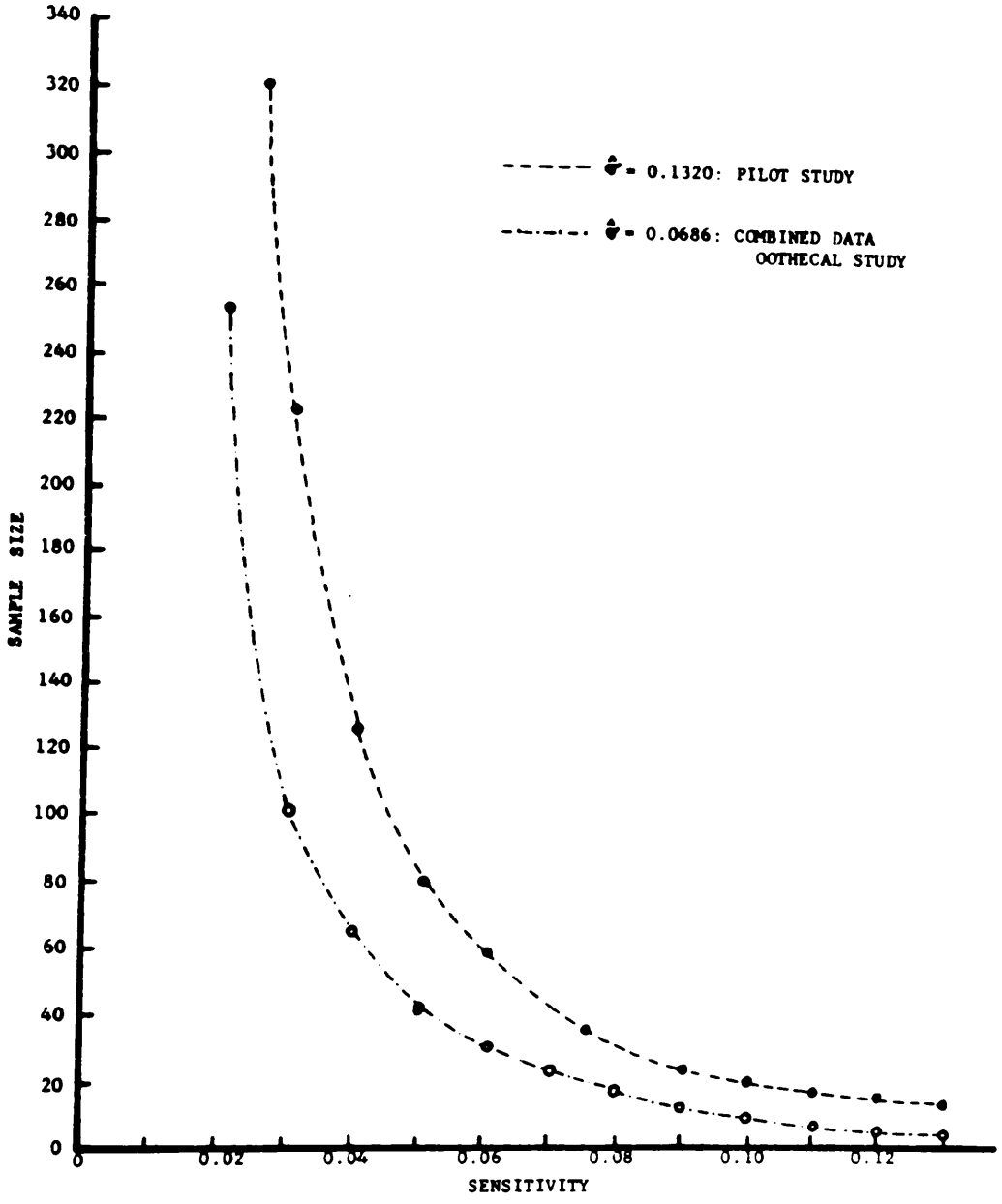

Figure 2. Graph of sample size necessary to achieve levels of sensitivity calculated for standard deviations from the pilot study and from the successive oothecal samples.

desired level of sensitivity and n, sample size. Ideally, one would wish to pick a sample size on the portion of the curve asymptotic to the Y axis. In this case the sample sizes are prohibitively high and yield a sensitivity much greater than necessary. A sample size of thirty insects was chosen as a compromise between sensitivity and practicability. As the sampling progressed it became apparent that the sample standard deviation was going to be lower than that of the preliminary sample and that a lower level of significance (0.1) would be acceptable. For these reasons the sample size was reduced to twenty cockroaches per group yielding a sensitivity of 0.1 at the 0.05 level and 0.09 at the 0.1 level of probability.

Successive oothecae study:

The mean chiasma frequencies of the first, second, and third oothecal groups are compared in Table 1. The means decrease with successive oothecae. This decrease is significant when all three means are compared by analysis of variance procedures, $P < 0.0027$, when the extremes are compared $P < 0.0007$, and when the second and third oothecal means are compared, $P < 0.0229$. The comparison of first and second oothecal means was inconclusive but bordered on significance, $P < 0.1950$ for H_o: $\hat{u}$ 1st oothecae = $\hat{u}$ 2nd oothecae. Both the ranges of the first oothecal means and the third oothecal means overlap the range of the second oothecal means to a large extent.

In terms of chiasmata per bivalent the first oothecal mean can be conceptualized as three bivalents invariably having a chiasma in each arm and a 66% chance of one of the other eight bivalents having

TABLE 1

COMPARISON OF MEAN CHIASMA FREQUENCIES PER BIVALENT OF VPI STRAIN *Blattella germanica* MALES

PARAMETER	FIRST OOTHECAE	SECOND OOTHECAE	THIRD OOTHECAE
N	20 Insects	20 Insects	20 Insects
MEAN WITH STANDARD ERROR	1.3325 ± .0184	1.2999 ± .0165;a	1.2525 ± .0113;b
RANGE	1.1846 - 1.4990	1.1462 - 1.4531	1.1710 - 1.3473
VARIANCE	0.00677	0.00543	0.00255
COEFFICIENT OF VARIATION	6.177%	5.668%	4.033%

Means followed by the same letter are not statistically different $P < 0.1$.

two chiasmata. The second oothecal mean can be interpreted as three bivalents with two chiasmata and a 30% chance of one other bivalent having two chiasmata. As implied by the analysis of variance procedure, the third oothecal mean is conceptually quite different. In this case two bivalents contain two chiasmata with the probability of a third being 78%.

The variance components of the data show a decrease similar to the oothecal means. In the progression from first to third oothecae, the sample variance drops precipitously. The third oothecal variance is about one third the first oothecal variance. The F test was used in comparisons of the individual variance components. For the hypothesis that the variances are equal the following probabilities have been calculated: first and second oothecal variances - $P < 0.6341$; first and third oothecal variances - $P < 0.0393$; and second and third oothecal variances - $P < 0.1085$. Tested as a group by Bartlett's test for homoscedasticity the three sample variances proved to be homogeneous but marginally so, X^2 calculated = 4.4087 and $X^2 \cdot 1[2] = 4.605$. The F_{max} test was more conclusive in its indication of homogeneous variances. The apparent conflicts in these results are due to differences in the calculation of the appropriate statistics, most specifically in the method of comparison - one to one or by group. Bartlett's test for homoscedasticity is highly sensitive to departures from normality (Sokal and Rohlf, 1969, p. 375) and accordingly each sample was tested using the Kolmogorov-Smirnov, g_1, and g_2 statistics. All three samples proved to be normally distributed (Figs. 3-5).

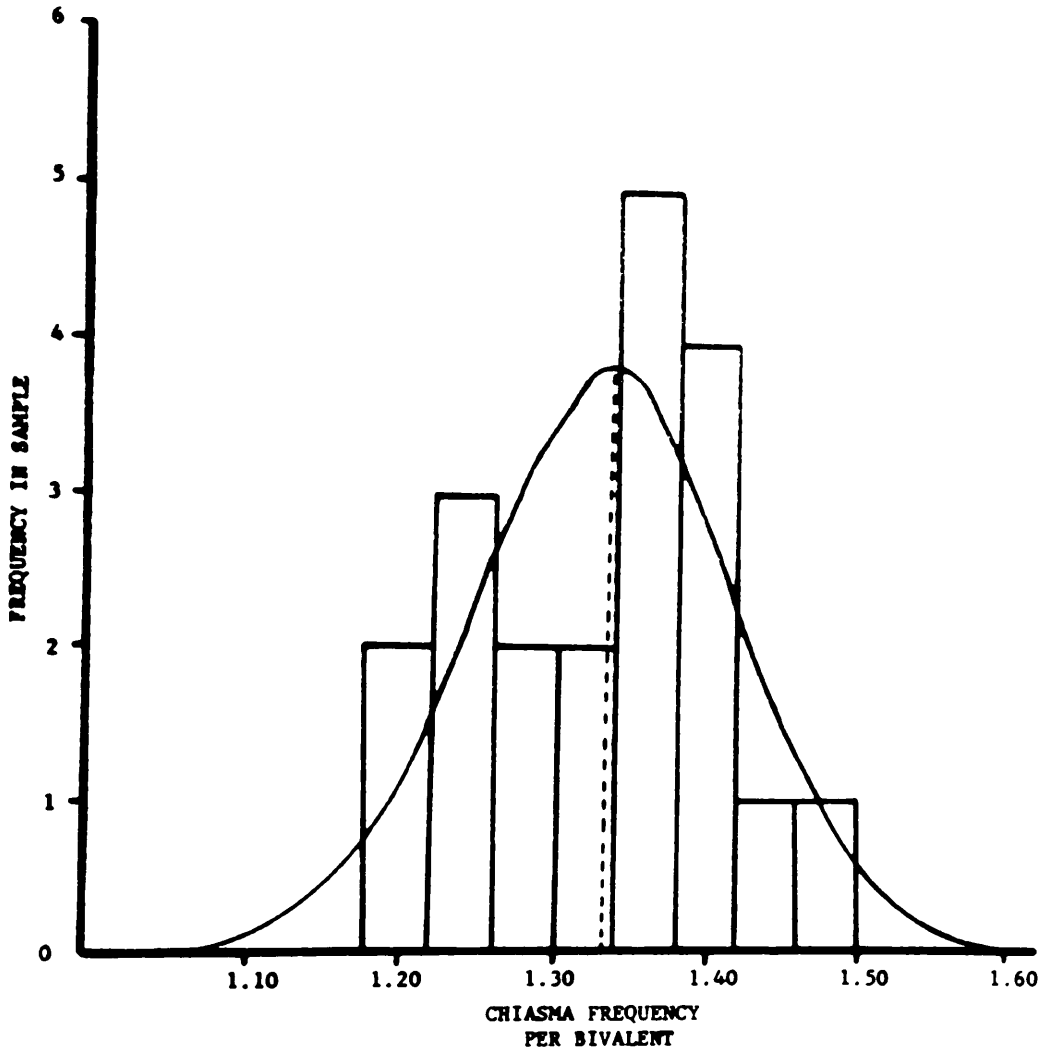

Figure 3. Distribution of the first oothecal sample of the VPI strain. Histogram - Actual frequency distribution. Curve - Calculated normal distribution corresponding to the mean and standard deviation of data.

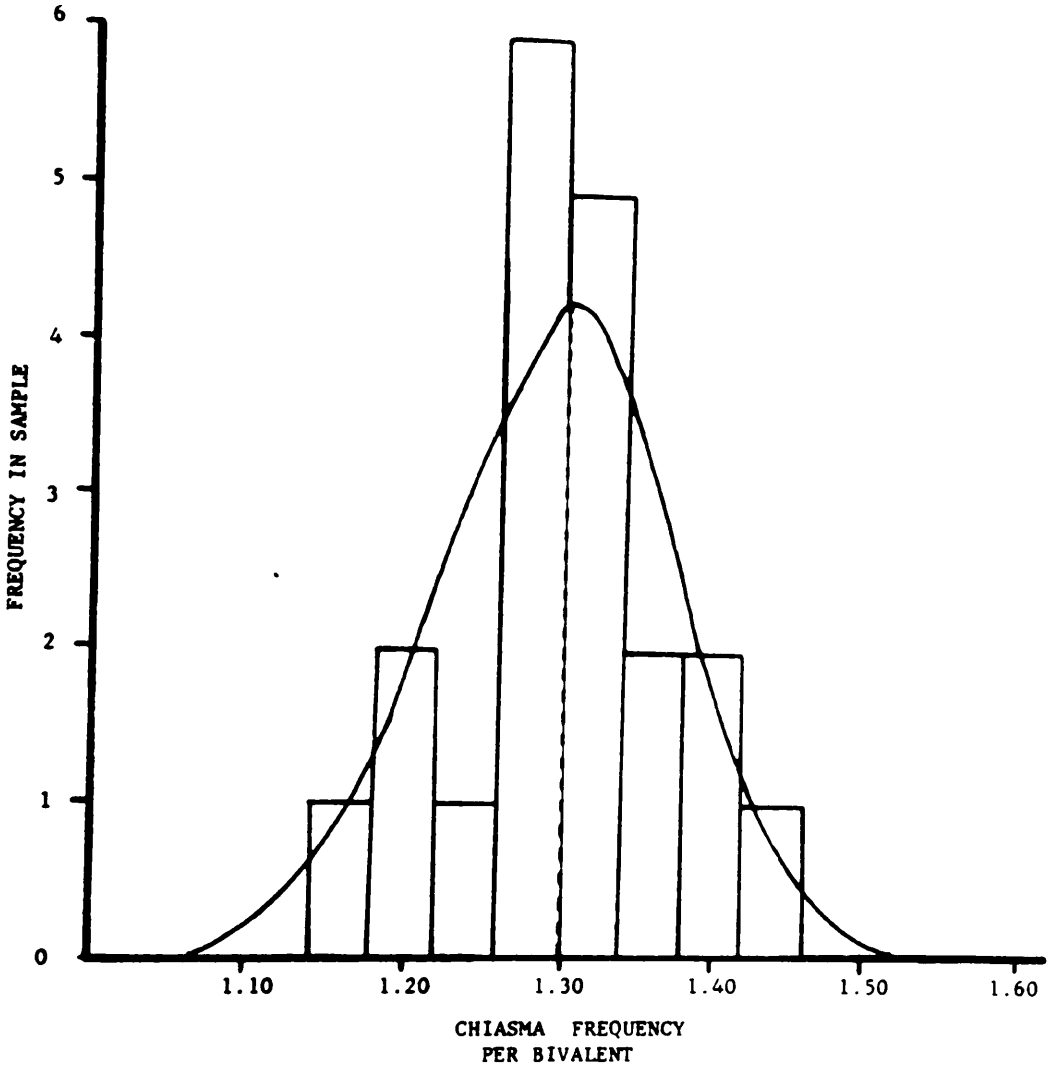

Figure 4. Distribution of the second oothecal sample of the VPI strain. Histogram - Actual frequency distribution. Curve - Calculated normal distribution corresponding to the mean and standard deviation of the data.

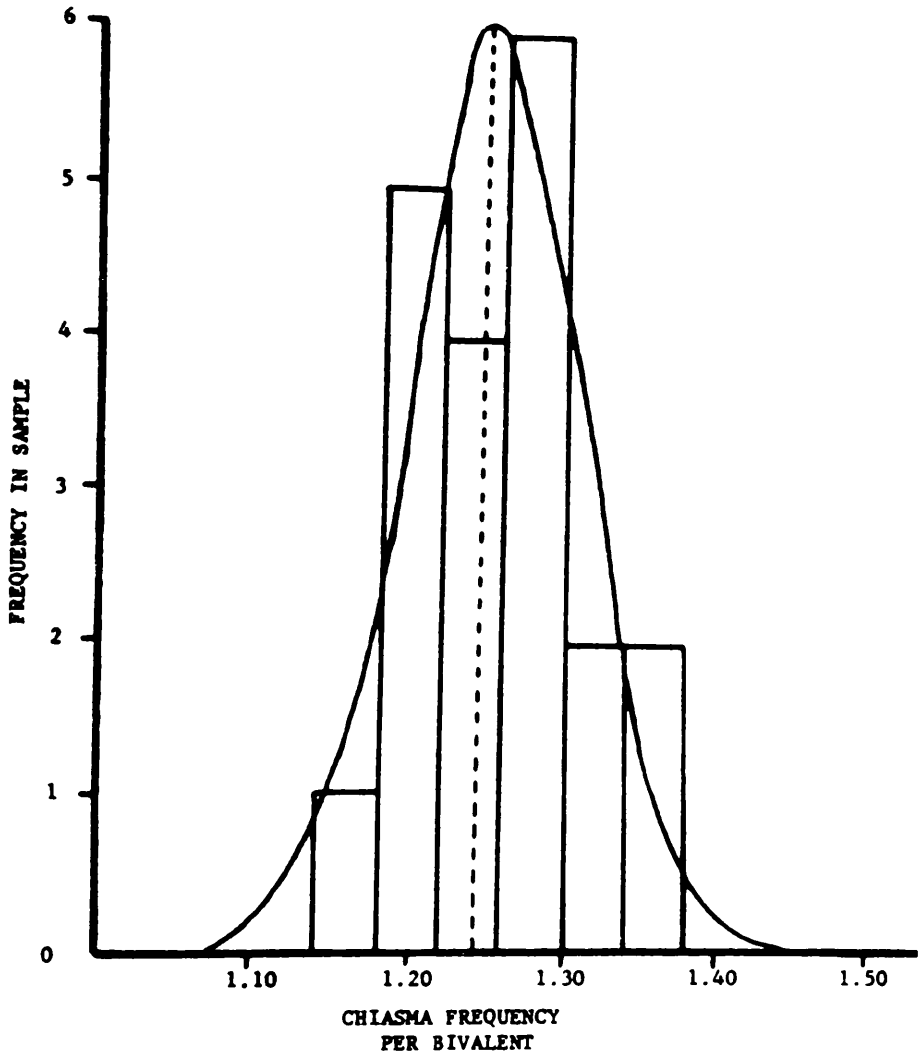

Figure 5. Distribution of the third oothecal sample of the VPI strain. Histogram - Actual frequency distribution. Curve - Calculated normal distribution corresponding to the mean and standard deviation of the data.

The means of individual cockroaches were plotted sequentially to determine if any trends in tabulation had occurred and to validate the optimal sample size calculations (Fig. 6). For all three oothecal series a general downward trend is evident. Both the second and third oothecal plots appear to have stabilized but the downward trend is still apparent in the first oothecal plot. Accordingly, a runs test (Sokal and Rohlf, 1969, p. 622) was used to detect deviations from randomness in trends in the individual means as they compare to the final sample mean. In all three cases the individual means were shown to have random deviations, $P < 0.05$.

The standard deviations of the samples taken from individual insects are compared in Table 2. These data are a measure of variability of chiasma frequency within a single insect and are reported as a mean for the groups compared. An analysis of variance showed the three statistics to be heterogeneous, $P < 0.0108$. Comparisons of the individual oothecal standard deviations by analysis of variance yielded the following results: $\hat{s}$ first oothecae $\neq \hat{s}$ second oothecae, $P < 0.0274$; $\hat{s}$ first oothecae $\neq \hat{s}$ third oothecae, $P < 0.0144$; $\hat{s}$ second oothecae $= \hat{s}$ third oothecae, $P < 0.4770$ for the hypothesis that the standard deviations are equal.

The variances of the oothecal standard deviations present a picture dissimilar to that of the variances of the oothecal means. Where the variances of the means decrease with successive oothecae and with decreased mean chiasma frequency, the variances of the oothecal standard deviations first drop from a high in the first oothecal sample to a low in the second oothecal sample. The variance then rises

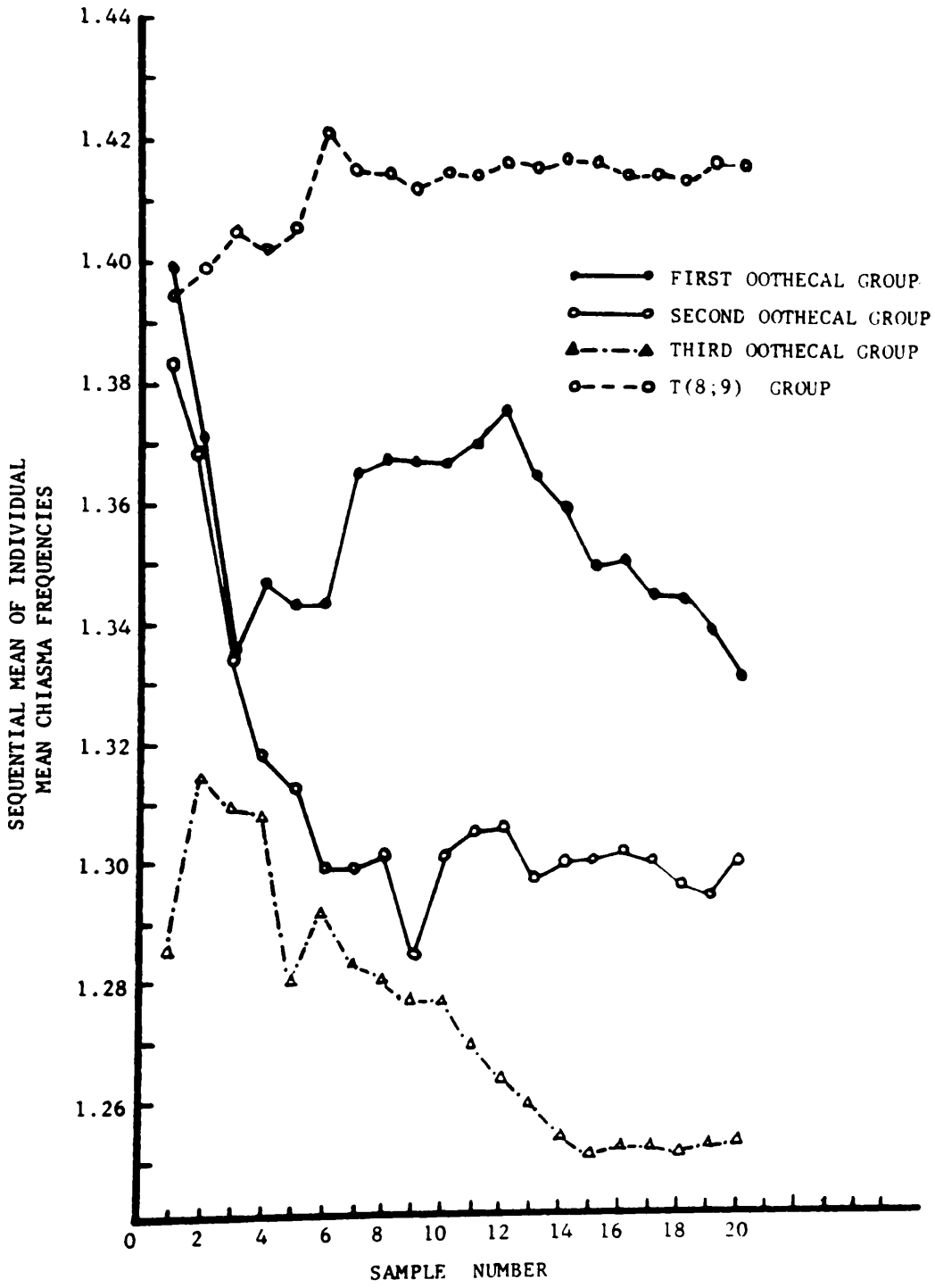

Figure 6. Plot of sequential mean chiasma frequency for three oothecal samples and T(8;9).

TABLE 2

COMPARISON OF THE MEAN STANDARD DEVIATIONS OF THE CHIASMA FREQUENCIES OF INDIVIDUAL VPI STRAIN B. germanica MALES

PARAMETER	FIRST OOTHECAE	SECOND OOTHECAE	THIRD OOTHECAE
N	20 Insects	20 Insects	20 Insects
MEAN WITH STANDARD ERROR	0.12297 ± .004;a	0.11080 ± .002;b	0.10823 ± .003;b
RANGE	.05830 - .15181	.09590 - .13410	.08600 - .13346
VARIANCE	0.0003954;a	0.0000786;b	0.0001785;c
COEFFICIENT OF VARIATION	16.303%	8.000%	12.345%
DISTRIBUTION	Leptokurtotic	Normal	Normal

Mean standard deviations followed by the same letter are not statistically different $P < 0.05$.

Variances are all significantly different $P < 0.1$.

to an intermediate level in third oothecal sample. The variances of the oothecal standard deviations are all significantly different as determined by the F test: $\hat{s}^2$ first oothecae $\neq$ $\hat{s}^2$ second oothecae, $P < 0.0009$; $\hat{s}^2$ first oothecae $\neq$ $\hat{s}^2$ third oothecae, $P < 0.0912$; $\hat{s}^2$ second oothecae $\neq$ $\hat{s}^2$ third oothecae, $P < 0.0816$.

Both the standard deviations from the second and third oothecal samples are normally distributed. However, the first oothecal sample is leptokurtotic and clumped slightly about the mean as described by the g_1 and g_2 statistics.

Plots of the sequential mean of the standard deviations of the oothecal samples show a generally declining pattern but contain enough fluctuations such that bias in sampling was not significant (Fig. 7). Runs tests for all three oothecal samples showed a pattern of random deviation from the mean standard deviation.

Translocation study:

Cytologically T(8;9) appears at diplotene as a large ring-of-four in most cells (Fig. 8). Pachytene figures seem to indicate that the breakpoints lie near the centromeres and that the arms are joined by chiasmata located distal to the breakpoints. Forty-nine insects were examined of which twenty had sufficient numbers of suitable arrested cells to make estimates of chiasma frequency, a success rate of 46.5%. In these twenty insects a total of 1348 cells were counted, $\bar{x} = 67.4 \pm 2.19$ cells per insect. Of this total number of cells, 67 showed only three chiasmata in the translocation quadrivalents, i.e. 4.97% with a chain-of-four. No cells exhibited disassociation into

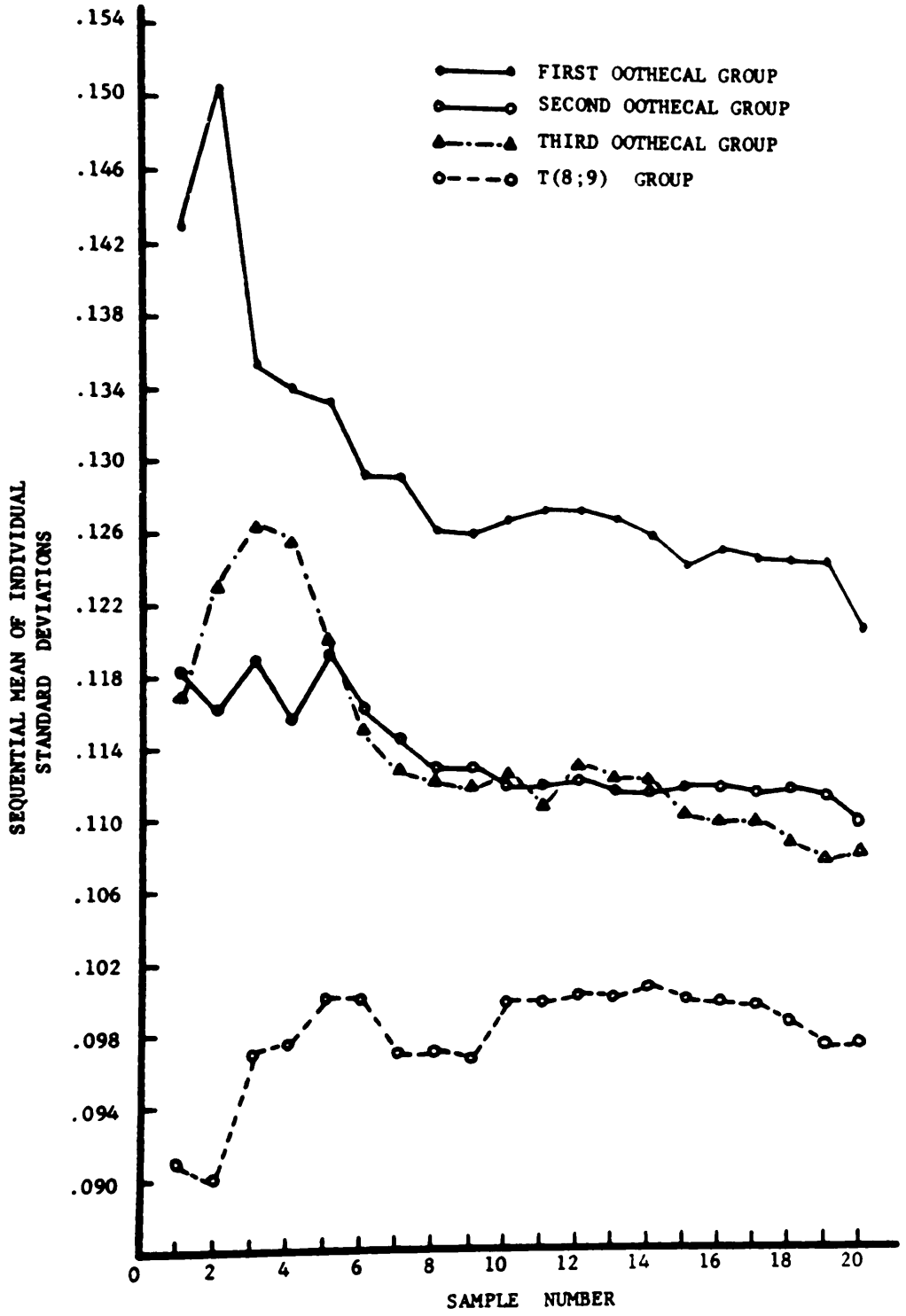

Figure 7. Plot of sequential mean standard deviations of individual cockroaches.

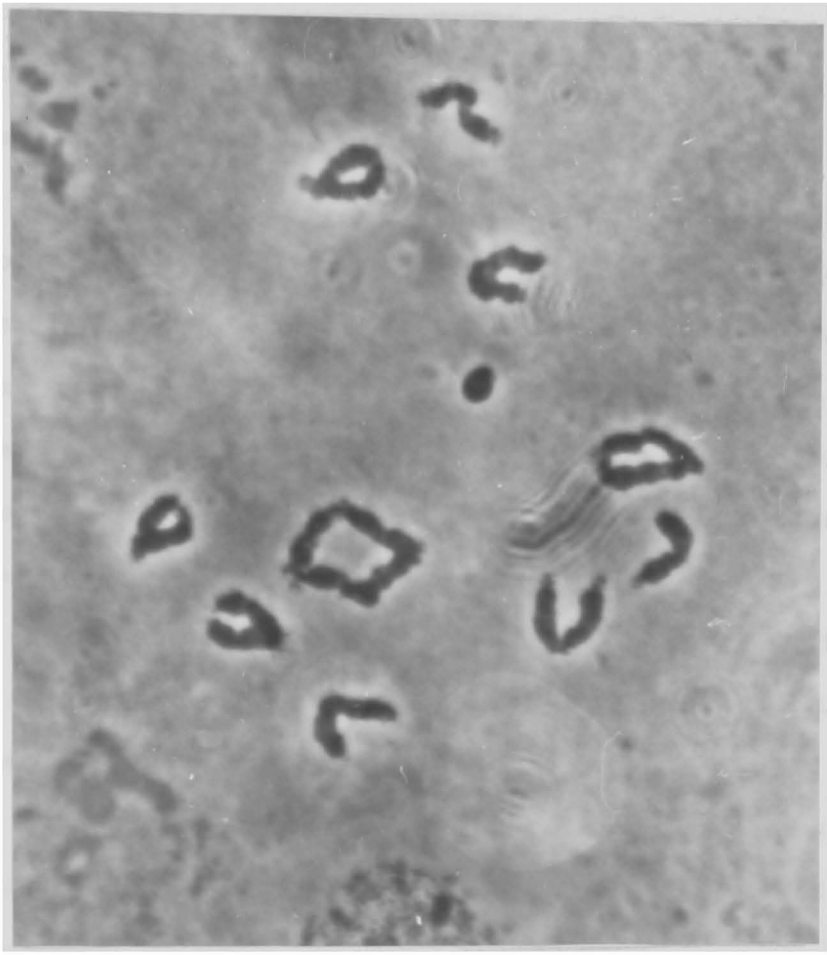

Figure 8. Blattella germanica spermatocyte at diplotene bearing a translocation, T(8;9).

bivalents or univalents. Terminalization of the chiasmata in the ring-of-four appeared to be slower than in the normal bivalent associations. This may be due to later formation or a more interstitial position of the chiasmata associated with the translocation in contrast to a more terminal position in normal bivalents.

As the insects used in this investigation were all drawn from the production of first oothecae, the statistics obtained were compared to those of the VPI strain first oothecal sample. A comparison of the mean chiasma frequency per bivalent is contained in Table 3. The mean chiasma frequency per bivalent of T(8;9) is significantly elevated in comparison to the VPI strain first oothecal mean chiasma frequency, $P < 0.0001$, F test. The variance and associated measures of sample variation (range, coefficient of variation, and standard error of the mean) are all lower for T(8;9). The variances are significantly different at the 0.0001 level.

The distribution of the individual mean chiasma frequencies presents an ambiguous picture. The Kolmogorov-Smirnov statistic indicates that the means are normally distributed at the 0.01 level. However, the histogram depicting the data, Fig. 9, appears skewed as the sample is deficient in the left tail of the curve. The g_1 statistic indicates this, $g_1 \neq 0$, $P < 0.01$. To generate this curve the class size used to group the data was reduced from .04 chiasmata per bivalent to .02 chiasmata per bivalent. The Kolmogorov-Smirnov statistic cannot account for this as it is not a comparative statistic and only considers the number of observations in each class. The g_2, kurtosis statistic, is sensitive to the continuous nature of the

TABLE 3

COMPARISON OF CHIASMA FREQUENCIES PER BIVALENT OF VPI AND TRANSLOCATION STOCK *Blattella germanica* MALES

PARAMETER	VPI FIRST OOTHECAE	T(8;9) FIRST OOTHECAE
N	20 Insects	20 Insects
MEAN WITH STANDARD ERROR	1.3325 .0184	1.4176 .0062
RANGE	1.1846 - 1.4990	1.3823 - 1.4986
VARIANCE	0.00677	0.00078
COEFFICIENT OF VARIATION	6.177%	1.968%

The means are statistically different $P < 0.001$.

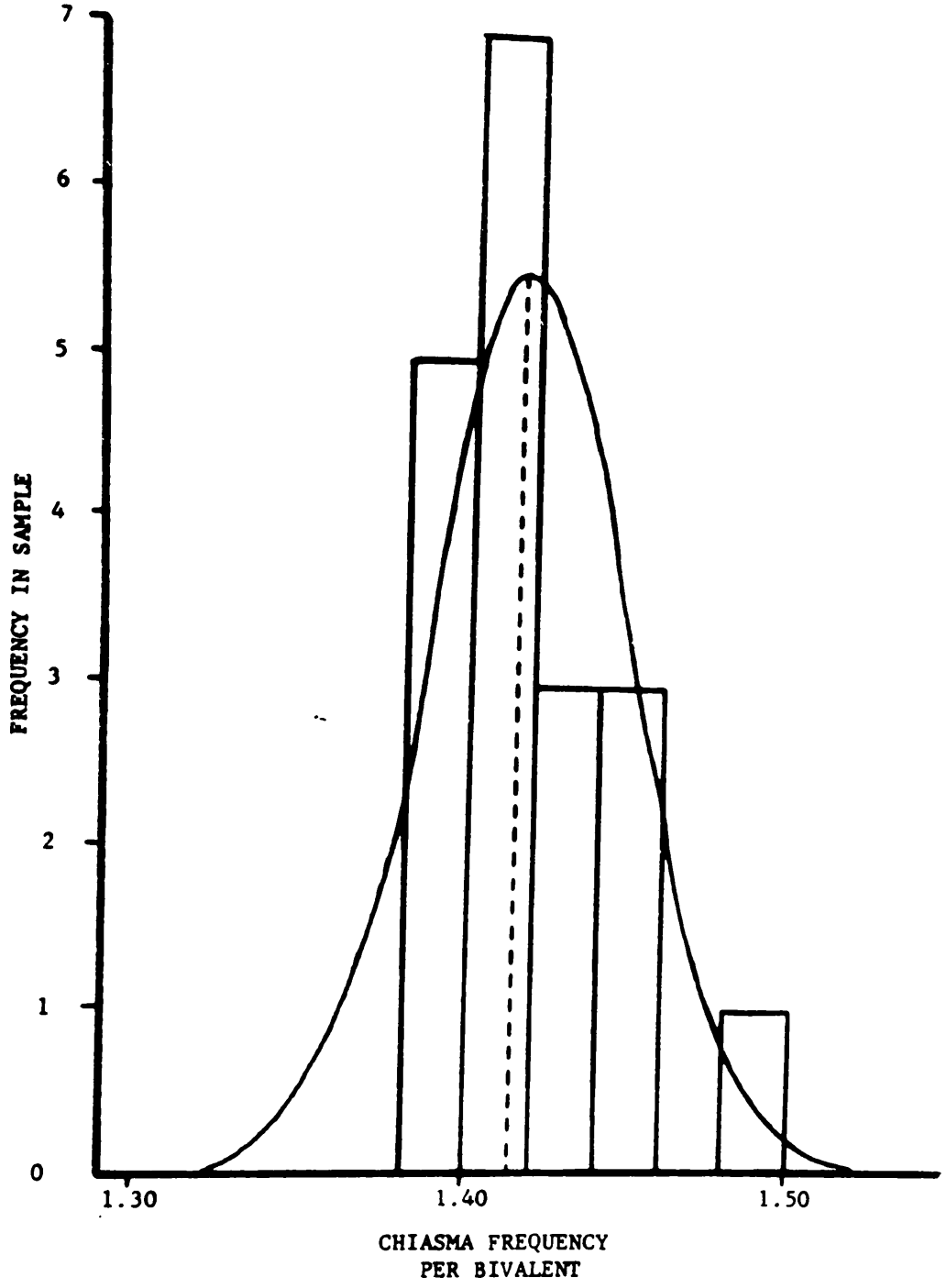

Figure 9. Distribution of the first oothecal sample of T(8;9) stock. Histogram - Actual frequency distribution. Curve - Calculated normal distribution corresponding to the mean and standard deviation of the data.

distribution as it is a parametric statistic. It indicates a clumped, leptokurtotic distribution, $g_2 \neq 0$, $P < 0.02$.

In an attempt to isolate the cause of the increased mean chiasma frequency observed in the translocation stock, the frequency per bivalent statistic was converted to total chiasmata per cell. This was done by multiplying the mean frequency per bivalent for each individual by the number of bivalents, 11, and in the case of the translocation subtracting appropriate numbers of chiasmate when chains-of-four were observed. The chiasma frequencies of the two bivalents (8;9) involved in the translocation could be estimated by subtracting chiasmata from the individual means until a rough equivalency with the chiasmata per cell of the first oothecae sample of the VPI strain was obtained. As all bivalents in B. germanica contain one chiasma, two chiasmata were subtracted from the cell totals to eliminate all "extra" chiasmata associated with the translocation. This would simulate a frequency of one chiasma per bivalent for chromosomes eight and nine. When compared to the VPI first ootheca data the two means were found to be significantly different, $P < 0.0001$. As expected the untransformed translocation mean per cell also differed from the first oothecae mean. When one chiasma was subtracted from the mean total per translocation cell a very close approximation to the mean chiasmata per cell for the VPI stock was obtained, $F = 0.09$, $P < 0.7666$. See Table 4.

TABLE 4

MEAN TOTAL CHIASMATA PER CELL: COMPARISON OF VPI FIRST OOTHECAE AND A TRANSLOCATION T(8;9)

GROUP	MEAN TOTAL CHIASMATA PER CELL	
FIRST OOTHECAE VPI	14.657 + 0.202	Normal meiosis
T(8;9)	15.594 + 0.069	Four chiasmata usually associated with bivalents 8 and 9
T(8;9) - 2 chiasmata per cell	13.594 + 0.069	Two chiasmata usually associated with bivalents 8 and 9
T(8;9) - 1 chiasma per cell	14.594 + 0.069	Three chiasmata usually associated with bivalents 8 and 9

Correlation of map distances with chiasma frequency:

The mean chiasma frequency per bivalent was converted to mean chiasma frequency per cell and then divided by two to yield a mean number of cross-overs per cell. The total number of map units in the genome was then calculated by multiplying by 100. Division by 11 yields a mean of 66.6255 map units per chromosome. This agrees well with the total map distance of 65 units reported in linkage group ten. This is the only group in which the mutant markers cover most of the chromosome. The total known map distance of the genome is considerably less than that calculated from cytological data. This was expected as several linkage groups contain only one or two closely linked mutant markers.

DISCUSSION

The results of this study indicate that further investigations of chiasma frequency in B. germanica would be profitable. The reduction in chiasma frequency variability achieved with controlled rearing conditions indicates that environmental parameters such as temperature, humidity, photoperiod, or other salient factors may have an impact on recombination in B. germanica. It would be interesting to compare field collected populations with the VPI strain. Although the German cockroach's association with man provides relatively stable conditions, vicissitudes such as insecticides, predators, fluctuating food supply, social stresses, etc., still exist. Wild B. germanica probably produce more recombinant genotypes than lab reared individuals to compensate for environmental uncertainty.

The reduction in variability achieved by controlled rearing conditions also allows smaller samples to be taken to obtain the same sensitivities indicated in the pilot study with twenty cockroaches per group. A sample size of ten cockroaches per group would be necessary for standard deviations approaching 0.1 as in the successive oothecae study. This will allow more extensive sampling, especially with regard to studies comparing different mutant or translocation stocks.

This study was based solely on examination of spermatocytes of B. germanica males. Suitable techniques for studying meiosis in general and chiasma frequency specifically in female B. germanica have not yet been developed. In general there seems to be little or no

sex difference in recombination, suggesting that chiasma frequency in females is equivalent to that in males. Sex differences in the disjunction of certain translocations as well as those found in studies of linkage group ten mutants raise the likelihood of sex differences in chiasma position (Ross and Cochran, 1975a).

Successive oothecae study:

The data show a trend in the progression from the first to third oothecal samples. Mean chiasma frequency declines and the distribution becomes much narrower about the mean. This trend can clearly be seen in Figures 3-5. It must be remembered that what has been measured is the chiasma frequency in the primary spermatocytes of male cockroaches that emerged from either a female's first, second, or third ootheca. The effects of this reduction in mean chiasma frequency would not be realized until the F_2 generation, the "grandchildren" of the original female cockroaches. Therefore, the amount of variability released into a male cockroach's offspring is in part determined by that male's order of birth or the age of its mother.

Reduced chiasma frequencies in aging oocytes is a well known phenomenon (Henderson and Edwards, 1968; Speed, 1977). Reduced recombinant genotypes have been noted in the offspring of older B. germanica females (Ross and Cochran, 1975a). However, there is no evidence to suggest that this reduction is heritable. It is hard to conceive of a mechanism that would confer heritibility on an age dependent trait such as chiasma frequency in oocytes.

What particular adaptive advantage can be attached to this trend of lower chiasma frequencies? It must be assumed that chiasma frequency itself is an adaptive trait especially in reference to recombination (Bodmer and Parsons, 1962). If chiasmata had the sole function of maintaining associations of chromosomes after the dissolution of the synaptonemal complex, no double or multiple chiasma events would be expected to occur except in the case of gross chromosome mutations such as translocations. The widespread occurrence of two chiasmata in bivalents of B. germanica is indicative of their value in producing cross-overs, genetic recombination, above their role in mediating desynapsis.

All serious conceptualizations of the crossing-over process require breakage and reunion of the chromatids and synthesis of new DNA. This breakage, repair, and synthesis process is subject to errors leading to point and/or frame-shift mutations. Errors in chiasma formation have been implicated in the formation of U-type exchanges producing acentric fragments and anaphase bridges (Jones, 1968; Giraldez and Lacadena, 1978). These are serious consequences as euchromatic regions are generally involved (Bostock and Summer, 1978; Emerson, 1969). Anaphase bridges have been noticed in B. germanica spermatocytes but their consequences have not been investigated. It is doubtful that it would be of adaptive value to an organism to form chiasmata above an optimal level because of the possibility of producing a genetically defective germ cell. Since error and mutation production are probabilistic events, a range of chiasma frequencies about the mean can be found even in highly adapted or phenotypically

canalized species.

If the first oothecal mean chiasma frequencies are adaptive because of the recombinants produced as a result, it follows that the reduction in chiasma frequency in the second and third oothecal groups is also adaptive. The adaptive value in this case is a reduction of the amount of recombination produced. If a female cockroach has survived to produce one ootheca it may be advantageous for her future offspring to reduce recombination in order to preserve favorable gene sequences and to reduce the possibility of producing an error through faulty chiasma formation. As subsequent oothecae are produced the probability that the parental genotypes were of a superior nature would increase. It would become more and more advantageous to their offspring to pass on maternal and paternal gene sequences relatively intact.

If chiasma frequency is a neutral character, as claimed by Shaw (1972) for Schistocerca, a far greater amount of variation would be expected in B. germanica. Table 1 shows the coefficients of variation are all in the range of four to six percent of the mean. This is not excessive. The distributions of the mean chiasma frequencies are all normal and tend toward leptukurtosis in the third oothecal data. If the recombination value of chiasma formation is low or selectively neutral, a distribution skewed heavily toward one chiasma per bivalent would result. A platykurtic curve, more observations in intermediate ranges in proportion to the mean and tails of the curve, would be expected if chiasma frequency was random with the constraints of a lower mechanical level and a higher level produced through interference.

One notable aspect of the T(8;9) data is the far lower variance of the T(8;9) means as compared to the first oothecal sample of the VPI strain (Table 1). A portion of this reduction in the variance is due to the shape of the T(8;9) distribution, Figure 7. The histogram is skewed toward the lower end of the distribution. This is a function of an artificially high lower limit imposed by the regular formation of ring-of-four associations. The tendency to be skewed narrows the distribution and thereby lowers the variance. Another possibility concerns genetic control of chiasma formation. Genotypic control of chiasma frequency has been studied in relatively few species; even less is known concerning the nature of genes that determine or influence variability in chiasma frequency. The lower variance associated with T(8;9) may possibly provide evidence of such genes. Translocation stocks of B. germanica are by nature highly inbred. Inbreeding in the T(8;9) stock may have resulted in greater degrees of homozygosity for such genes than found in the laboratory wild-type stock.

Table 4 indicates that the translocation had an intrachromosomal effect of raising chiasma frequency in bivalents 8 and 9 by one chiasma. That is to say, normally bivalents 8 and 9 have three chiasmata between them. Of the complement of eleven autosomal bivalents, 8 and 9 are the fifth and fourth longest, respectively. The first oothecal data indicate two bivalents generally have two chiasma and a third bivalent in 66% of the cells. It appears that double chiasmata are not correlated with increasing chromosome length in B. germanica as hypothesized by Henderson (1963) and Fox (1973) for

Schistocerca and Fogwill (1958) for Lilium and Fritillaria.

Henderson (1963) found, in spite of an overall positive relationship between bivalent length and chiasma frequency, that mid-length bivalents had a slightly higher chiasma frequency than expected from their proportional length. Klasterska et al (1974) discovered extreme localization of chiasmata in the grasshopper, Bryodema tuberculata, through the use of meiotic C-banding, which darkly stains areas of constitutive heterochromatin. They found that chiasmata were limited in the position they occupy by blocks of heterochromatin, especially large centric blocks. The chiasmata formed preferentially on the distal margins of these blocks. Thus, chiasmata were restricted to distal locations on the bivalents with large heterochromatic blocks as a result. Morgan (1978) found a similar situation in the newt, Triturus cristatus. Chiasmata were absent from one arm-pair of the longest bivalent in the karyotype. C-banding techniques have shown this arm to be "heteromorphic", composed almost entirely of heterochromatin.

While only chromosome-banding techniques, applied to meiotic cells, are likely to resolve the question of bivalent specific crossing-over in B. germanica, some speculation is possible. The morphology of chromosome 12 as depicted by Ross and Cochran (1975b) is suggestive of large heterochromatic blocks in one arm. Ross and Cochran (1975b) reported that chiasmata failed to form in the darkly staining arm on occasion, although it was the other arm-pair that frequently opened into a chain formation in translocations. It was noted in this study and others (Ross, pers. communication) that some of the ring bivalents

indicative of a double chiasma event were very small. It is likely that B. germanica does not conform to the classical chromosome length-chiasma frequency hypothesis.

In T(8;9) there is no apparent evidence of significant inter-chromosomal effect on chiasma frequency as has been reported in many other organisms (Lucchesi and Suzuki, 1968). This may be either a characteristic of this translocation only or be of much wider occurrence in B. germanica. Only further investigations using the other twenty translocation stocks will begin to resolve this question.

Conclusions:

This demonstration that chiasma frequency can be readily analyzed opens many new avenues for continued cytogenetic studies of B. germanica. The variability is sufficiently low for detection of significant differences between individuals and groups without extensive sampling. However, the genetic basis for this relatively low variability remains to be elucidated. The tendency for close linkage between mutant markers in this species is accounted for by the indication that chiasmata are terminal or sub-terminal in this species (Fig. 1). Eventually the analysis of chiasma frequency and position may shed light on the evolution of B. germanica, a species belonging to the most ancient extant group of winged insects. This may be especially valuable in a comparative sense as many of the organisms used in investigations of this kind are of far more recent origin and tend to exist in patchy habitats. This study also opens the way for more extensive investigations of the relationship bewteen chiasma

frequency and disjunction in translocation heterozygotes. There are few other organisms suitable for such studies outside of certain plant species, e.g. grasses, rye, and maize.

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VITA

Clifford Bennett Keil was born June 17, 1952 in Garfield Heights, Ohio. He attended North High School in Eastlake, Ohio and South Broward High School in Hollywood, Florida from which he graduated in 1970. Mr. Keil did his undergraduate work at the University of Michigan in the School of Natural Resources. He received his Bachelor of Science in Wildlife Management and Forestry in May, 1976. The summer of 1976 was spent in the employ of the University of Michigan as a research associate. He came to the Department of Entomology at V.P.I. and S.U. in September, 1976 and received a research assistantship in June, 1977.

BASIC STUDIES OF CHIASMA FREQUENCY IN MALE *Blattella germanica* (L.)

by

Clifford Bennett Keil

(ABSTRACT)

This investigation represents the first thorough examination of chiasma frequency in the primitive orthopteroid insect, *Blattella germanica* (L.). The data obtained are useful both in advancing our knowledge of cockroach genetics and in stimulating comparative cytogenetic studies. A pilot study was conducted to ascertain appropriate sample sizes and to assess the feasibility of more extensive investigations.

Experimental animals were reared in a constant temperature and humidity chamber and segregated according to whether they emerged from a first, second, or third ootheca. The testes of third to fourth instar male nymphs were removed, stained with acetic orcein, and examined for suitable numbers of cells at diplotene-diakinesis. Analysis of the chiasma frequency data showed a decrease in mean chiasmata per bivalent: 1.3325 ± 0.0184 for the first oothecal group, 1.2999 ± 0.0165 for the second oothecal group, and 1.2525 ± 0.0113 for the third oothecal group. The variances decline with the means.

The chiasma frequency of a stock heterozygous for a reciprocal translocation involving two mid-sized chromosomes, eight and nine, was compared to the wild-type frequencies. In comparison with the

first oothecal group, an elevated chiasma frequency, 1.4176 ± 0.0062 chiasma per bivalent, was found. The translocation had the effect of raising chiasma frequency in the two bivalents involved. No inter-chromosomal effect on chiasma frequency was observed, due to the presence of the translocation. It was noted that in B. germanica, chiasma frequency is probably not positively correlated with bivalent length.